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CERTIFICATION REPORT

The certification of the catalytic activity concentration of alpha-amylase in ERM®-AD456/IFCC

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Abstract

This report describes the production of ERM®-AD456/IFCC, which is a material certified for the catalytic activity concentration of alpha-amylase. This material was produced following ISO 17034:2016 [ ] and is certified in accordance with ISO Guide 35:2017.
The starting material was pancreatic alpha-amylase purified from human tissue. The enzyme was diluted in a buffered solution which was filled into glass vials and lyophilised.
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017.
The material was characterised by an interlaboratory comparison of laboratories of demonstrated competence and adhering to ISO/IEC 17025.
Uncertainties of the certified values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) [ ] and include uncertainties related to possible inhomogeneity, instability and characterisation.
The material is intended for the assessment of method performance of the primary reference measurement procedure (PRMP) for the catalytic activity concentration of alpha–amylase at 37 °C established by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). In addition the material can also be used as trueness control or external quality control material for routine measurement systems if commutability has been proven for the assay concerned. As with any reference material, it can be used for establishing control charts or in validation studies. The CRM is available in glass vials containing lyophilised material from 1 mL of alpha-amylase solution which were sealed under an atmosphere of nitrogen. The minimum amount of sample to be used is 5 µL after reconstitution of the whole content of 1 vial in 1 mL.

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM®-AD456/IFCC, which is a material certified for the catalytic activity concentration of alpha-amylase. This material was produced following ISO 17034:2016 [1] and is certified in accordance with ISO Guide 35:2017 [2].

The starting material was pancreatic alpha-amylase purified from human tissue. The enzyme was diluted in a buffered solution which was filled into glass vials and lyophilised.

Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017 [2].

The material was characterised by an interlaboratory comparison of laboratories of demonstrated competence and adhering to ISO/IEC 17025 [3].

Uncertainties of the certified values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for the assessment of method performance of the primary reference measurement procedure (PRMP) for the catalytic activity concentration of alpha–amylase at 37 °C established by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [5,6]. In addition the material can also be used as trueness control or external quality control material for routine measurement systems if commutability has been proven for the assay concerned. As with any reference material, it can be used for establishing control charts or in validation studies. The CRM is available in glass vials containing lyophilised material from 1 mL of alpha-amylase solution which were sealed under an atmosphere of nitrogen. The minimum amount of sample to be used is 5 µL after reconstitution of the whole content of 1 vial in 1 mL.

The following values were assigned:

	Catalytic activity concentration	
	Certified value [2)]	Uncertainty [3)]
Alpha-amylase[1)]	274 U/L 4.58 µkat/L	7 U/L 0.12 µkat/L

[1)] Catalytic activity concentration of alpha-amylase in the reconstituted material, as obtained by the PRMP for the measurement of catalytic activity concentration of alpha-amylase at 37 °C from the IFCC.

[2)] Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified values and their uncertainty are traceable to the International System of units (SI). Values were converted from U/L into µkat/L by multiplication with the factor $f = 0.01667$.

[3)] The uncertainty is the expanded uncertainty of the certified value with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008. "

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Glossary

ANOVA	Analysis of variance
b	Slope in the equation of linear regression $y = a + bx$
BCR®	One of the trademarks of CRMs owned by the European Commission; formerly Community Bureau of Reference
BELAC	"Belgische Accrediatie-instelling": national accreditation body of Belgium
c	Mass concentration $c = m / V$ (mass / volume)
CLSI	Clinical and Laboratory Standards Institute
CNAS	China National Accreditation Service
CRM	Certified reference material
DAkkS	"Deutsche Akkreditierungsstelle GmbH": national accreditation body of the Federal Republic of Germany
EC	European Commission
EDTA	Ethylenediaminetetraacetic acid
ENAC	"Entidad Nacional de Acreditación" : the national accreditation Body of Spain
ERM®	Trademark of European Reference Materials
EU	European Union
GUM	Guide to the Expression of Uncertainty in Measurements *[ISO/IEC Guide 98-3:2008]*
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
kat/L	Katal per liter
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable
PIPES	piperazine-N,N′-bis(2-ethanesulfonic acid)
PRMP	Primary reference measurement procedure
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RSD	Relative standard deviation
r^2	Coefficient of determination of the linear regression

s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of Units
RM Unit	Reference Materials Unit of Directorate F
RvA	"Raad voor Accreditatie" national accreditation body of the Netherlands
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
t_i	Time point for each replicate
$t_{\alpha, df}$	Critical t-value for a t-test, with a level of confidence of 1-α and df degrees of freedom
t_{sl}	Proposed shelf life
u	standard uncertainty
U	expanded uncertainty
U/L	Units per litre
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	combined standard uncertainty; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_Δ	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel"

	is added as appropriate
u_t	Standard uncertainty of trueness
V	Volume
$\overline{x}$	Arithmetic mean
$\overline{x}_{ref}$	Arithmetic mean of results of reference samples
α	significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

The protein enzyme alpha-amylase is present in large amounts in the digestive tract where it catalyses the hydrolysis of large, alpha-linked polysaccharides (such as starch and glycogen) into glucose. The enzyme is mainly secreted by the exocrine pancreas and the salivary glands which each produce their specific isoenzyme. In normal circumstances the concentration alpha-amylase in the blood is low but lesions in the organs producing the enzyme can lead to a large increase. The catalytic activity of (pancreatic) alpha-amylase in serum is frequently measured in medical laboratories for detection and diagnosis of acute pancreatitis [7] and pancreatic trauma [8].

The catalytic activity of an enzyme is a property that is measured by the rate of a specified chemical reaction under certain experimental conditions. The measurement of this property is very important in clinical chemistry, but the standardisation of catalytic activity measurements is challenging as a number of parameters influence the enzyme activity (e.g. temperature, pH, substrate nature and concentration, activators, inhibitors). Therefore, the measurement results are heavily dependent on the measurement procedure used to attain them. This led to the development of universally recognised measurement procedures for enzymes commonly measured in clinical chemistry, such as the primary reference measurement procedures (PRMP) for the measurement of catalytic activity concentrations of enzymes at 37 °C from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [9].

The regulation (EU) 2017/746 of the European Parliament and of the Council on *in vitro* diagnostic medical devices requires traceability of the assigned values of calibrants and control materials to reference measurement procedures and/or reference materials of higher order.

In collaboration with members of the IFCC Committee for Reference Systems of Enzymes and the IFCC Working Group for Pancreatic Enzymes, the Reference Materials Unit of the Joint research Centre (JRC) developed a CRM certified for the catalytic activity concentration of alpha-amylase. This material, ERM-AD456/IFCC, is intended to be used as a quality control material for the PRMP for the measurement of catalytic activity concentrations of alpha-amylase at 37 °C from the IFCC [5,6]. The homogeneity and the stability of ERM-AD456/IFCC were demonstrated and the certified values were assigned using the PRMP in an interlaboratory comparison of expert laboratories.

