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CERTIFIED REFERENCE MATERIAL

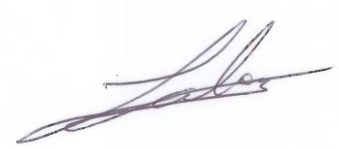
Name of the material :	**Glucose, creatinine, total Glycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, urea and uric acid in human frozen serum**
Code of the CRM :	**LNE CRM Bio 302a**
Organism responsible of the emission of the certificate :	**LNE**
Lot number :	**A20210426D**
Sample number :	**302B4C3 (example)**
Certification date :	**26 April 2021**
Issue date :	**26 April 2021**

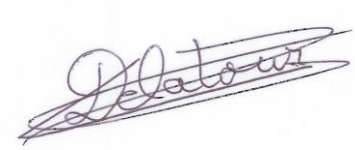
Head of the Department
Biomedical and organic Chemistry

Béatrice LALERE

The Technical Officer

Vincent DELATOUR





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1. DESCRIPTION ET IDENTIFICATION

The Certified Reference Material LNE CRM Bio 302a corresponds to a pool of Human Frozen Serum that were prepared by Solomon Park Research Laboratories (Kirkland, WA, USA) according to the Clinical Laboratory Standards Institute (CLSI) C37-A guidelines describing the preparation and validation of commutable frozen human serum pools as Secondary Reference Materials for Cholesterol Measurement [1]. For glucose, creatinine, total cholesterol, total glycerides, HDL-C, LDL-C, urea and uric acid, the concentrations derived from naturally occurring levels of the analytes in donors that were selected to this purpose. Each unit of LNE CRM Bio 302a comprises one 0.7 mL vial for which the concentration of the following parameters has been certified : glucose, creatinine, total glycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, uric acid and urea. Sera are stored in 1mL plastic vials with screw-cap. After thawing, samples appear as a transparent yellow liquid.

LNE CRM Bio 302a is a Certified Reference Material produced in compliance with requirements of ISO 17034 : 2016 [2].

2. VALIDITY OF THE CERTIFIED AMOUNT-OF-SUBSTANCE RATIO

The certified concentrations are valid, within the measurement uncertainty specified, until **31 December 2022** if storage and transportation recommendations are met (See section “Storage”) and if conditions of use are respected (See section “Operating instructions”). After that period, the materials will be subject to a new certification campaign. If the certified concentrations are still valid, the validity of LNE CRM Bio 302a will be extended. If the materials exhibit insufficient stability, users will be informed and a new certificate will be released and sent to them. The certified concentration values of LNE CRM Bio 302a are invalid if the serum materials have been deteriorated or are mishandled.

3. INTENDED USE

LNE CRM Bio 302a is an internationally certified reference material intended for use as quality control material to assess the measurement bias or measurement uncertainty of established or new measurement procedures for the determination of creatinine, glucose, total cholesterol, total glycerides, HDL-cholesterol and LDL-cholesterol in human serum.

Given that the 98/79/EC Directive and the 2017/746 regulation do not apply to internationally certified reference materials, LNE CRM Bio 302a is not considered as an in vitro diagnostic medical device and doesn’t require CE marking. Still, LNE CRM Bio 302a should not supersede the control materials needed to establish or verify performances of in vitro diagnostic medical devices.

4. SAFETY PRECAUTIONS

LNE CRM Bio 302a is intended for in-vitro diagnostic use only. It should be handled as biohazardous material capable of transmitting infectious disease. The supplier of the serum has reported that each donor unit of serum used in the preparation of this product has been tested by an FDA-approved method and found non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV). Since no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material, this human blood-based product should be handled and disposed according to the commendations of the concerned local and national legislation and regulations for potentially infectious human or blood specimen.

