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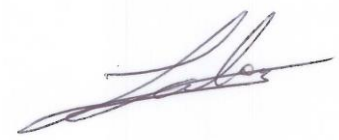
CERTIFIED REFERENCE MATERIAL

Name of the material :	**HbA_{1c} in lyophilized hemolysates**
Code of the CRM :	**LNE CRM HbA1c 401**
Organism responsible of the emission of the certificate :	**LNE**
Batch number :	**A20210427D**
Sample number :	**N/A**
Certification date :	**27 April 2021**
Issue date :	**27 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 HbA1c 401 corresponds to lyophilized human blood hemolysates that were produced by Aalto Scientific Ltd. (Carlsbad, CA, USA). Blood collection was performed in agreement with human subject protection laws and following an IRB approved protocol. Each unit of the certified reference material LNE CRM HbA1c 401 contains lyophilized human blood hemolysates intended to be reconstituted with 0.5 mL of water (See Section 5), resulting in a solution for which the amount-of-substance fraction (IFCC unit [HbA1c/(HbA1c+HbA0)]) has been certified. LNE CRM HbA1c 401 is a secondary standard produced in compliance with requirements of ISO 17034:2016 [1]. LNE CRM HbA1c 401 is listed in JCTLM Database as Higher-order Reference Material [2].

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

The certified amount-of-substance ratio (IFCC unit [HbA1c/(HbA1c+HbA0)]) is valid, within the measurement uncertainty specified, until **31 December 2022** if storage and transportation recommendations are met (See section 6) and if conditions of operating instructions are respected (See section 5). After that period, the materials will be subject to a new certification campaign. If the certified amount-of-substance ratio is still valid, the validity of LNE CRM HbA1c 401 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 amount-of-substance ratio of LNE CRM HbA1c 401 is invalid if the material is deteriorated or mishandled.

3. INTENDED USE

LNE CRM HbA1c 401 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 HbA_{1c} in hemolysate or whole blood.

Given that the 98/79/EC Directive and the 2017/746 regulation do not apply to internationally certified reference materials, LNE CRM HbA1c 401 is not considered as an in vitro diagnostic medical device and doesn't require CE marking. Still, LNE CRM HbA1c 401 should not supersede the control materials needed to establish or verify performances of in vitro diagnostic medical devices.

4. SAFETY PRECAUTIONS

LNE CRM HbA1c 401 is intended for in-vitro diagnostic use only. It should be handled as biohazardous material capable of transmitting infectious disease. The product manufacturer has reported that each donor unit of blood 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 HbA1c 401 should be removed from the refrigerator and allowed to stand at room temperature (between 18°C and 25°C) for 15 to 30 minutes. Materials should then be reconstituted with 0.5 mL of ultrapure water and left to equilibrate for 30 minutes at room temperature (between 18°C and 25°C). Once re-suspended, materials should be gently mixed by repeated inversion of the vials (about 20 times, being careful not to generate foam). LNE CRM HbA1c 401 ought to be used rapidly after reconstitution and can only be stored up to 3 days between 2 and 8°C after reconstitution. Materials are intended for a unique use. Stability of the reconstituted materials at -80°C and at room temperature has not been investigated and the certified amount-of-substance ratio is not to be considered valid if materials have been stored in such conditions. Similarly, the certified amount-of-substance ratio is not to be considered valid for open bottles and materials that have been stored in any other conditions than those assessed during the short-term stability study (see section 6). The certified amount-of-substance ratio is not valid either for samples that are diluted.

6. STORAGE

LNE CRM HbA1c 401 should preferably be shipped on cooling elements under controlled temperature conditions between 2 and 8°C. Otherwise, materials can be shipped at ambient temperature if transportation does not exceed 1 week and if temperature doesn't exceed +24°C. Upon receipt, materials should be stored away from light between 2 and 8°C until analyses.

7. COMMUTABILITY TESTING

Sample commutability was assessed through an extensive study that took place between end May and early June 2017. It involved seventeen of the most used method for the quantification of HbA1c in clinical laboratory [3]. Commutability was assessed according to the difference in bias approach described in the IFCC recommendations on commutability assessment [4, 5]. For each routine method, the difference between the bias of the routine method against the IFCC RMP using the EQA material under investigation, and the mean bias of the same routine method against the IFCC RMP was calculated on a panel of 22 fresh clinical specimens. This bias difference was then compared with a commutability criterion that corresponds to the minimum commutability need for clinical purposes [6]. In this study, HbA1c concentrations were ln-transformed and a fixed acceptance criterion of 6% was used [3]. Results show that LNE CRM HbA1c 401 is commutable to fresh, unpooled human whole blood clinical specimens for most of the field methods studied (Table 1).

LNE CRM HbA1c 401 was found non-commutable for the Siemens DCA Vantage, the Abbott Architect enzymatic HbA_{1c} assay, the BioRad Variant II NU and the Afinion AS100 methods. Inconclusive results were additionally observed for Tosoh G8 and Trinity Hb 9210 methods. For these methods, it is not recommended to use LNE CRM HbA1c 401 to estimate bias. Diluting the material is forbidden since the impact of material dilution has not been assessed.

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Table 1: Results of the commutability assessment of LNE HbA1c CRM for the 17 methods involved in the commutability study. C: Commutable; NC: Non-commutable; I: Inconclusive.

