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REFERENCE MATERIALS

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IRMM/IFCC-456

Report

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Catalytic concentration of α-amylase determined by IFCC reference method at 37 °C

IRMM/IFCC-456

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Directorate General
Joint Research Centre

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Mission

The mission of the Institute for Reference Materials and Measurements is to promote a common European measurement system in support of EU policies, especially internal market, environment, health and consumer protection standards.

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ABSTRACT

The Institute for Reference Materials and Measurements and the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) characterised and certified a reference material under the name and number IRMM/IFCC-456. This report describes the certification of the catalytic concentration of α-amylase in a purified lyophilised material of human origin (pancreas) in a human serum albumin matrix when measured by the IFCC reference method at 37 °C. The catalytic concentration of amylase in reconstituted material is certified to 546 ± 19 U/L or 9.1 ± 0.3 μkat/L. It is the intention that the reference material should be used to control and optimise the performance of enzyme measurements, verify the comparability of results from different laboratories and be used as a reference material for manufacturers of reagents and diagnostic kits.

GLOSSARY

ANOVA ..... analysis of variance
BCR ..... Community Bureau of Reference
CRM..... certified reference material
DEAE..... diethylaminoethyl
DGKC ..... Deutsche Gesellschaft für Klinische Chemie
EDTA..... ethylene diamino tetraacetic acid
GUM..... Guide to the Expression of the Uncertainty in Measurement
HIV ..... human immunodeficiency virus
HBsAg..... hepatitis B surface antigen
IFCC..... International Federation for Clinical Chemistry and Laboratory Medicine
IRMM ..... Institute for Reference Materials and Measurements
ISO..... International Organisation for Standardisation
MSA..... mean square among groups (ANOVA)
MSW..... mean square within groups (ANOVA)
PIPES..... 1,4-piperazine diethanesulfonic acid
RSD..... relative standard deviation
SI..... international system of units
SOP..... standard operating procedure
WG-CCE..... working group for calibrators in clinical enzymology
s..... standard deviation
s_{betw}..... standard deviation between certification laboratories (ANOVA)
s_{wb}..... standard deviation within groups from the hom. study (ANOVA)
s_{bb} ..... standard deviation between groups from the hom. study (ANOVA)
u_{bb}..... standard uncertainty of homogeneity
u^*_{bb}..... upper limit for inhomogeneity from homogeneity study
u_{char}..... standard uncertainty of the characterisation
U_{CRM}..... expanded (k=2) combined uncertainty of the CRM
u_{lts}..... standard uncertainty of stability during storage
u_{sts}..... standard uncertainty of stability during transport

DEFINITIONS

Catalytic activity (z) of an enzyme is a property quantified by the catalysed rate of conversion of a specified chemical reaction, produced in a specified assay system.

Unit of catalytic activity: katal (kat) = mole per second (mol/s). For any measurement procedure 1 U = 1 µmol/min = 16.67 nmol/s = 16.67 nkat.

Catalytic (activity) concentration (b) is the catalytic activity of the component (enzyme) divided by the volume of the original system containing the enzyme (not the assay system).

Unit of catalytic (activity) concentration: 1 kat/L = 10^3 mol · s^{-1} · m^{-3}, 1 U/L = 0.01667 µkat/L.

CONTENTS

1. INTRODUCTION

Human serum α-amylase (1,4-α-D-Glucan-glucano-hydrolases, EC 3.2.1.1) is a monomer protein consisting of 511 amino acids. The acinous cells of pancreas and the saliva glands are the major tissue sources of serum α-amylase. Lower concentrations are found in skeletal muscle, small intestine and other tissues. α-Amylase deriving from pancreas or saliva differ by the content of carbohydrates resulting in molecular weights of the enzymes between 50 kD (pancreas) and 51 kD (saliva). α-Amylase catalyses the degradation of carbohydrates with α1→4 bound glucose units like amylose, amylopectin and glycogen. Increased α-amylase catalytic concentrations in human serum and urine occur under various diseases with affection of the pancreas or the parotid glands.