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5. OPERATING INSTRUCTIONS

Prior to use, LNE CRM Bio 302a should be removed from the freezer and allowed to stand at room temperature (between 18°C to 25°C) until thawed and gently mixed by repeated inversion of the vials (about 20 times, being careful not to generate foam). After the material is thawed to room temperature, it should be used immediately. LNE CRM Bio 302a is intended for a unique use : the certified concentrations are not be valid for opened bottles and for materials that have been thawed and re-frozen or that have been stored more than 12 hours at temperatures higher than +4°C because the stability of the analytes subjected to such conditions has not been investigated. The certified concentrations are not valid for samples that have been diluted.

6. STORAGE

LNE CRM Bio 302a is shipped frozen on dry ice or at –30°C if transportation doesn't exceed 1 week. Upon receipt, it should be stored away from light at or -80°C ± 20°C until analysis.

7. COMMUTABILITY TESTING

Samples commutability was assessed through a commutability study that involved 7 clinical laboratories using the most popular field methods in France: Roche Cobas, Siemens Vista, Abbott Architect, Beckman DxC, Ortho-CD Vitros, Beckman AU, Siemens Advia and Siemens EXLThe same 33 clinical specimens consisting in fresh non-modified sera were measured in triplicate in the same analytical sequence as LNE CRMs. The clinical specimens were selected so that concentrations of the different parameters bracket those of LNE's CRM. Clinical specimens were collected on Day 1, stored at +4 ± 3°C until shipment on Day 2 and measured on Days 3 and 4. The statistical analysis was performed according to the difference in bias approach described in the IFCC recommendations on commutability assessment [3, 4]. Acceptance criteria were 7% for glucose, 8.2% for creatinine, 8.5% for total cholesterol, 8% for triglycerides, 11.1% for HDL-C, 15.0% for LDL-C, 9.0% for urea and 7.7% for uric acid. Results of the commutability assessment show that for the different analytes, LNE CRM Bio 302a is commutable for the main routine methods except Ortho CD Vitros methods for glucose and creatinine, Beckman DxC methods for creatinine and total glycerides, Siemens Advia method for urea and Siemens Vista method for HDLc, for which commutability can be considered questionable (Table 2). The complete experimental results and data analysis are detailed in the certification report that can be made available upon request.

Table 2 : Commutability results LNE CRM Bio 302a

C stands for commutable, NC non commutable and I means that the statistical analysis was inconclusive. NA means that commutability could not be assessed.

LNE CRM Bio 302a	Glc	Creat	TC	TG	HDL	LDL	Uric Acid	Urea
Siemens Vista	C	C	C	C	I	C	C	C
Roche Cobas	C	C	C	C	C	C	C	C
Beckman DxC	C	I	C	I	C	NA	NA	C
Ortho CD Vitros	I	I	C	C	C	C	C	C
Abbott Architect	C	C	C	C	C	NA	C	C
Siemens Advia	C	C	C	C	C	C	C	I
Beckman AU	C	C	C	C	C	NA	C	C
Siemens EXL	C	C	C	C	C	NA	C	C

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8. HOMOGENEITY TESTING

Homogeneity testing of LNE CRM Bio 302a was performed on one sub-sample taken from fifteen bottles and four sub-samples taken from each bottle. Measurements were performed on a Siemens Vista analyzer (University Hospital Clermont-Ferrand, France). The sample size taken for homogeneity testing was about 2µL. No significant differences in the between and within-bottle variances were found using F-test at 95 % confidence level. The Student's t-test also indicated no significant differences between the means obtained from between and within bottle analyses. The materials were thus considered as sufficiently homogeneous. Results of the homogeneity study are detailed in the certification report that is available upon request.

9. STABILITY TESTING

Stability testing of LNE CRM Bio 302a was performed over 35 months at -80°C, 12 months at -20°C and 4 days at +4°C and +25°C. When stored at -80°C, the slope of the fitted regression line was found to be insignificant at 95% confidence level [5] and the serum materials were considered sufficiently stable. Moreover, it has been shown by other National Metrology Institutes that pools of human serum produced according to CLSI C37-A guidelines and stored at temperatures inferior to –60°C are stable over several years. The materials are stable for 12 months when stored at -20°C and 4 days when stored at +4°C. Results of the stability study are detailed in the certification report that is available upon request.

