Standardisation, accreditation and harmonisation

Cod: 1499

EFFECT OF CALIBRATION METHODS ON AUTOMATED IMMUNOASSAY ACCURACY AND STABILITY

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BACKGROUND: The accuracy and stability of automated immunoassays are dependent on the calibration curve used for converting instrument measurements to analyte concentrations. We investigated the calibration effects of using multiple calibrator measurements on assay accuracy and stability for three assays (luteinizing hormone (LH), total triiodothyronine (TT3) and vitamin B12) performed on the Beckman Coulter Access 2 analyzer.

METHODS: Thirty data sets were collected over forty-three days for six levels of calibrators and three levels of serum quality control pools. Each data set was obtained by performing four replicates of calibrator levels S0, S1, S4 and S5 and three replicates of levels S2 and S3. One replicate for three levels of quality control materials (QC1, QC2, and QC3) also were collected for comparison. Percent of bias offset between average of three consecutive days after calibration and target values were calculated to measure accuracy. The maximum number of days before the calibrations exceeded the defined calibration limits also were calculated using time regression analysis with 5% tolerance limit for each of three calibration procedures (CC1, CC2 and CC3). These procedures were based on 12, 34 and 56 calibrator measurements over 1, 2, or 3 days, respectively.

RESULTS: Time regression analyses of LH (sandwich assay) showed a negative slope, while TT3 and vitamin B12 (competitive assays) had positive slopes. The bias offset values for LH were 2.26%, 1.44% and -0.29% using CC1, CC2 and CC3, respectively. The stability of LH was artificially longer with more positive bias offset values: >43, 39 and 36 days. The bias offset values for TT3 were -2.49%, 1.49%, and -0.50% using CC1, CC2 and CC3, with stability of 32, 20, 30 days respectively. The bias offset values for vitamin B12 were 2.44%, 0.91%, and -0.50% using CC1, CC2 and CC3 with stability of 4, 9, 12 days, respectively. The assay stability days were consistent with bias offset values.

CONCLUSIONS: Accuracy was improved (decreased bias offset values) when assays were calibrated with more calibrator measurements. Measurements of calibrator stabilities for these assays were dependent on the calibration bias offset. These improvements in accuracy could have good impact on clinical practice.

Standardisation, accreditation and harmonisation

Cod: 1500

MEASUREMENT UNCERTAINITY OF TOTAL PROTEINS AND ALBUMIN

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BACKGROUND: The uncertainty of measurement (UM) is non-negative parameter characterizing the dispersion of quantity values being attributed to a measurand based on the information used (VIM III, 2.26), which serves, to characterize a range of values for the true value of the measurand. Reporting the UM is required of laboratories accredited to ISO/IEC 17025 and ISO/IEC 15189 and is important mostly to make patient results comparable irrespective of where the testing is done. Aim of the study: to estimate UM for serum total proteins and albumin in our laboratory.

METHODS: top-down approach was used as a method estimation of UM where data from internal quality control and EQAS were included as follows: estimation of within-laboratory ucertainity ($\%u_{rw}$) from intralab imprecision (reproducibility and repeatability) as well as calculations of $\%u_{bias}$, combined uncertainty $\%u = (\%u^2_{rw} + \%u^2_{bias})1/2$ and expanded UM (with a 95% confidence).

RESULTS: Results were similar for both control levels used and higher values are presented. These are: for total proteins - $\%u_{rw}$=5,3, %u=6,1 and expanded UM 8,3 (g/L); for albumin- $\%u_{rw}$=5,9, %u=5.5 and expanded UM (g/L)=4,1.

CONCLUSIONS: In cases where no demands have been published, a guiding principle that was accepted in this study could be that expanded MU should be approximately equal to, or less than 2 times the reproducibility. Our results showed just a little bit higher expanded UM. So, the main goal that arose from the study was to reduce the uncertainty. A few recommendations could be accepted and implemented: use of calibration facilities with the smallest uncertainties, corrections to compensate for known errors, checking the measurements by repeating them and check calculations.