The preparation and certification of enzyme reference materials namely gamma-glutamyltransferase (BCR-319), alkaline phosphatase (BCR-371), creatine kinase BB (BCR-299), alanine aminotransferase (BCR-426), prostatic acid phosphatase (BCR-410) and lactate dehydrogenase isoenzyme 1 (BCR-404), creatine kinase MB (BCR-608) and pancreatic α-amylase (BCR-476, [1]) have been organised on behalf of he Community Bureau of Reference (BCR) of the European Commission in the past. With the exception of α-amylase, these enzymes have been certified for measurements at 30 °C. The continuous development of new methods by manufacturers based on different substrates, temperature, pH, cofactors etc. causes difficulties related to interpretation of interlaboratory results. On the 1998 IFCC General Conference in Seville, the working group for calibrators in clinical enzymology (WG-CCE) and the manufactures of diagnostic enzyme kits agreed to change the measurement temperature for the IFCC reference procedures from 30 °C to 37 °C. This has resulted in a co-operation project between the Institute for Reference Materials and Measurements (IRMM), IFCC and diagnostic companies with the objective to prepare new standard operating procedures (SOPs) and certify – amongst others (IRMM/IFCC-452 to 455; [2-5], previously BCR-319, -404, -426 and -608) – the material for α-amylase. The new IFCC recommended method for α-amylase is optimised with regard to both kinetic reactions and technical aspects of the measurements.

Recent regulatory initiatives [6, 7] will have a major impact on the design of test systems, including those for enzyme measurements. European legislation foresees that manufacturers include in the technical documentation of their test systems "*adequate performance evaluation data showing the performances claimed by the manufacturer and supported by a reference measurement system with information on the reference methods, the reference materials, the known reference values, the accuracy and measurement units used*" [6]. Also ISO 17025 [7] requires testing laboratories to perform measurements in a way that they are either traceable to the SI or to a specified standard, as it is the case for the determination enzymatic activity.

2. PARTICIPATING LABORATORIES

Purification of the enzyme

Universidad Autónoma de Barcelona, Departamento de Bioquímica y Biologia Molecular, Bellaterra (ES)

Lyophilisation and ampouling

National Institute for Biological Standards and Control, Potters Bar (UK)

Homogeneity and stability studies

Universidad Autónoma de Barcelona, Departamento de Bioquímica y Biologia Molecular, Bellaterra (ES)
National Institute for Biological Standards and Control, Potters Bar (UK)
Medizinische Hochschule Lübeck, Institut für Klinische Chemie, Lübeck (DE)
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Characterisation exercise

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Evaluation

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Project management

The IRMM/IFCC-456 was produced and certified in a close co-operation between the above mentioned institutions on behalf of IRMM and IFCC in the frame of a project on standardisation of enzyme measurement initiated by WG-CCE. The work of certification was co-ordinated by the German Society of Clinical Chemistry (Deutsche Gesellschaft für klinische Chemie, DGKC), Reference Institute of Bioanalysis (DE) and the IRMM (BE).

3. PREPARATION OF THE MATERIAL

The preparation of the lyophilised material is described in detail in the original certification report from 1995 [1]. However, a short summary of the main steps involved in the purification and characterisation of the enzyme is given below.

3.1 Choice of the source of the enzyme

α-Amylase in human tissues and blood plasma shows two different isoenzymes, namely "pancreatic" and "salivary" amylases. The diagnostic significance of serum and urine α-amylase measurements is in the diagnosis of acute pancreatitis, where an increase of the pancreatic isoenzyme is usually found. As large quality differences in α-amylase derived from malt, bacteria, porcine and human sources have been demonstrated, it was decided to use material of human origin. Human pancreas was chosen for the right isoenzyme and because a relatively small mass of tissue can yield high amounts of purified enzyme. The use of human tissue was done according to the ethical guidelines at the time of production.

3.2 Extraction and purification of the enzyme

Tissue was obtained according to the Tissue was homogenised in phosphate buffer. Amylase was purified by ammonium sulphate precipitation and chromatography successively on DEAE-Sephacel® , CM-Sepharose® and Sephadex G-75®.