10. CERTIFIED VALUES

The certified concentrations for glucose, creatinine, total glycerides, total cholesterol, HDL-C and LDL-C in LNE CRM Bio 302a are provided in Table 3. Each certified concentration value is the mean of 6-18 measurements determined with the reference measurement procedures described hereafter (see section "Analytical methods"). The final result is expressed as the certified concentration value ± the expanded uncertainty. The associated measurement uncertainty of each certified concentration value was estimated in accordance to ISO/IEC Guide 98-3:2008 [6]. The uncertainty provided with each certified value is an expanded uncertainty that is calculated as $U = ku_c$, where u_c is the combined uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. For the certified values shown in Table 3, $k = 2$. Total glycerides results are expressed as triolein in mmol per liter. Results of the certification campaign are detailed in the certification report that is available upon request.

Table 3 : Certified concentrations for glucose, creatinine, total glycerides, total cholesterol, LDL-C and HDL-C in LNE CRM Bio 302a

Parameter	Certified Value
Glucose	7.12 ± 0.11 mmol/L
Creatinine	86.6 ± 1.6 µmol/L
Total Glycerides	1.45 ± 0.04 mmol/L
Total Cholesterol	5.55 ± 0.11 mmol/L
HDL Cholesterol	1.47 ± 0.08 mmol/L
LDL Cholesterol	3.48 ± 0.04 mmol/L
Urea	5.46 ± 0.08 mmol/L
Uric acid	356.0 ± 6.4 µmol/L

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11. ANALYTICAL METHODS

For glucose [6], total glycerides [7] and total cholesterol [8], values have been certified by reference measurement procedures relying on Gas Chromatography Isotope Dilution mass Spectrometry (GC-IDMS) listed in JCTLM database. For HDL-C and LDL-C, values were certified at the CDC by Beta Quantification with cholesterol being determined by the Abell Kendall method [9]. For creatinine and uric acid, values have been certified by reference measurement procedures relying on Liquid Chromatography Isotope Dilution mass Spectrometry (LC-IDMS) listed in JCTLM database.

LNE is accredited according to standards ISO EN 17025 [12] and ISO EN 15195 [13] for the measurement of glucose, creatinine, total cholesterol, total glycerides, LDL-C and HDL-C. LNE has CMCs (Calibration and Measurement Capabilities) for the measurement of glucose, creatinine and total cholesterol in human frozen serum [14]. LNE is recognized as a Reference Measurement Services Provider by the JCTLM for the measurement of glucose, creatinine, total cholesterol, LDL-C and HDL-C in frozen human serum.

The molar concentrations mentioned in Table 3 were calculated from mass fractions, the measured serum density of 1,0260 ± 0,0020 and the relative molecular masses of the analytes [113.12 (creatinine), 180.16 (glucose), 386.65 (cholesterol), 885.43 (triolein),] 60.056 g / mol (urea), 168.1103 g / mol (uric acid)].

12. METROLOGICAL TRACEABILITY

The certified concentration values are traceable to the SI through the use of metrological procedures and the following certified reference materials used as primary calibrators: NIST SRM 917c for glucose determination, NIST SRM 914a for creatinine determination, NIST SRM 911c for cholesterol determination, NIST SRM 1951c and NIST SRM 909c for total glycerides determination, NIST SRM 912a for urea determination and NIST SRM 913b for uric acid determination.