Standardisation, accreditation and harmonisation

Cod: 1501

STUDIES OF REFERENCE METHOD FOR URIC ACID -DIRECT SPECTROSCOPY

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BACKGROUND: Evaluation of the UV spectrophotometry of serum uric acid method, which to be recommended as the initial determination of (UV spectrophotometry) reference method; And application of this method with isotope dilution mass spectrometry (ID-MS) method for measuring precision and accuracy results were compared.

METHODS: Determination of the United States by certified reference material NIST (SRM), uric acid, a substance made national standard recovery experiments, participated in the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference laboratory room between the ratio of activities (RELA), verify the accuracy of this method and reliability.

RESULTS: UV spectrophotometric determination of uric acid detection range up to 1200μmol/L, coefficient of variation (CV) <1.1%, CV(day) <1.8%, the recovery was 99.5% ~ 100.3%. The average relative bias measured SRM was 0.25%; measured the RELA samples (A / B), the results with isotope dilution mass spectrometry (ID-MS) method of the relative bias ranged from 1.5% to 2.5 %.

CONCLUSIONS: The UV spectrophotometry to be recommended as a reference method determination of uric acid.

Standardisation, accreditation and harmonisation

Cod: 1502

COMPARISON OF HBA1C IN EDTA AND EDTA - FLUORIDE TUBES

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BACKGROUND: HbA1c estimation has become the gold standard for the long-term control of the glycaemic state of diabetic patients.It's common to co-request HbA1c and glucose (fasting and post prandial). The HbA1c concentration may trigger a revision of the dosage of drugs, guided by fasting glucose and post prandial glucose concentrations as well as during diagnosis of diabetes. The IFCC document on the standardization of HbA1c measurements uses EDTA blood and this has become the standard procedure in most laboratories where as fluoride tubes are standard for glucose estimation due to its antiglycolytic effect. In this study we employ the CLSI recommendation EP9 and EP15 to determine the degree of agreement between HbA1c concentration measured in EDTA, and EDTA+fluoride tubes.

METHODS: Vacutainers® used were Potassium EDTA and Sodium Fluoride-Sodium EDTA. Venous blood, 2 mL, was collected in each tube from 104 patients and HbA1c was measured by HPLC on Bio-Rad D10. Interactive spreadsheets developed for Microsoft Excel and based on innate statistical functions were used.

RESULTS: Bland Altman plots showed mean difference between the EDTA-fluoride and EDTA tubes was 0.4 mmol/mol (SEM 0.13 mmol/mol). The regression calculated using Deming regression, the slope was 1.00 and intercept 0.14 over the whole measuring interval. The imprecision study by ANOVA using EP 15 showed within series imprecision of 0.47 mmol/mol, the between run uncertainty 0.20 mmol/mol and combined uncertainty 0.50 mmol/mol. The results were the same for both tubes. The concentration in the EDTA tubes was 53.0 mmol/mol, and in the EDTA-fluoride tubes 54.1 mmol/mol ($p<0.01$).

CONCLUSIONS: Data analysis shows that there is a statistically significant difference between the results attributable to the tubes but these difference is too small to be clinically relevant. Paired requests for HbA1c and glucose concentrations are common. We show that HbA1c can be measured using hemolysates equally well from EDTA tubes and EDTA-fluoride tubes. This will contribute to savings of materials/consumables and inconveniences for patients and staff. This can have a significant impact on healthcare costs and patient comfort considering the high and rising prevalence of Diabetes.

Standardisation, accreditation and harmonisation

Cod: 1503

THE DEVELOPMENT OF ENZYMOLOGY REFERENCE SYSTEM IN CHINA

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BACKGROUND: A document was carried out by the EU in vitro diagnostic apparatus in 1998 and was implemented in 2002 (Directive 98/79/EC, it requires a measurement result should be traceable to a higher reference material or a reference method. After the year 2003, 5 standard documents were carried out by ISO, such as ISO15193 and so on. To achieve a target that the results of various laboratories in medical institutions are consistent in China. Six laboratories such as the Ministry of Health Clinical Laboratory Center started to construct enzymology reference laboratories and enzymology reference system. Currently 4 laboratories had been accredited by CNAS, and one of them entered the JCTLM list. The enzymology reference system has been established in China.