1.2 Choice of the material

Ideally, the material should contain human pancreatic alpha-amylase without significant contaminations from human salivary alpha-amylase. The natural presence of salivary alpha-amylase in human serum underscores the need for an artificial background solution to which human pancreatic alpha-amylase is added.

The starting material was human pancreatic alpha-amylase purified from human tissues by Lee Biosolutions (Missouri, USA). The enzyme was provided in lyophilised form and solubilised in a buffered solution containing human serum albumin. This starting material and the background solution were selected based on the outcome of a commutability study carried out by the JRC [10]. The same background solution was also used for the production of IRMM/IFCC-456, the previous CRM for alpha-amylase, and the lyophilised material has shown to be stable for more than 20 years.

The aim of the production process was to obtain a material that after reconstituted with 1 mL of distilled/deionised water would have a catalytic activity concentration of about 300 U/L.

1.3 Design of the CRM project

A commutability study including five routine measurement procedures was completed to select the most appropriate starting material and background solution for the production of ERM-AD456/IFCC [10].

The material was certified by interlaboratory comparison. Data from expert laboratories using the PRMP for the catalytic activity concentration of alpha-amylase at 37 °C from the IFCC [5] and adhering to the important procedural details were used [6].

The homogeneity and stability of the material were assessed using an automated version of the PRMP. The reagent solutions, incubation time, delay time, measurement time and spectrometric parameters were according to the official (and manual) version of the PRMP. Only the volumes were reduced to suite the instrument's specifications, however, the volume fractions of the sample, of the reagent solution and of the start reagent solution were identical to those used in the official PRMP. A correction of evaporation effects during the measurement series was applied. This automated version of the PRMP has a high throughput (> 120 measurements in 1 run) and the method showed a low average relative standard deviation (RSD) during the commutability study [10].

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre (JRC), Directorate F – Health, Consumers and Reference Materials, Geel, BE
(accredited to ISO 17034:2016 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

Biosystems, S.A., Barcelona, ES

2.3 Homogeneity and stability studies

*Medizinische Hochschule Hannover, Institut für Klinische Chemie, Kalibrierlaboratorium II, Referenzinstitut für Bioanalytik, Hannover, DE
(measurements under the scope of ISO/IEC 17025:2005 and ISO15195:2003 accreditation, DAkkS No. D-K-15117-02-00)

2.4 Characterisation

The laboratories are listed below in alphabetic order. This order does not necessarily correspond to the ranking of the laboratories L01 - L11 described in the Tables of this report.

*Beijing Aerospace General Hospital, Reference Laboratory, Beijing, CN
(measurements under the scope of ISO/IEC 17025:2005 and ISO 15195:2003 accreditation, CNAS No. L5536)

Biosystems, S.A., Barcelona, ES

*Guangdong Provincial Hospital of Chinese Medicine, Medical Calibration Laboratory, Department of Laboratory Medicine, Guangzhou, CN
(measurements under the scope of ISO/IEC 17025:2005 and ISO 15195:2003 accreditation, CNAS No. L6192)

LabWest/HagaZiekenhuis, Klinisch Chemisch en Hematologisch Laboratorium, Den Haag, NL
(meaurements under the scope of NEN-EN-ISO 15189:2012 accreditation, RvA No. M027)

*Maccura Biotechnology Co., Ltd., Reference System Department, Chengdu, CN
(measurements under the scope of ISO/IEC 17025:2005 and ISO 15195:2003 accreditation, CNAS No. L6172)

*Medical System Biotechnology Co. Ltd, Reference Laboratory, Ningbo, CN
(measurements under the scope of ISO/IEC 17025:2005 accreditation, CNAS No.L8377)

*Medizinische Hochschule Hannover, Institut für Klinische Chemie, Kalibrierlaboratorium II, Referenzinstitut für Bioanalytik, Hannover, DE
(measurements under the scope of ISO/IEC 17025:2005 and ISO15195:2003 accreditation, DAkkS No. D-K-15117-02-00)

Ospedale San Raffaele, Servizio di Medicina di Laboratorio, Laboratorio di Standardizzazione par la Chimica Clinica, Milano, IT

Roche Diagnostics GmbH, Penzberg, DE

*Shanghai Center for Clinical Laboratory, Shanghai, CN
(measurements under the scope of ISO/IEC 17025:2005 and ISO 15195:2003 accreditation, CNAS No. L6730)

*Universitat Autònoma de Barcelona, Departament de Bioquímica i de Biologia Molecular, Laboratori de Referència d' Enzimologia Clínica, Barcelona, ES
(measurements under the scope of ISO/IEC 17025:2005 and ISO 15195:2003 accreditation, ENAC No. 195/LC10.141)

*Listed in the JCTLM database for reference measurement services

3 Material processing and process control

3.1 Origin and purity of the starting material

Purified alpha-amylase from human pancreatic tissues (product number 120-16) was obtained from Lee Biosolutions (Missouri, USA). The enzyme was provided in lyophilised form and stored at -20 °C until use. The presence of 4 contaminating enzymes (i.e. alkaline phosphatase, aspartate aminotransferase, lipase and protease) was checked by Lee Biosolutions and for each contaminating enzyme the relative catalytic activity concentration was no more than 0.01 % of the total catalytic activity concentration.

3.2 Processing and processing control

The processing of the CRM was done by Biosystems S.A. (Barcelona, ES).

Based on the catalytic activity concentration of the alpha-amylase powder as determined by Lee Biosolutions a calculated amount of the powder was dissolved in a buffered solution to obtain a solution with a catalytic activity concentration around 300 U/L. The solution contained: 50 mmol/L NaCl, 0.5 mmol/L EDTA, 1.5 mmol/L $CaCl_2$, 30 g/L human albumin, and 25 mmol/L PIPES (i.e. piperazine-N,N′-bis(2-ethanesulfonic acid)) at pH 7.

The solution was filled into colourless glass vials in portions of 1.0 mL during one continuous process. The filling order of the vials was reverse in comparison with the numbers indicated on the vials; the vial with the highest number was filled first and the vial with number 1 was filled at the end. The filling process was controlled by measuring the mass of the filled solution in 20 test vials that were placed at regular intervals throughout the filling process (see graph in Annex A). The average mass of the filled solutions was 0.995 g and the standard deviation (*s*) was 0.009 g. There was no trend according to the filling sequence at a 99 % confidence level.

The filled vials were chilled at -50 °C and then lyophilised. The material in the vials was submitted to secondary desiccation at 35 °C for 11 hours. Afterwards the vials were filled with pure dry nitrogen and closed using rubber stoppers. Finally the vials were closed with a white screw cap and stored at -20 °C. The vials were dispatched on dry ice to the JRC for final storage.