3.3 Choice and addition of the matrix followed by lyophilisation

A freeze-dried material was preferred to ensure long-term stability. Human serum albumin (30 g/L) in combination with PIPES-buffer (25 mmol/L, pH 7), NaCl (50 mmol/L) EDTA (0.5 mmol/L) and $CaCl_2$ (1.5 mmol/L). PIPES was chosen as buffer because it was found that this buffer does not affect the commutability.

3.4 Production control

- Infectious diseases: The material was obtained at autopsy from subjects believed to have been free from pancreatic and transmissible diseases. Sera of the subjects were free of hepatitis B s antigen and HIV 1+2 antibodies. The final material was also tested and found negative for HIV and HBsAg.
- Contaminating enzymes: Catalytic activities of α-glucosidase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, gamma-glutamyltransferase, trypsin, chimotrypsin, protease and alkaline phosphatase were not detectable. Trace amounts of lipase and lactate dehydrogenase were measured.
- Purity: Polyacrylamide-gel electrophoresis of the purified enzyme in the presence of sodium dodecyl sulphate showed one major band of relative molar mass 57500 g/mol and one minor band of relative molar mass of 63100 g/mol. Traces of other proteins could only be identified by overloading the gel. Non-denaturing gel-electrophoresis of the purified material showed one major band that was identified to be pancreatic α-amylase by western blotting using a monoclonal antibody (anti-pancreatic α-amylase). Isoelectric focusing of the purified α-amylase on agarose and on acrylamide showed a major band with an isoelectric point at 7.0
- Water content: The water content of 3 ampoules was measured by the coulometric Karl Fischer method as 0.103 % (mean). The results confirmed that adequate drying had taken place.
- Testing of filling procedure: 271 Ampoules taken at regular intervals throughout the filling procedure had a mean mass of contents of 1.0141 g with a range 1.0118 to 1.0164 g. There was no evidence for a general trend in the variation of mass throughout the filling procedure.
- Residual oxygen content. The oxygen content of six ampoules was measured by mass spectrometry. Oxygen volume fractions ranged from 0.14 to 0.3 %.

3.5 Commutability

A short overview over the studies performed for the certification using the previous method are given. A more detailed description can be found in [1]. It should be pointed out that these data do not per se prove commutability of the material at the new measurement conditions. Further commutability studies will be organised later.

The effect of the pH on the α-amylase catalytic activity was studied using the purified pancreatic enzyme and a human serum sample. The pH effect profile was very similar for the pancreatic preparation and the human sample.

Commutability of the material concerning the measurement temperatures 30 °C and 37 °C was tested. Catalytic activity of a serum pool and IRMM/IFCC-456 was determined in 4 replicates each at 30 °C and 37 °C. Conversion factors obtained were 1.42 for the serum pool and 1.40 for IRMM/IFCC-456 (RSD between 0.2 and 0.4 %), indicating commutability for the previous measurement conditions.

4. MOLAR ABSORPTION COEFFICIENT OF 4-NITROPHENOL

The measurement is based on the absorption by 4-nitrophenol. As no value for the molar absorption coefficient in the reaction mixture conditions is available in the literature, it was decided to determine the absorption coefficient as well. This is especially important as this coefficient is very pH dependent. A change of pH 7.00 to 7.03 gives a 3 % higher amylase value.

4-Nitrophenol was dried according to a specified procedure. All laboratories reported values for the molar absorption coefficient in NaOH within ± 0.6 % of the value specified by Sigma (4-nitrophenol, spectrometric grade, order # 104-8; ε_{401nm} in 0.01 M NaOH = 1840 m^2mol^{-1} ± 1 %). Traceability of spectrophotometry was assured through nitrophenolate solutions certified by the German Metrology Institute (Physikalisch-Technische Bundesanstalt) and by checking the wavelength calibration using a mercury vapour lamp or a holmium oxide filter or a holmium containing solution. An aqueous stock solution of 4-nitrophenol was diluted. The diluent was equal to solution 2 as described in the SOP for the reference procedure for amylase, except that the pH value was adjusted to 7.00. Water or serum, respectively was used as the volume fraction of sample. The molar absorption coefficients of samples with and without serum were determined on two days each. After examination of the results, it was decided to remove the results of one laboratory from the set because of its high standard deviation caused by insufficient pH control. The results of the accepted determinations are shown in Table 1.