METHODS: Summarize the number of laboratories which had participated in IFCC RELA activity, the number of laboratories which had been accredited by CNAS, and the information from website platform. And to introduce the development of enzymology reference system in China through the information from these three aspects.

RESULTS: 1. From 2006 to 2012, the number of laboratories which run enzymology reference methods and participate in RELA are 6, 9, 8, 9, 13, 17, 13. 2. As of December 2013, 4 laboratories had been accredited by CNAS, and one of them entered the JCTLM list. 3. The number of certified reference materials increased from 0 to 24 from the information of Chinese standard material platform site from the year 2008 to 2014(include ALT, AST, LDH, CK, ALP, AMY, GGT).

CONCLUSIONS: China has built a complete enzymatic reference system.

Standardisation, accreditation and harmonisation

Cod: 1504

IN COMPLIANCE WITH 15189 STANDARD: INTRODUCTION OF AUTOMATIC INSTRUMENTS FOR DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE

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BACKGROUND: Assessment of erythrocyte sedimentation rate (ESR) by Westergren has been the working method at the Surgical Emergency Laboratory in our clinic for decades. However, concerning the bio-hazard safety requirements, this reference method is inconvenient for routine work for middle and large scale laboratories. Our goal was to introduce a safe, closed, automated system which generates data accessible by LIS, in compliance with ISO 15189 standard. We compared the results obtained for ESR by automatic instruments Ves Matic Easy, Terumo and Alifax with the reference Westergren method, as well as the technical performance of the instruments and turnaround time. The results from the evaluation procedure would be used as guidance for choosing the optimal working method in our laboratory.

METHODS: For method comparison 95 fasted blood samples were used (65 man and 33 women, aged 33-82 years) collected early morning in appropriate EDTA and citrate vacuum tubes. All samples were analyzed up to two hours after phlebotomy. ESR was measured by automatic instruments (Ves Matic Easy, Terumo and Alifax) in parallel with Westergren method. Standard T-test was used for data comparison.

RESULTS: There was no significant difference between the mean values of ESR obtained by Ves Matic Easy instrument and Westergren (p=0,5760), Terumo instrument and Westergren (p=0.4456) and Ves Matic Easy and Terumo instruments (p=0,13876). The only significant difference was found between values from Ves Matic Easy and Alifax instruments (p=0,00248).

CONCLUSIONS: Concerning the safety aspects, we found all three automated, closed methods superior to Westergren. As for the waste disposal, Ves Matic Easy and Terumo instruments generate solid waste, while Alifax technology generates liquid one, in addition with daily washing maintenance. Statistical comparison of Ves Matic Easy and Terumo instruments data with the reference method indicated reliable ESR test results. These data were taken in consideration in the process of managerial decision making while implementation of ISO 15189 Standard.

Standardisation, accreditation and harmonisation

Cod: 1505

LABORATORY MEDICINE BEST PRACTICE GUIDELINE: VITAMINS A, E AND THE CAROTENOIDS IN BLOOD

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BACKGROUND: Despite apparent method similarities between laboratories there appear to be confounding factors inhibiting uniform reporting and standardisation of vitamin assays. The Australasian Association of Clinical Biochemists (AACB) Vitamins Working Party (VWP), in conjunction with the RCPA Quality Assurance Program (RCPAQAP) has formulated guidelines to improve performance, reproducibility and accuracy of fat soluble vitamin results. The aim of the guidelines is to identify critical pre-analytical, analytical and post-analytical components of the analysis of vitamins A, E and carotenoids in blood to promote best practice and harmonisation.