4 Homogeneity

A key requirement for any reference material aliquoted into units is equivalence between those units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value, but it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO 17034:2016 [1] requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit inhomogeneity is therefore necessary to determine the minimum sample intake.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified value of the CRM is valid for all vials of the material, within the stated uncertainty.

The number of vials selected exceeds the cube root of the total number of vials produced. The 20 vials were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 20 groups (with a similar number of vials) and one vial was selected randomly from each group.

Each vial was reconstituted with (1.00 ± 0.01) mL of distilled H_2O at 20 – 22 °C and the mass of the added H_2O was recorded to the nearest 0.1 mg. After reconstitution, four independent samples were taken from each selected vial, and analysed by an automated version of the PRMP for the catalytic activity concentration of alpha-amylase at 37°C from the IFCC. The measurements were performed under repeatability conditions, and in a regular sequence eliminating a correlation between the vial number and the position in the measurement sequence. A potential analytical drift could therefore be separated from a trend in the filling sequence. The obtained measurement results were corrected for the reconstitution volume calculated from the recorded mass assuming a water density of 0.997992 g/mL (i.e. the density of H_2O at 21 °C [11]). The results are shown as a graph in Annex B.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling sequence or the analytical sequence were observed at a 95 % confidence level.

The dataset was assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means. No outlying individual results and outlying unit means were detected. All the data were retained for statistical analysis.

Quantification of between-unit inhomogeneity was undertaken by analysis of variance (ANOVA), which separates the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples were representative for the whole vial.

Evaluation by ANOVA requires mean values per vial, which follow at least a unimodal distribution and results for each vial that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per vial was visually tested using histograms and normal probability plots. This inspection confirmed that individual results and vial means followed normal distributions.

It should be noted that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and are therefore subject to random fluctuations. Therefore, the mean of squares between groups ($MS_{between}$) can be smaller than the mean of squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit

variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [12]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 1}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}\sqrt[4]{\frac{2}{v_{MSwithin}}}}}{\bar{y}} \qquad \textbf{Equation 3}$$

MS_{within}	mean of squares within-unit from an ANOVA
$MS_{between}$	mean of squares between-unit from an ANOVA
$\bar{y}$	mean of all results of the homogeneity study
n	mean number of replicates per unit
$v_{MSwithin}$	degrees of freedom of MS_{within}

The results of the evaluation of the between-unit variation are summarised in Table 1. The resulting value from the above equation was converted into a relative uncertainty.

Table 1: Results of the homogeneity study

ERM-AD456/IFCC	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{bb,rel}$ [%]
Catalytic activity concentration of alpha-amylase	1.16	0.94	0.25	0.94

The homogeneity study showed no outlying unit means or trends in the filling sequence. Therefore the between-unit standard deviation can be used as estimate of u_{bb}. As u^*_{bb} sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus should be used in an analysis. Using sample sizes equal or above the minimum sample intake guarantees the certified values within their stated uncertainty.

Homogeneity and stability experiments were performed using a 5 µL sample intake. This sample intake gives acceptable repeatability, demonstrating that the within-unit inhomogeneity no longer contributes to analytical variation at this sample intake.

5 Stability

Time, temperature, light (including ultraviolet radiation) and water content were regarded as the most relevant influences on the stability of the materials. The influence of ultraviolet or visible light was minimised by storing the material in the dark and dispatched in boxes, thus removing any possibility of degradation by light. The water content was reduced by freeze-drying to obtain a stable material. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish the conditions for storage (long-term stability) as well as the conditions for dispatch of the materials to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C can be reached and stability under these conditions must be demonstrated, if the samples are to be transported without any cooling.

The stability studies were carried out using an isochronous design [13]. In this approach, samples were stored for a particular length of time at different temperature conditions. Afterwards, the samples were moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples were analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

5.1 Short-term stability study

For the short-term stability study, samples were stored at -20 °C, 4 °C, and 18 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to -70 °C. Four vials per storage time were selected using a random stratified sampling scheme.

After reconstitution, 3 samples were taken from each vial and measured by the automated version of the PRMP for the catalytic activity concentration of alpha-amylase. The measurements were performed under repeatability conditions, and in a regular sequence eliminating a correlation between the storage time and the position in the measurement sequence. A potential analytical drift could therefore be differentiated from a trend over storage time. The obtained measurement results were corrected for the reconstitution volume assuming a temperature of 21 °C.

The data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test with a confidence level of 99 %. No outlying individual results were found.

The data were evaluated against storage time, and regression lines of catalytic activity concentration of alpha-amylase versus time were calculated, to test for potential increases/decreases of the catalytic activity concentration due to shipping conditions. The slopes of the regression lines were tested for statistical significance. None of the trends was statistically significant at a 95 % confidence level for any of the temperatures.

The results of the measurements are shown in Annex C.

The material shall be shipped under cooled conditions, to avoid exposure to higher temperatures than those tested.

5.2 Long-term stability study

For the long-term stability study, samples were stored at -20 °C for 0, 4, 8 and 12 months. The reference temperature was set to -70 °C. Five vials per storage time were selected using a random stratified sampling scheme. After reconstitution, four samples were taken from each vial and measured by an automated version of the PRMP for the catalytic activity concentration of alpha-amylase. The measurements were performed under repeatability conditions, and in a regular sequence eliminating a correlation between the storage time and the position in the measurement sequence. A potential analytical drift could therefore be separated from a trend over storage time. The results were corrected for the volume of water added during the reconstitution step assuming a temperature of 21 °C.

Regression analysis was performed to evaluate a potential trend in the analytical sequence. A significant (99 % confidence level) trend in the analytical sequence was visible, pointing at a changing parameter, e.g. a signal drift in the analytical system. The correction of biases, even if they are statistically not significant, was found to combine the smallest uncertainty with the highest probability to cover the true value [14]. Correction of trends is therefore expected to improve the sensitivity of the subsequent statistical analysis through a reduction in analytical variation without masking a potential trend over storage time. As the analytical sequence and the storage time were not correlated, the significant trend was corrected as shown below:

$$x_{i\_corr} = x_i - b \cdot i \qquad \textbf{Equation 4}$$

b slope of the linear regression
i position of the result in the analytical sequence

The trend-corrected dataset was screened for outliers using the single and double Grubbs test at a confidence level of 99 %. One outlying individual result was found. As no technical reason for the outlier could be found all data were retained for statistical analysis.

The data were plotted against storage time and linear regression lines of catalytic activity concentration versus time were calculated. The slope of the regression lines were tested for statistical significance (loss/increase due to storage). No significant trend was detected at a 95 % confidence level.

The results of the long-term stability measurements are shown in Annex D.