13. CERTIFICATE REVISION RECORDS

Date	Name of responsible	Reason and content of the revision
04/11/2016	Vincent DELATOUR	Revision A : initial certificate
01/07/2019	Vincent DELATOUR	Revision B : - Extension of the period of validity - Revision of the intended use section
16/02/2021	Vincent DELATOUR	Revision C : - Extension of the period of validity - Revision of certified values for LDLc and HDLc
26/04/2021	Vincent DELATOUR	Revision D : - Extension of the period of validity - Correction of the molecular masses of urea and uric acid

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14. REFERENCES

[1] CLSI. Preparation and validation of commutable frozen human serum pools as secondary reference materials for cholesterol measurement procedures; approved Guideline. CLSI document C37-A.Wayne, Pennsylvania: CLSI; 1999.

[2] ISO - International Organization for Standardization. ISO 17034 : General requirements for the competence of reference material producers; 2016.

[3] Miller WG, Schimmel H, Rej R, Greenberg N, Ceriotti F, Burns C et al. IFCC working group recommendations for assessing commutability part 1: General Experimental Design. Clin Chem. 2018 ;64(3):447-454.

[4] Nilsson G, Budd JR, Greenberg N, Delatour V, Rej R, Panteghini M, Ceriotti F, Schimmel H, Weykamp C, Keller T, Camara JE, Burns C, Vesper HW, MacKenzie F, Miller WG. Recommendations for Assessing Commutability Part 2: Using the Difference in Bias between a Reference Material and Clinical Samples. Clin Chem. 2018;64(3):455-464.

[5] ISO/IEC Guide 98-3:2008 Incertitude de mesure -- Partie 3: Guide pour l'expression de l'incertitude de mesure

[6] Delatour V, Lalere B, Saint-Albin K, Peignaux M, Hattchouel JM, Dumont G, De Graeve J, Vaslin-Reimann S, Gillery P. Continuous improvement of medical test reliability using reference methods and matrix-corrected target values in proficiency testing schemes: application to glucose assay. Clin Chim Acta. 2012; 413(23-24):1872-8. doi: 10.1016/j.cca.2012.07.016.

[7] Edwards SH, Stribling SL, Pyatt SD, Kimberly MM. Reference measurement procedure for total glycerides by isotope dilution GC-MS. Clin Chem. 2012;58(4):768-76. doi: 10.1373/clinchem.2011.177063

[8] Heuillet M, Lalere B, Peignaux M, De Graeve J, Vaslin-Reimann S, Pais De Barros JP, Gambert P, Duvillard L, Delatour V. Validation of a reference method for total cholesterol measurement in human serum and assignation of reference values to proficiency testing samples. Clin Biochem. 2013; 46(4-5):359-64. doi: 10.1016/j.clinbiochem.2012.11.026.

[9] Myers, GL; Cooper, G.R.; Greenberg, N.; Kimberly, M.M.; Waymack, P.P.; Hassemer, D.J.; Standardization of Lipid and Lipoprotein Measurements; In Handbook of Lipoprotein Testing, 2nd ed., Rifai, N.; Warnick, G.R.; Dominiczak, M.H., Eds.; American Association for Clinical Chemistry (AACC), Washington, D.C., 717–748

[10] Stokes P, O'Connor G. Development of a liquid chromatography-mass spectrometry method for the high-accuracy determination of creatinine in serum. Chromatogr. B., 2003, 794, 125-136. doi : 10.1016/S1570-0232(03)00424-0

[11] Dodder NG, Tai SS, Sniegoski LT, Zhang NF, Welch MJ. Certification of creatinine in a human serum reference material by GC-MS and LC-MS. Clin Chem. 2007 ; 53(9):1694-9. doi : 10.1373/clinchem.2007.090027

[12] ISO - International Organization for Standardization. ISO 17025 : general requirements for the competence of testing and calibration laboratories; 2017.

[13] ISO - International Organization for Standardization. ISO 15195 : laboratory medicine- requirements for reference measurement laboratories; 2018.

[14] http://kcdb.bipm.org/appendixC/QM/FR/QM_FR_10.pdf (accessed in July 2019).

End of the certificate