METHODS: These best practice guidelines have been developed with reference to the Centers for Disease Control (CDC) "Laboratory Medicine Best Practices: Developing an Evidence-Based Review and Evaluation Process". The CDC document cites an evaluation framework for generating best practice recommendations that are specific to laboratory medicine. The recommendations proposed herein, were generated from a comprehensive literature search and the extensive combined experience of the AACB VWP members. They were formulated based on comparison between an impact assessment rating and strength of evidence and were classified as either: (1) strongly recommend, (2) recommend, (3) no recommendation for or against, or (4) recommend against.

RESULTS: Fifty recommendations have been formed based on an exhaustive literature review and implementation of the CDC model for laboratory best practice.

CONCLUSIONS: These best practice recommendations represent the consensus views, in association with peer reviewed evidence of the AACB VWP, towards best practice for the collection, analysis and interpretation of vitamins A, E and carotenoids in blood.

Standardisation, accreditation and harmonisation

Cod: 1506

ESTABLISHMENT OF A COMMON CALIBRATOR FOR MASS SPECTROMETRY ANALYSIS OF SERUM TESTOSTERONE

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BACKGROUND: In 2010 the Mass Spectrometry Harmonisation Working Group was established under the auspices of the Asian and Pacific Federation of Clinical Biochemistry (APFCB) to progress the harmonisation of mass spectrometry based clinical laboratory methods. The first project is to pursue the harmonisation of the measurement of serum steroids, with serum testosterone as the key analyte. Here we describe the outcome of the first two project goals: 1. provide detailed information on the serum testosterone LC-MSMS methods used in Asian-Pacific clinical biochemistry laboratories; and 2. harmonise serum testosterone results through a common calibrator.

METHODS: All laboratories participated in the RCPA Quality Assurance Programs (QAP) for serum testosterone. In 2013 a detailed questionnaire was sent to participating laboratories in conjunction with a seven level common calibrator set (Biocrates), RCPAQAP material and two de-identified human serum samples. Samples were analysed by each laboratory's (n=8) routine LC-MSMS method on two separate occasions. Target values were assigned by the National Measurement Institute of Australia (NMIA). ANOVA was performed to determine if recalculation led to a change in results.

RESULTS: The questionnaire demonstrated significant commonality in the LC-MSMS methods. Most laboratories prepared samples by LLE and one used SPE. Consistent MRM's were seen (289→109 and 289→97) for the testosterone quantifier/qualifier. The source of the laboratory's calibrator, number of levels and deutrated sites on the internal standard differed. Recalculation of the human testosterone samples against the common calibrator demonstrated a significant change ($p<0.05$) in results for the male serum (NMIA assigned value 16.19 nmol/L) whereas the change for the female serum (NMIA assigned value 0.57 nmol/L) was not statistically significant for the group.

CONCLUSIONS: This pilot demonstrates the utility of the Biocrates material as a common calibrator. Reference values for future calibrator batches will be made traceable to SI units. To ensure alignment with other harmonisation initiatives NIST SRM971 will be used as a QC material. Continued monitoring, and on-going method improvements will be achieved through collaboration and participation in the RCPAQAP.

Standardisation, accreditation and harmonisation

Cod: 1507

COMPARISON OF DIFFERENT IMMUNO-LUMINOMETRIC METHODS

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BACKGROUND: Quantitative automated luminometric immunoassays for the determination of Cancer Antigen 125 (CA125), Alpha-Fetoproteine (AFP), Total Prostate-Stimulating Antigene (t-PSA), Free Prostate-Stimulating Antigene (f-PSA), Ferritin, Prolactine, Parathormone (PTH), Luteinizing hormone (LH), Follicle Stimulating hormone (FSH) were compared with a Architect i2000SR (Company: ABBOTT GmbH & Co. KG Max-Planck-Ring 2, Wiesbaden, Germany) and a LIAISON XL (Company: DiaSorin Deutschland GmbH Von-Hevesy-Str. 3, Dietzenbach, Germany).