The material can be stored at -20 °C.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during dispatch and storage were estimated, as described in [15]. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum \left(t_i - \bar{t}\right)^2}} \cdot t_{tt} \qquad \textbf{Equation 5}$$

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum \left(t_i - \bar{t}\right)^2}} \cdot t_{sl} \qquad \textbf{Equation 6}$$

S_{rel} relative standard deviation of all results of the stability study
t_i time elapsed at time point *i*
$\bar{t}$ mean of all t_i
t_{tt} chosen transport time (1 week at 18 ºC)
t_{sl} chosen shelf life (12 months at -20 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 18 °C studies. The uncertainty describes the possible change during a dispatch at 18 °C lasting for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the -20 °C studies. The uncertainty contribution describes the possible degradation during 12 months storage at -20 °C.

The results of these evaluations are summarised in Table 2.

Table 2: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 18 °C and one week; $u_{lts,rel}$ was calculated for a storage temperature of -20 °C and 12 months

ERM-AD456/IFCC	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
Catalytic activity concentration of alpha-amylase	0.15	0.47

The material showed no significant degradation for transport at 18 °C or lower, but evidence of stability above 18 °C was not obtained. Cooled shipment is therefore necessary to ensure that the temperature stays within the tested conditions.

After the certification study, the material will be included in the JRC's regular stability monitoring programme, to control its further stability.

6 Characterisation

The material characterisation is the process of determining the property value of a reference material.

This was based on an interlaboratory comparison of expert laboratories, i.e. the catalytic activity concentration of alpha-amylase of the material was determined in different laboratories. All participants applied the official (and manual) version PRMP for the measurement of catalytic activity concentration of alpha-amylase at 37 °C from the IFCC [5,6]. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

Eleven laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of catalytic activity concentration measurements of alpha-amylase using the PRMP. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

Before participating in the characterisation study, the 11 laboratories demonstrated their technical competence in a feasibility study. Each laboratory received 3 vials from 2 independent control materials (6 vials in total) which had to be measured with the PRMP. All laboratories were requested to provide detailed information about the calibration of their instruments and the purity of the used reagents.

Based on the provided information it was concluded that each laboratory preformed the measurements in compliance with the PRMP as described in [5,6]. The method performance of each laboratory was evaluated based on the intermediate precision (repeatability and day-to-day variation) of the measurement results. For all 11 laboratories this intermediate precision was low enough to achieve a precision ≤ 3 % in the final characterisation study.

The reproducibility of the PRMP among the different laboratories was also evaluated by calculating the RSD between the lab means for both test materials and by testing for outlying means using the Grubbs test. The RSD between the lab means was ≤ 3.5 % and there were no outlying lab means at 99 % confidence level.

6.2 Study setup

Each laboratory received five vials of ERM-AD456/IFCC and was requested to provide 15 measurement results, three per vial. The vials for material characterisation were selected using a random stratified sampling scheme covering the whole batch. The measurements on the five vials had to be spread over at least two days to ensure intermediate precision conditions. Each vial had to be reconstituted with (1.00 ± 0.01) mL of distilled H_2O at 20-22 °C and the mass of the added H_2O should be recorded to the nearest 0.1 mg. After reconstitution, three independent samples should be taken from each vial and measured within 4 hours after reconstitution.

Laboratories were requested to provide detailed information about the calibration of their instruments and the purity of the used reagents to prove compliance with the PRMP as described in [5,6]. In addition, laboratories were also requested to give estimations of the expanded uncertainties on the mean value of the 15 results. No approach for the estimation was prescribed, i.e. top-down and bottom-up were regarded as equally valid procedures.

6.3 Methods used

All laboratories used the PRMP for the catalytic activity concentration of alpha-amylase at 37 °C from the IFCC [5,6].

6.4 Evaluation of results

The characterisation study resulted in 11 datasets and an estimate of the expanded uncertainty was provided for 9 of these datasets. Ten laboratories provided measurement results that were not corrected for the reconstitution volume. The masses recorded during the reconstitution step were used to correct these results assuming a temperature of 21 °C, unless another temperature was reported by the laboratory. All individual results of the participants are displayed in tabular and graphical form in Annex E.

6.4.1 Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- compliance with the PRMP: proper calibration of relevant instruments (pH meter, balance, spectrophotometer, thermometers and pipettes) and use of reagents with the highest purity.
- compliance with the analysis protocol: measurements performed on at least two days.
- method performance: repeatability (i.e. within-vial standard deviation ≤ 1.5 %)

Based on the above criteria, none of the datasets were rejected as not technically valid.

6.4.2 Statistical evaluation

The statistical evaluation of the datasets was based on the mean result obtained for each vial. The statistical evaluation of the datasets was based on the mean result obtained for each vial. The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 3.

Table 3: Statistical evaluation of the technically accepted datasets for ERM-AD456/IFCC. *p*: number of technically valid datasets

ERM-AD456/IFCC	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [U/L]	*s* [U/L]	$s_{between}$ [U/L]	s_{within} [U/L]
Catalytic activity concentration of alpha-amylase	11	None	None	yes	274.45	5.88	5.74	2.80

The laboratory means follow a normal distribution. There are no outlying lab means and variances. The datasets are therefore consistent and the mean of laboratory means is a good estimate of the true value. The standard deviation between laboratories is considerably larger than the standard deviation within laboratories, showing that confidence intervals of replicate measurements are unsuitable as estimate of measurement uncertainty.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means (Table 4).

Table 4: Uncertainty of characterisation for ERM-AD456/IFCC

ERM-AD456/IFCC	*p*	Mean [U/L]	*s* [U/L]	$u_{char,rel}$ [%]
Catalytic activity concentration of alpha-amylase	11	274.45	5.88	0.65

7 Value Assignment

A certified value for the catalytic activity concentration of alpha-amylase was assigned to ERM-AD456/IFCC.

Certified values are values that fulfil the highest standards of accuracy. Procedures at the JRC, Directorate F require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 4 was assigned as certified value.

The assigned uncertainty consists of uncertainties relating to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1), and potential degradation during transport, u_{sts}, and long-term storage, u_{lts} (Section 5). The different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,\ rel}$) with a coverage factor *k* given as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2 + u_{char,rel}^2} \qquad \textbf{Equation 7}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1
- u_{sts} *and* u_{lts} were estimated as described in Section 5.3

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor *k* of 2 was applied, to obtain the expanded uncertainties. The certified value expressed in U/L and its uncertainty is summarised in Table 5.

Table 5: Certified value expressed in U/L and its uncertainty for ERM-AD456/IFCC

	Certified value [U/L]	$u_{char,rel}$ [%]	$u_{bb,rel}$ [%]	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]	$U_{CRM,\ rel}$ [1)] [%]	U_{CRM} [1)] [U/L]
Catalytic activity of alpha-amylase	274	0.65	0.94	0.15	0.47	2.48	7

[1)] Expanded (*k* = 2) and rounded uncertainty.

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

The catalytic activity concentration of alpha-amylase is a method-defined measurand and can only be obtained by following the procedure specified in the PRMP at 37 °C of the IFCC [5,6]. Adherence to this procedure was confirmed by the detailed information on instrument calibration and reagent purity provided by the participating laboratories. The measurand is therefore operationally defined by the PRMP.