Tab. 1: Results accepted for the determination of the molar absorption coefficient of 4-nitrophenol in serum; ratio = ratio of the mean result with serum divided by the mean result without serum

	ε with serum [m^2mol^{-1}]			ε without serum [m^2mol^{-1}]]			ratio
	day 1	day 2	mean	day 1	day 2	mean	
lab 1	1012.7	1020.7	1016.7	1030.3	1031.1	1030.7	0.986
lab 2	1055.1	1049.0	1052.0	1059.1	1073.0	1066.1	0.987
lab 3	962.2	961.9	962.1	966.6	963.0	964.8	0.997
lab 4	1074.0	1060.3	1067.2	1023.7	998.7	1011.2	1.055
lab 6	997.3	1002.7	1000.0	981.1	983.3	982.2	1.018
lab 7	984.6	989.3	987.0	1006.5	1004.8	1005.7	0.981
lab 9				1034.6	1041.2	1037.9	
lab 10	996.7	994.3	995.5	999.2	996.8	998.0	0.997
lab 11	1001.3	1043.1	1022.2	992.3	1003.3	997.8	1.024
mean ± s			1013 ± 34			1010 ± 31	1.006 ± 0.025

As can be seen in Tab. 1, the results from the determinations with or without addition of serum do not differ significantly, neither for individual laboratories nor for the means of all laboratories. Therefore, the results of all data can be pooled and a molar absorption coefficient of 1012 m^2mol^{-1}(mean of the two means) shall be used from now on.

5. CHARACTERISATION MEASUREMENTS

5.1 Certification procedure

The certification was based on the agreement between the results obtained in the different laboratories, all of them using the same SOP to measure the catalytic concentration at 37 ° C [8]. In advance to the certification campaign, the laboratories volunteered to perform a training exercise by analysing 5 different commercial enzyme solutions. By this exercise it was possible to ensure the validity of the SOP for measuring enzyme as well as the performance of the individual laboratories. The protocol contained information about instructions for calibration of pipettes, specifications and instructions for the calibration and verification of apparatus used in measurement, specifications for reagents and procedure for the preparation of solutions, specifications for the measurement conditions for the procedure and calculation of results. The measurement principle is as follows:

$$ENM + H_2O \xrightarrow{\alpha\text{-}amylase} 4,6\text{-}Etylidene\text{-}GP\text{-}Gx + 4\text{-}Nitrophenyl\text{-}GP\text{-}G(7-x)$$

$$4\text{-}Nitrophenyl\text{-}GP\text{-}G(7-x) + (7-x)H_2O \xrightarrow{\alpha\text{-}glucosidase} (7-x)glucose + 4\text{-}Nitrophenol$$

ENM: 4,6-Ethylidene(G1)-4-nitrophenyl(G7)-α-(1→4)-D-maltoheptaoside (substrate)

GP: α-(1→4)-D-glucopyranosil

G1 and G7 denote the glucosyl moieties (numbered from the non reducing end) of the oligosaccharide.

The change of the concentration of 4-nitrophenol is measured photometrically.

Each participant received 7 vials of the lyophilised amylase material together with samples for internal quality control, the SOP, the reconstitution procedure and data sheets for reporting results and the requested information. Three vials were reconstituted on each of two days, and one measurement of catalytic concentration of amylase on each vial was performed on the day of reconstitution. The remaining vial was a spare sample. Vials containing commercially available lyophilised sera were distributed together with the CRM to have an independent means of quality assessment. All vials were shipped on dry ice to the participants on July 2000 and were delivered in less than 2 days.

5.2 Quality assurance

Traceability of gravimetry, volumetry and thermometry was demonstrated and documented. Traceability of spectrophotometry was assured through nitrophenolate solutions certified by the German Metrology Institute (Physikalisch-Technische Bundesanstalt) and by checking the wavelength calibration using a mercury vapour lamp or a holmium oxide filter/holmium containing solution.