	LNE CRM HbA1c 401
Beckman UniCel DxC 700 AU	C
Roche Cobas C-513	C
Siemens DCA Vantage	NC
Abbott enzymatic	NC
BioRad D10	C
BioRad D100	C
BioRad VII NU	NC
BioRad VII Turbo	C
Menarini HA 8160	C
Tosoh G8	I
Tosoh G11	C
Tosoh Gx	C
Alere Afinion AS100	NC
Trinity Hb 9210	I
Sebia Capillarys 2	C
Sebia Capillarys 3	C
Sebia Minicap	I

8. HOMOGENEITY TESTING

Homogeneity testing of LNE CRM HbA1c 401 was performed on four sub-sample taken from seven bottles. Measurements were performed on a Sebia Capillarys 3 analyzer (University of Reims Champagne Ardennes, faculty of medicine, Reims, France). No significant differences in the between and within-bottle variances were found using F-test at 95 % confidence level. Thus, the LNE CRM HbA1c 401 materials were considered as sufficiently homogeneous. Results of the homogeneity study are detailed in the certification report that is available upon request [7].

9. STABILITY TESTING

Short term stability testing for LNE CRM HbA1c 401 was performed in different storage conditions, when in lyophilized form and once reconstituted in ultrapure 18.2 mΩ water. The slope of the fitted regression line over time was found to be insignificant at 95% confidence level [2] and the materials were considered sufficiently stable for the condition presented in Table 2. Results of the stability study are detailed in the certification report that is available upon request [7].

Table 2 : Summary of the short-term stability of LNE HbA1c CRMs 401

Ambient (lyophilized, 21 ± 3°C)	4°C (after reconstitution)
12 days	3 days

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10. CERTIFIED VALUE

A certified value is a value for which a laboratory has a high confidence in terms of accuracy since all potential sources of uncertainty have been taken into account [8]. The certified amount-of-substance ratio [HbA1c/(HbA1c+HbA0)] in LNE CRM HbA1c 401 is provided in Table 3. The certified amount-of-substance ratio value is the mean of 18 measurements determined by using the reference measurement procedure described hereafter (see section 11). The final result is expressed as the certified amount-of-substance ratio value ± the expanded uncertainty. The associated measurement uncertainty of each certified amount-of-substance ratio value was estimated in accordance to ISO/IEC Guide 98-3:2008 [9]. 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 the certified value shown in Table 3, $k = 2$. Results of the certification campaign are detailed in the certification report that is available upon request [7].

Table 3 : Certified amount-of-substance ratio (IFCC unit [HbA1c/(HbA1c+HbA0)]) in LNE CRM HbA1c 401

	LNE CRM HbA1c 401
[HbA1c/(HbA1c+HbA0)]	32.5 ± 1.8 mmol/mol (k=2)

This certified value was found coherent with value obtained by Instand e.V. (Düsseldorf, Germany) during an interlaboratory comparison with LNE. Results of the interlaboratory comparison are detailed in the certification report that is available upon request [7].

11. ANALYTICAL METHODS

LNE HbA_{1c} CRMs 401 was characterized using the LC-MS reference method endorsed by the IFCC and listed in the JCTLM database [10].

LNE is accredited according to standards ISO EN 17025 [11] and ISO EN 15195 [12] for the measurement of HbA1c in human haemolysates and whole blood. LNE is recognized as a Reference Measurement Services Provider by the JCTLM for the measurement of amount-of-substance ratio [HbA1c/(HbA1c+HbA0)] in human hemolysates and whole blood.

12. METROLOGICAL TRACEABILITY

The certified amount-of-substance ratio value is traceable to the SI through the use of a higher order metrological procedure and through the use of certified reference materials ERM/IFCC AD-500 as calibrators.

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13. CERTIFICATE REVISION RECORDS

Date	Name of responsible	Reason and content of the revision
22/05/2018	Vincent DELATOUR	Revision A : initial certificate
01/07/2019	Vincent DELATOUR	Revision B : - Inclusion of listing in JCTLM database - Extension of the period of validity - Revision of the intended use section - Update of references
01/12/2020	Vincent DELATOUR	Revision C : - Extension of the period of validity
27/04/2021	Vincent DELATOUR	Revision D : - Extension of the period of validity

14. REFERENCES

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

[2] Joint Committee for Traceability in Laboratory Medicine; available at https://www.bipm.org/jctlm/ (accessed July 2019).

[3] Delatour V, Clouet-Foraison N, Jaisson S, Kaiser P, Gillery P. Trueness assessment of HbA1c routine assays: are processed EQA materials up to the job? Clin Chem 2019. doi: 10.1515/cclm-2019-0219.

[4] 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.

[5] 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.

[6] Korzun WJ, Nilsson G, Bachmann LM, Myers GL, Sakurabayashi I, Nakajima K, et al. Difference in bias approach for commutability assessment: Application to frozen pools of human serum measured by 8 direct methods for HDL and LDL cholesterol. Clin Chem. 2015;61:1107–13.

[7] Delatour V, Clouet-Foraison N, Lalere B, Vaslin-Reimann S. Certification of HbA1c in lyophilized hemolysates. Certification report. 2018.

[8] ISO - International Organization for Stardardization. NF ISO/CEI Guide 99: International vocabulary of metrology — Basic and general concepts and associated terms. (VIM).

[9] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).

[10] Jeppsson J, Kobold U, Barr J, Hoelzel W, Hoshino T, Miedema K, et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002; 35:78–89.

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

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

End of the certificate