Quantity value

Traceability of the obtained results is based on the traceability of all relevant input factors. Instruments in individual laboratories were verified and calibrated with tools ensuring traceability to the International System of units (SI). Consistency in the interlaboratory comparison supports the assumption that all relevant input factors were covered. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Many measurement procedures include one or more steps which select specific (or specific groups of) analytes from the sample for the subsequent whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions that define this concept. For instance, the CLSI Guideline C30-A [16] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant. For instance, CRMs intended to be used to establish or verify metrological traceability of routine clinical measurement procedures must be commutable for the routine clinical measurement procedures for which they are intended to be used.

A commutability study was carried out in which a trial batch of the starting material for ERM-AD456/IFCC was compared with 30 frozen serum pools [10]. Each serum pool was prepared from about 10 single donation sera to obtain sufficiently large volumes which could be aliquoted and distributed to the participating laboratories. Frozen serum pools have been used in EQA schemes for the catalytic concentration of α-amylase before and it has been shown that they behave like single donation sera for several routine methods.

The serum pools and the trial batch of ERM-AD456/IFCC were measured with five different routine clinical measurement procedures and the automated version of the PRMP for the catalytic activity concentration of alpha-amylase from the IFCC. The list of the routine clinical measurement procedures included in the study is available in Annex F. Two different

approaches were used to assess the commutability: a linear regression analysis with a 95 % prediction interval as described in CLSI EP30-A [16] and a difference in bias analysis as described in the recommendations of the IFCC Working Group on Commutability [17]. The commutability criterion for the difference in bias analysis was set at 3.7 %. The results of this study are shown in Annex F.

The trial batch of ERM-AD456/IFCC had a good commutability profile for all tested routine methods with the same substrate as the PRMP. The applied commutability criterion of 3.7 % in this study is sufficient to prove that ERM-AD456/IFCC is suitable as trueness control or as external quality control material for the relevant routine methods. A CRM intended to be used in the calibration hierarchy of routine measurement systems might require a stricter commutability criterion and smaller uncertainty on the commutability assessments than obtained in this study. In case the customer intends to use ERM-AD456/IFCC as a calibrant for a routine measurement procedure it is recommended to perform an additional commutability study with a stricter commutability criterion and more replicate measurements for ERM-AD456/IFCC (>6).

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply. The human pancreatic alpha-amylase and the human serum albumin used in the production of the material have been tested and found negative for Hepatitis B surface antigen, HIV 1&2, and Hepatitis C antibodies. However, the product must be handled with adequate care as any material of human origin. It is intended for *in vitro* analysis only.

9.2 Storage conditions

The materials should be stored at (-20 ± 5) °C in the dark. After reconstitution, the material must be used within four hours.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially for opened vials.

9.3 Reconstitution

To make it ready for use, the material has to be reconstituted according to the following procedure:

1. Remove the vial from the freezer and let equilibrate to room temperature (20-25 °C).
2. Tap the vertically positioned vial gently to ensure that the lyophilised material is at the bottom of the vial. Remove the screw cap.
3. Weigh the vial together with the rubber stopper and record the mass to the nearest 0.1 mg (i.e. m_1).
4. Carefully lift the rubber stopper, avoiding the loss of lyophilised material.
5. Reconstitute with (1.00 ± 0.01) mL distilled water (20-22 °C) and carefully close the vial again with the rubber stopper.
6. Weigh the vial after adding the water and record the weight to the nearest 0.1 mg (i.e. m_2). Calculate the mass of the water added during the reconstitution (m_{H2O}) by subtracting m_1 from m_2. Use this m_{H2O} to calculate volume of the added water ($v_{H2O)}$ taking into account the temperature dependent density.

7 The catalytic concentration of alpha-amylase in the solution, corrected for the reconstitution volume, can be obtained by multiplying the certified value with $v_{intended}/v_{H2O}$ with $v_{intended}$ the volume intended to be added (1.0000 mL)

8. Dissolve the lyophilised material by gently swirling and carefully invert the vial at least five times. Allow to stand at room temperature for 10 minutes.
9. It is recommended to store the vials cold (2-8 °C) if not measured directly. Carefully invert the vial five times just before starting the measurements.
10. The catalytic activity concentration of amylase must be measured within 4 hours following the reconstitution.

9.4 Minimum sample intake

The minimum sample intake representative for the catalytic activity concentration of alpha-amylase in ERM-AD456/IFCC is 5 µL as this was the sample intake for the homogeneity and stability studies.

9.5 Use of the certified value

The main purpose of the material is to assess method performance of the PRMP for the catalytic activity concentration of alpha-amylase at 37 °C from the IFCC [5,6]. In addition the material can also be used as trueness control or external quality control material for routine measurement systems if commutability has been proven for the assay concerned. As any reference material, it can be used for establishing control charts or in validation studies.

Use as a calibrant

It is not recommended to use this material as calibrant. If used nevertheless, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty. When the material is used as a calibrant in a routine measurement procedure the commutability should be verified for the assay concerned.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes [18].

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine the measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty ($u_{\Delta,}$) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_{meas} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 %.