5.3 Components of CRM uncertainty

Uncertainty of the CRM was estimated according to the Guide to the Expression of Uncertainty in Measurement (GUM) [9]. For this, estimates of the uncertainty of the characterisation (u_{char}), homogeneity (u_{bb}) and stability (u_{lts}) are required as described by Pauwels *et al.* [10]. Uncertainty of the characterisation was derived from the certification measurements. An estimation for the inhomogeneity was derived from the homogeneity study of the original material. For assessment of stability, data from the original stability study and results from stability monitoring afterwards were available. Evaluation and combination of these uncertainties was done analoguous to IRMM/IFCC-452 to –455 [11].

6. RESULTS

6.1 Certification collaborative study

The results submitted were subjected to a technical scrutinisation. One laboratory had measured at a pH outside of the range specified by the SOP. It was therefore decided not to use these results. Another laboratory was consistently high on both days for the quality control samples. It was decided not to include these results for the calculation of the certified value.

The individual results together with laboratory averages, standard deviations and RSDs from the 7 laboratories accepted are given in Table 2 (results rounded to the same decimal position). The results were obtained from single measurements of each of six vials that were performed in November 2000.

Table 2. Individual results accepted for the certification.

Laboratory	Experimental results [U/L]						Mean ± s [U/L]	RSD [%]
lab 01	554.0	550.6	551.4				552.0 ± 1.8	0.3%
lab 02	560.6	562.3	560.1	556.4	559.7	562.2	560.2 ± 2.1	0.4%
lab 06	546.0	537.6	541.7	538.0	539.1	540.1	540.4 ± 3.1	0.6%
lab 07	549.9	549.9	549.8	550.5	548.4	553.5	550.3 ± 1.7	0.3%
lab 08	561.1	511.6	543.8	531.9	537.6	526.4	535.4 ± 1..7	3.1%
lab 10	550.5	535.8	554.7	541.9	533.7	541.6	543.0± 8.2	1.5%
lab 11	540.3	538.8	540.6	541.3	543.8	542.7	541.3 ± 1.8	0.3%

An analysis of variance (ANOVA) was performed, the results of which are shown in Table 3. The results show that the variation of laboratory averages can be mainly attributed to differences between the averages while variation within the laboratories is only a minor contribution, as its influence is devided by the square root of the number of measurements per laboratory.

Table 3. Results of the statistical analysis of accepted results

mean of means		546.1 U/L
standard deviation between means *s*		8.5 U/L
number of laboratories *n*		7
standard error of the mean of means		0.59 %
ANOVA	s within laboratories	1.39 %
	s between laboratoriess	1.47 %

The uncertainty from the interlaboratory study is referred to as u_{char} and comprises uncertainties from between-laboratory reproducibility and within-laboratory repeatability. The standard error of the mean ($\frac{s}{\sqrt{n}}$) was used as the best estimation of the combined effect of these influences. The total u_{char} amounts to 3.10 U/L (0.59 %).

6.2 Stability

Two data sources were available for the assessment of uncertainty of stability, namely the original stability study and the results from stability monitoring. In the original stability study, samples were stored up to 857 days at –20, 20, 37 and 45 °C. After this period, activity of sample stored at 45 °C was still 92 % of the activity of the samples stored at –20 °C, whereas

activity of the samples stored at +20 °C was 101 % of the sample stored at –20 °C. A yearly loss of 0.03 % at –20 °C was calculated.

Stability was monitored after 1.3, 2.2, 3.16, 4.06 and 4.09 years. Low results after 3.16 years were refuted by the later findings (two different laboratories) and were therefore not used for evaluation. The averages of the analyses are shown in Table 4.

Tab. 4: Results from the stability monitoring.

time [y]	n	average catalytic activity ± s [U/L]
0.00 (certification)	8	555.0 ± 12.0
1.33	12	557.0 ± 6.5
2.16	12	535.5 ± 3.1
3.16	12	456.3 ± 12.0
4.06	6	563.0 ± 8.5
4.09	6	542.3 ± 18.2

Uncertainty of stability was estimated by extrapolation of the standard error of the slope as described in [12]. The standard error of the slope was calculated as 0.58 % if the calculation was based on the mean of each time or as 0.26 % if the individual values were used. Seeing the apparent stability in the accelerated stability study, the latter value was regarded more realistic. Uncertainty of stability is therefore 1.32 % if estimated for a shelf life of five years.

Uncertainty of degradation during transport (u_{sts}) can be assumed negligible, as no degradation was found after storage at 45 °C for 31 days.

6.3 Homogeneity

Data for the assessment of homogeneity were taken from the earlier certification exercise. 20 ampoules had been analysed in triplicate on two days each. Several doubtful values were present in the dataset of day 1, so homogeneity was assessed using the data of day 2 only. Results of two days were pooled and an ANOVA over the data grouped by ampoule was performed. Grand mean, mean squares among and within groups (MSA, MSW), derived standard deviations within and between groups (s_{wb}, s_{bb}) and the upper limit for the inhomogeneity as given by the method repeatability (u^*_{bb}) according to [13] are shown in Table 5.

Tab. 5: Results of the ANOVA over the homogeneity study.

grand mean	432.4 U/L
MSA	75.9U^2/L^2
MSW	37.9 U^2/L^2
s_{wb}	1.42 %
s_{bb}	0.82 %
u^*_{bb}	0.39%

As the estimated standard deviation between units (s_{bb}) was larger than the upper limit for the inhomogeneity as given by the method repeatability, s_{bb} was used as estimate for between-unit inhomogeneity (u_{bb}).

6.4 Estimation of the combined uncertainty

The uncertainty of the CRM can be estimated by summation of the contributions of characterisation, homogeneity and stability [10]. The individual uncertainty components for characterisation, inhomogeneity and instability described in the equation below are added,

and multiplied by a coverage factor of 2 to give a combined expanded uncertainty. As uncertainties have the format of standard deviations, addition is done quadratically.

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2}$$

U_{CRM} ..... expanded uncertainty of the CRM
k ..... coverage factor
u_{char} ..... uncertainty of the certified property of the batch
u_{bb} ..... between-bottle inhomogeneity
u_{lts} ..... uncertainty of long-term stability (storage)
u_{sts} ..... uncertainty of stability during transports

Transport conditions are chosen that make the uncertainty of stability during transport negligible. u_{char} of the batch amounts to 0.59 %. u_{bb} and u_{lts} were estimated 0.82 % and 1.32 % respectively. Thus, expanded uncertainty U_{CRM} amounts to

$$U_{CRM} = 2 * \sqrt{0.59^2 + 0.82^2 + 1.32^2} = 3.32\% \triangleq 18.2\ U/L$$

7. CERTIFIED VALUE

The certified value and the corresponding expanded uncertainty are given below.

Catalytic concentration of α-amylase as determined by the 37 °C IFCC reference method:

546 ± 19 U/L

or

9.1 ± 0.3 µkat/L

The catalytic activity concentration in µkat/L was calculated by multiplication of the results in U/L with 0.01667. The certified value is valid until 11/2005. The validity can be extended if further evidence of the stability of the material is obtained. In contrast to previously certified materials for which the 95 % confidence interval of the mean of laboratory means was used as uncertainty, an expanded combined uncertainty according to the GUM was calculated this time. This included also influences of homogeneity and stability.

The results of the laboratory averages and the certified value with its expanded uncertainty estimated according to the GUM are given in Figure 1. The figure was obtained by estimating expanded uncertainties for the individual laboratories using the between laboratory standard deviation (s_{betw}) of the certification exercise from the ANOVA as the best estimate of the variation of the laboratory bias. Thus, laboratory uncertainty was calculated using $(2 * \sqrt{s_{betw}^2 + \frac{s_{lab}^2}{n_{lab}}})$, with s_{lab} and n_{lab} being the standard deviation and number of replicates in the individual laboratories.