Use in quality control charts

The materials can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

10 Acknowledgments

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Annexes

Annex A: Results from the processing control measurements

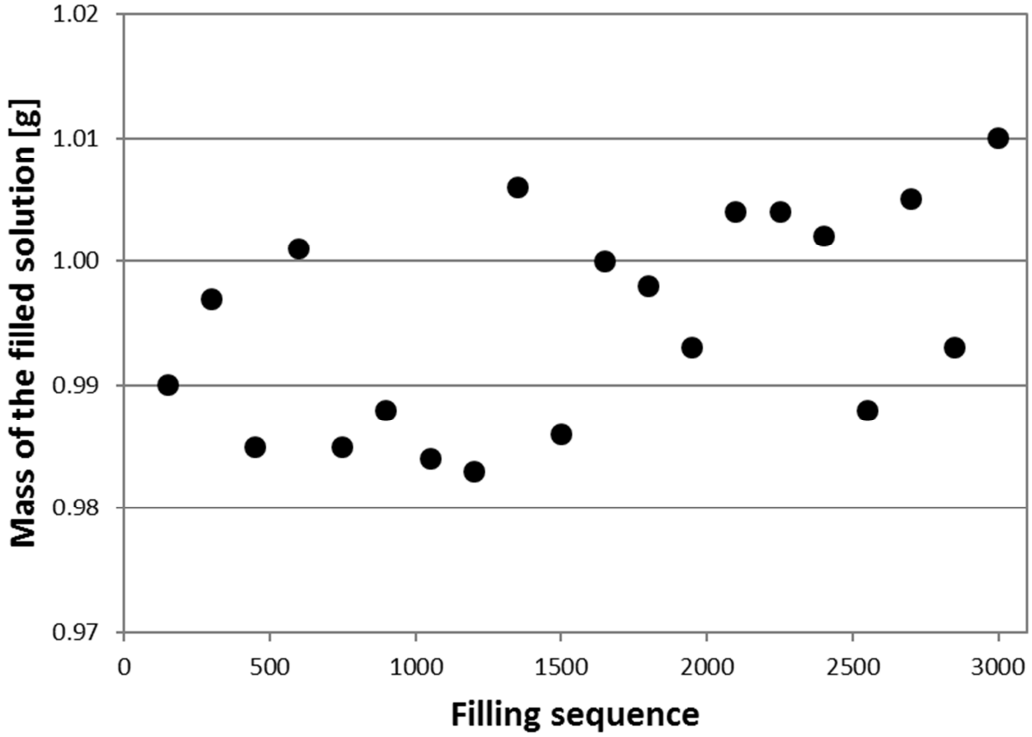

Figure A1: Weighing results of the mass of the filled solution in 20 test vials placed at regular intervals throughout the filling process of ERM-AD456/IFCC.

Annex B: Results of the homogeneity measurements

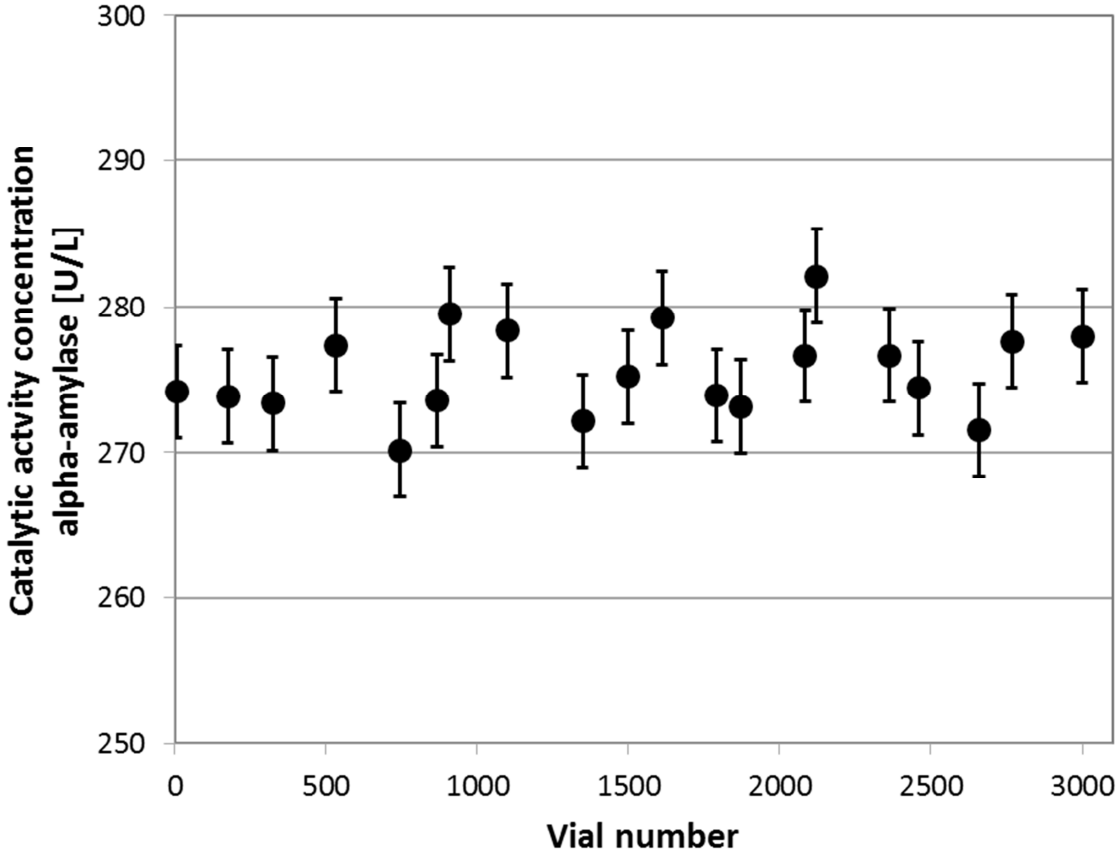

Figure B1: Homogeneity data of the catalytic activity concentration of alpha-amylase in ERM-AD456/IFCC as measured with an automated version of the PRMP from the IFCC. Shown are the averages per vial number and their 95 % confidence interval based on the within-group standard deviation as derived from a one-way ANOVA of all data grouped by vial number.

Annex C: Results of the short-term stability measurements

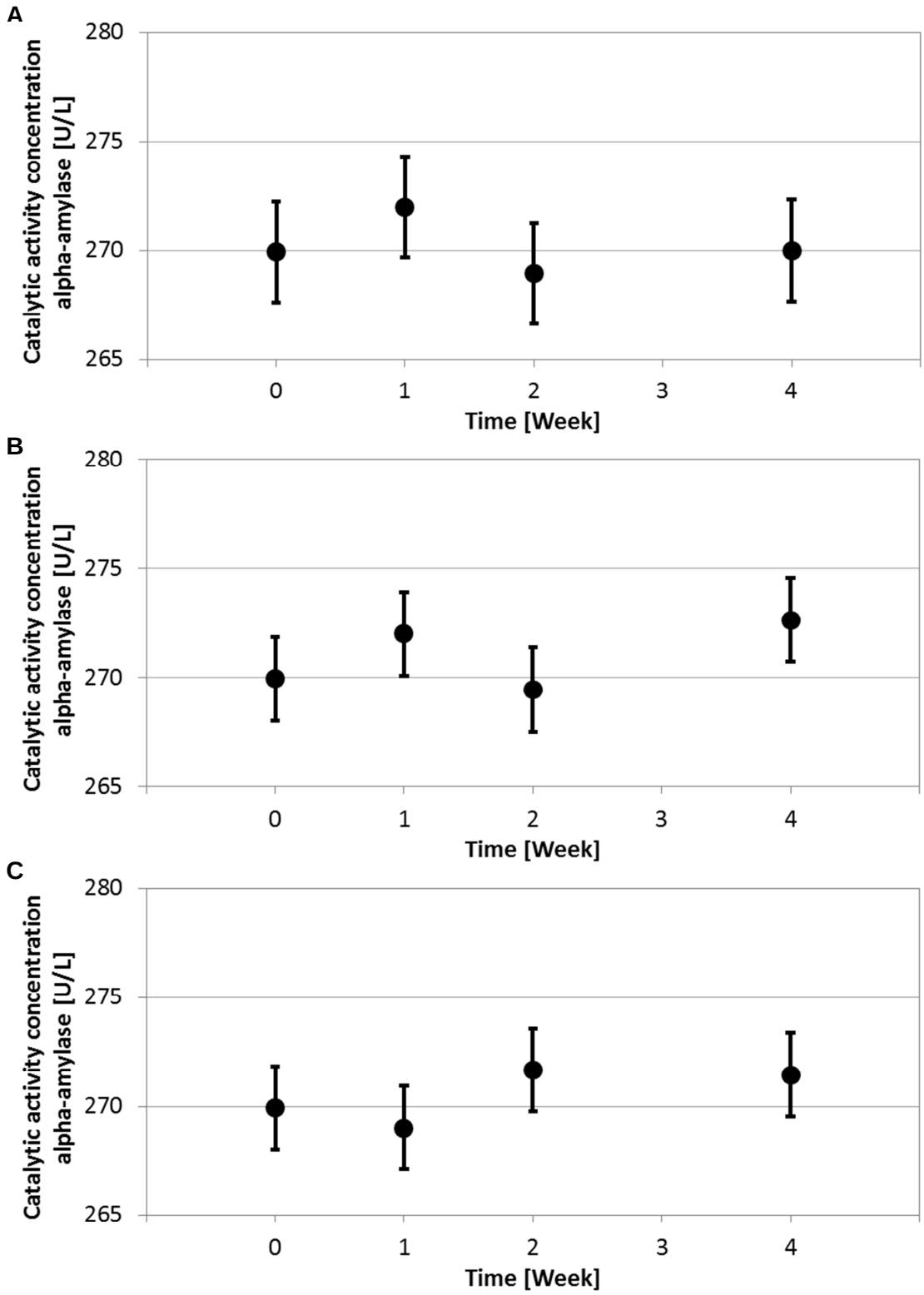

Figure C: Isochronous short-term stability measurements of the catalytic activity concentration of alpha-amylase stored at 18 °C in A, 4 °C in B and -20 °C in C. Measurements were performed with an automated version of the PRMP from the IFCC. Shown are the averages per time point and their 95 % confidence interval based on the within-group standard deviation as derived from a one-way ANOVA of all data grouped by time point.

Annex D: Results of the long-term stability measurements

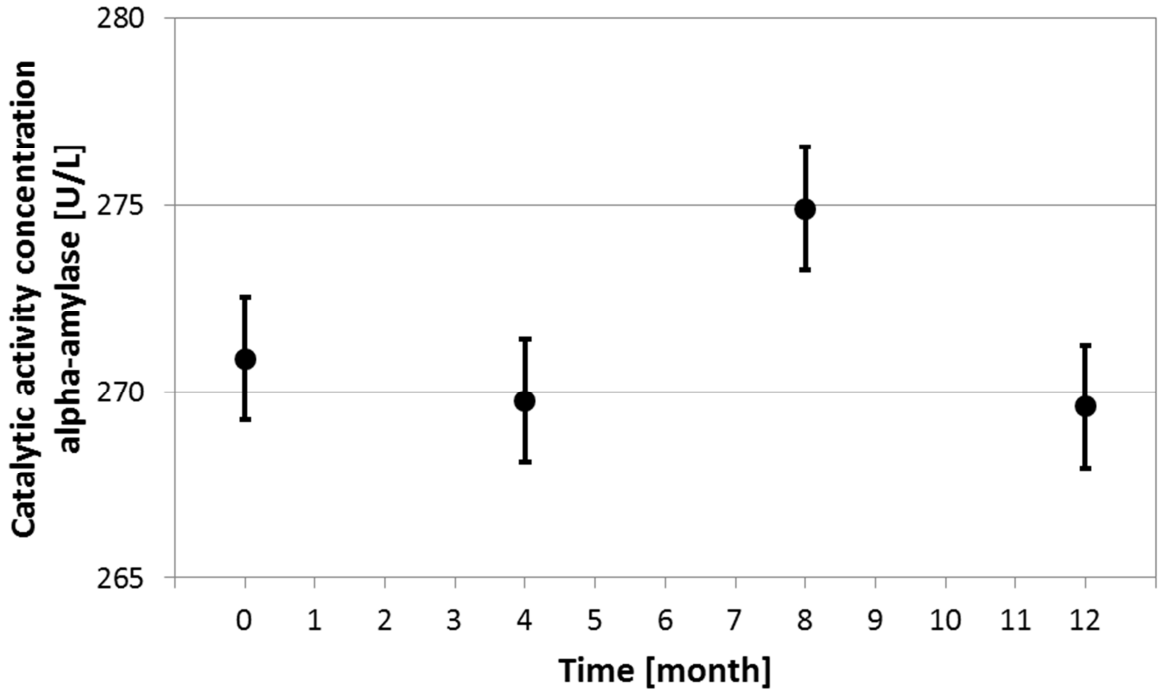

Figure D: Isochronous long-term stability measurements of the catalytic activity concentration of alpha-amylase stored at -20 °C. Measurements were performed with an automated version of the PRMP from the IFCC. Shown are the averages per time point and their 95 % confidence interval based on the within-group standard deviation as derived from a one-way ANOVA of all data grouped by time point.

Annex E: Results of the characterisation measurements

Table E1: All accepted individual results, the mean laboratory values and the expanded uncertainty (when provided by the laboratories) for the catalytic activity concentration of alpha-amylase in ERM-AD456/IFCC measured with the PRMP of the IFCC. For all but one laboratory (indicated by an asterisk) the reported results were not yet corrected for the reconstitution volume. These results were corrected assuming a H_2O temperature of 21 °C, unless another temperature was reported.

Lab code	replicate	Vial 1 [U/L]	Vial 2 [U/L]	Vial 3 [U/L]	Vial 4 [U/L]	Vial 5 [U/L]	Mean [U/L]	Expanded uncertainty [U/L]
L1	1	266.9	269.0	265.7	266.5	264.7	266.4	5.9
	2	265.8	268.3	267.2	266.4	260.4		
	3	268.2	268.2	272.2	265.0	261.4		
L2	1	263.1	262.2	264.8	263.5	264.4	265.2	Not provided
	2	259.0	269.1	261.4	271.7	265.2		
	3	263.6	267.3	263.2	268.7	270.5		
L3*	1	267.5	267.5	270.5	266.5	269.9	268.3	7.2
	2	272.1	265.8	267.5	268.7	269.6		
	3	269.3	267.0	267.4	265.3	269.5		
L4	1	268.8	266.5	271.7	270.9	268.3	270.6	11.4
	2	269.8	268.9	274.7	274.0	269.2		
	3	271.6	268.3	273.5	274.0	268.3		
L5	1	282.5	277.3	282.3	279.4	271.9	278.3	Not provided
	2	278.3	279.4	282.6	274.6	271.9		
	3	282.4	279.7	283.2	274.9	273.8		
L6	1	283.0	283.4	276.7	281.4	280.8	281.3	8.5
	2	284.2	281.6	277.9	282.2	281.2		
	3	283.7	282.5	277.3	282.8	280.7		
L7	1	275.4	281.0	271.6	281.3	280.1	277.8	6.7
	2	275.1	278.8	269.9	282.6	279.9		
	3	276.9	280.0	270.3	282.4	282.0		
L8	1	278.5	270.6	270.9	273.9	275.0	274.0	9.2
	2	276.9	272.5	271.4	274.7	275.5		
	3	278.0	271.4	270.9	274.7	274.7		
L9	1	283.0	279.3	276.3	276.5	281.3	279.6	5.9
	2	282.5	278.0	278.1	277.1	281.3		
	3	283.5	279.0	278.7	277.4	282.2		