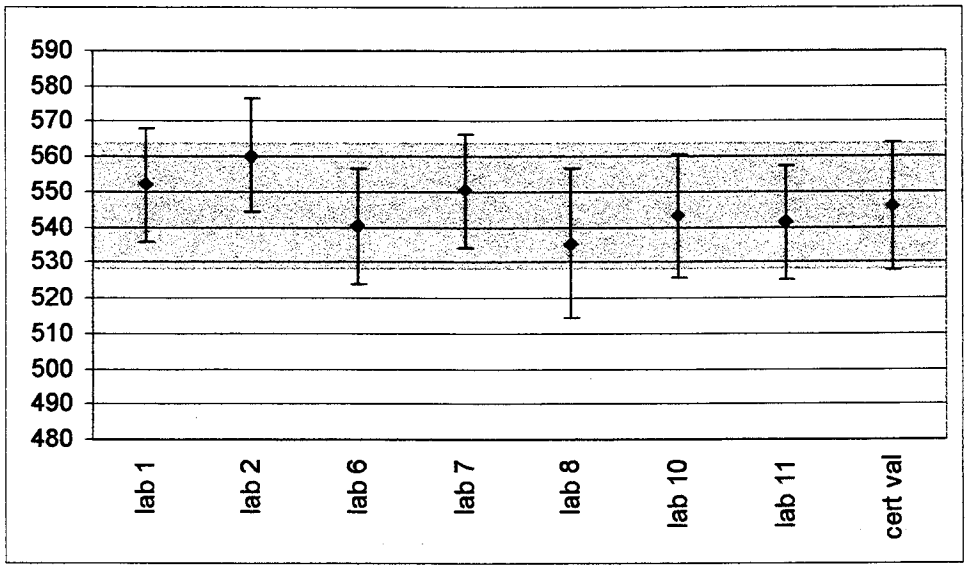

Fig. 1:. Laboratory averages, their expanded uncertainties (k=2), the certified value and its expanded uncertainty (k=2). The shaded area corresponds to the certified range.

8. USE OF THE CRM

8.1 Dispatch and instructions for use

Dispatch to the customer will be done under cooled conditions. Upon receipt by the customer, it is advisable to keep the material at – 20 °C but the material can be stored refrigerated (4 °C) for not longer than 3 months until used.

8.2 Reconstitution of the material

1. Take the ampoule out of the freezer and allow reaching room temperature.
2. Tap the vertically positioned ampoule gently to ensure that the lyophilised material is at the bottom of the ampoule.
3. Score the ampoule at the constriction with a sharp file and open, by applying a red-hot glass rod to the score for about 1 s, while holding the ampoule almost horizontally to prevent glass from entering the ampoule.
4. Weigh the ampoule with its contents to the nearest 0.1 mg.
5. Reconstitute by slow addition to the sides of the ampoule of (1.00 ± 0.01) ml distilled water (20-22 °C) with calibrated volumetric equipment. Note the temperature.
6. Weigh the ampoule after adding the water.
7. Vortex mix. Keep the reconstituted material at 4 °C.
8. Calculate the volume of water at 20 °C from the weight of the volume by multiplying with the factor z = 1.003. This factor take into account the temperature-dependent density and the buoyancy in the air.
9. The catalytic concentration of α-amylase must be measured within 8 hours following the reconstitution and the ampuls should not be stored for re-use.

8.3 Intended use

The material is intended to provide, when reconstituted, a solution with a known catalytic concentration of α-amylase that can be used for intra-laboratory quality control of the measurement procedure and to verify comparability of results from laboratories using this measurement procedure. The certified reference material can also be used for evaluation of in vitro test systems for α-amylase measurements by method comparison with the 37 °C IFCC reference measurement procedure. The material can also be used for the calibration of lower order procedures for measuring α-amylase activities provided they have the same or similar analytical specificities as the reference measurement procedure used for the certification. Commutability of the results from the undiluted material to dilutions has to be checked thoroughly before using the material at several concentration levels for calibration. Furhermore, traceability of the routine method to the IFCC reference method must be ensured.

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Abstract

The Institute for Reference Materials and Measurements and the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) characterised and certified a reference material under the name and number IRMM/IFCC-456. This report describes the certification of the catalytic concentration of α-amylase in a purified lyophilised material of human origin (pancreas) in a human serum albumin matrix when measured by the IFCC reference method at 37 °C. The catalytic concentration of amylase in reconstituted material is certified to 546 ± 19 U/L or 9.1 ± 0.3 μkat/L. It is the intention that the reference material should be used to control and optimise the performance of enzyme measurements, verify the comparability of results from different laboratories and be used as a reference material for manufacturers of reagents and diagnostic kits.

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