Table E1 (continued)

Lab code	replicate	Vial 1[U/L]	Vial 2 [U/L]	Vial 3 [U/L]	Vial 4 [U/L]	Vial 5 [U/L]	Mean [U/L]	Expanded uncertainty [U/L]
L10	1	276.8	277.5	274.7	278.9	273.4	277.4	6.6
	2	278.6	280.6	275.2	279.8	278.6		
	3	278.3	279.5	274.3	279.1	276.4		
L11	1	284.0	276.1	282.3	279.4	283.4	280.2	9.4
	2	283.4	277.0	280.2	280.6	281.2		
	3	278.2	281.2	277.8	277.3	280.3		

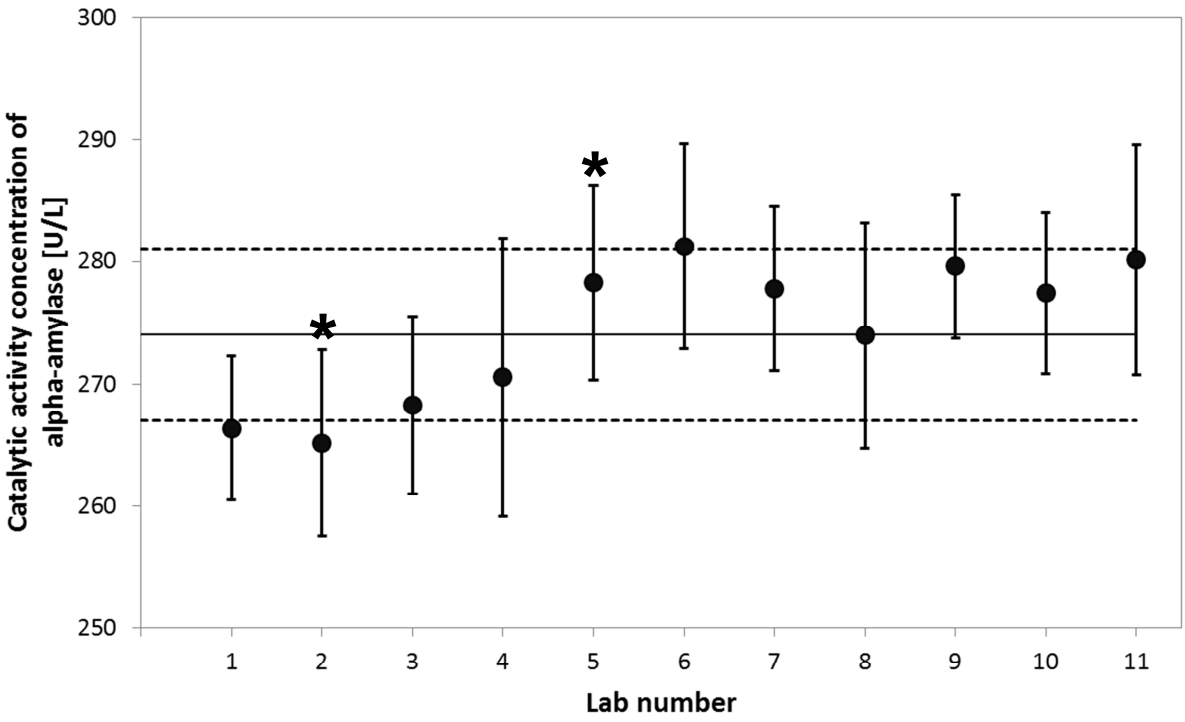

Figure E1: Graph showing the laboratory means for the enzymatic activity concentrations of alpha-amylase in ERM-AD456/IFCC measured with the PRMP from the IFCC. The error bars indicate the expanded measurement uncertainties as reported by the laboratories. Lab 2 and Lab 5 did not report the uncertainty associated with their measurement results. The average measurement uncertainty of the other participating laboratories was therefore used as an estimate of their measurement uncertainty (indicated by an asterisk). The solid black line represents the certified value, while the dashed lines represent the expanded uncertainty of the certified value.

Annex F: Results of the commutability study

Table F1: The measurement procedures (combination of platforms and reagents) used in the commutability study on the trail batch of ERM-AD456/IFCC. There are different substrates available to measure the catalytic activity concentration of alpha-amylase including: 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) and 4,6-ethylidene(G1)-4-nitrophenyl(G7)-α-(1→4)–D-maltoheptaoside (EPS).

Method code	Platform	Reagents or kit used	Substrate
Automated version of the PRMP	Konelab 30 i (Thermofisher)	Reagents according to the PRMP were used: α-glucosidase (Roche diagnostics) EPS (Roche diagnostics) HEPES (Sigma-Aldrich)	EPS
Abbott	Architect ci8200 (Abbott)	Abbott amylase kit Product code 7D58	CNPG3
Beckman	AU 5800 (Beckman Coulter)	Beckman Coulter AMY (alpha Amylase IFCC-EPS) Product code OSR6182	EPS
Biosystems	BA-400 (Biosystems)	Biosystems alpha-AMYLASE –EPS Product code 21534	EPS
Roche	Cobas c702 (Roche)	Roche alpha-amylase EPS ver.2 Product code 05167027 190	EPS
Siemens	Advia 2400 (Siemens)	ADVIA amylase Product code: 03031177	EPS

Table F2: The outcome of the commutability study performed on the trial batch of ERM-AD456/IFCC and 30 serum pools. The measurement results of the five routine measurement procedures were compared to the measurement results of the automated version of the PRMP for the catalytic activity concentration of alpha-amylase from the IFCC. Two different statistical approaches were used to assess the commutability: a linear regression analysis with 95 % prediction interval as described in CLSI EP30-A (indicated as CLSI) and a difference in bias analysis as described in the recommendations of the IFCC Working Group on Commutability (indicated as IFCC). The commutability criterion was set at 3.7 % for the difference in bias analysis.

	Statistical analysis	
Method code	**CLSI**	**IFCC**
Abbott	Non-commutable	Non commutable
Beckman	Commutable	Inconclusive result
Biosystems	Commutable	Commutable
Roche	Commutable	Commutable
Siemens	Commutable	Commutable

European Commission

EUR 29857 EN – Joint Research Centre – Directorate F – Health, Consumers and Reference Materials
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Author(s): Liesbet Deprez, Ingrid Zegers, Heinz Schimmel, Stefanie Trapmann
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