METHODS: The commercially available luminometric immunoassays were performed as described by the manufacturer. The luminometric immunoassays are monoclonal two-site immunoluminometric methods (sandwich principle). Antibody-coated tubes serve as solid phase. The light signal is directly proportional to the concentration of the measuring sample. The whole measuring procedure consists of pipeting of samples, incubation periods, washing cycles, and measurements. Twenty patient samples for each method have been measured parallel at ARCHITECT i2000SR [Comp.: ABBOTT GmbH & Co. KG Max-Planck-Ring 2, Wiesbaden, Germany] and at LIAISON XL [Comp.: DiaSorin Deutschland GmbH Von-Hevesy-Str. 3, Dietzenbach, Germany]. Pearson's Correlation Coefficient and Passing-Bablok-Correlation were used to evaluate the results mathematically.

RESULTS: Passing-Bablok-Regression and Pearsons Correlation Coefficient (R) were calculated for the following methods: t-PSA (Y = 0,9489x + 0,034; R=0,99997); fPSA (Y = 0,6225x + 0,1835 ; R=0,9745); AFP (Y = 1,1307x + 0.1626; R=0,99423); Ferritin (Y = 0,842x + 53,124; R=0,93395); Prolactine (Y = 0,7017x + 1,3913; R=0,99978); PTH (Y=0,6225x + 0,1835, R=0,9915); LH (Y=1,2019x + 0,679; R=0,98241); FSH (Y=1,265x + 1,2344, R=0,8875); CA125 (Y=1,1379x + 1,6201; R=0,99624).

CONCLUSIONS: The results showed a high degree of correlation and regression. The immunoassays at LIAISION XL can be accurately measured using the automated method which can be recommended.

Standardisation, accreditation and harmonisation

Cod: 1508

THE IMPACT OF THE REAGENTS ON THE CANDIDATE REFERENCE METHOD OF CHE

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BACKGROUND: German professor Schuman proposed CHE candidate reference method for measuring the catalytic activity, to looking for a candidate reference method meets the requirements of the reagents, the laboratory studied the key reagent butyrylcholinesterase and hexacyanoferrate(III) which commercially-available and summarized as follows.

METHODS: 1-Reagent blank absorbance of the raw materials of different manufacturers and the rate of change in absorbance at 405nm. 2-The effect of the key reagent materials on the catalytic activity measurements of CHE.

RESULTS: 1.Composing the CHE substrates-butyrylthiocholine of Sigma, Merk, Roche, Alfa and Tci five manufacturers with hexacyanoferrate(III) of Sigma, reagent blanks were 1.8145, 1.8149, 1.7258, 1.8147 and 1.809Abs respectively. The rate of change in absorbance at 405nm were -0.00445, -0.00295, -0.00345, -0.00350 and -0.00225. When the hexacyanoferrate(III) is produced by Alfa Company, reagent blanks were 1.8157,1.8160,1.7365,1.8152 and 1.8215Abs. The rate of change in absorbance at 405nm were -0.00575,-0.00265,-0.00250,-0.00270 and -0.00195. butyrylthiocholine produced by Roche could not meet the requirements of the candidate reference method. 2. Composing the CHE substrates-butyrylthiocholine of five manufacturers with hexacyanoferrate(III) of Sigma, 2200, 8700, 11700 U/L mixture of the three levels in the serum CHE catalytic activity were 2378, 2466, 2395, 2457 and 2451U/L; 8446, 8756, 8399, 8721 and 8747 U/L; 11520, 11920, 11490, 11946 and 11944 U/L. When the hexacyanoferrate(III) is produced by Alfa Company 2200, 8700, 11700 U/L mixture of the three levels in the serum the CHE catalytic activity was 2417, 2472, 2381, 2458 and 2481 U/L; 8472, 8787, 8409, 8753 and 8744 U/L;11605, 11968, 11491, 11875 and 11894 U/L.

CONCLUSIONS: The reagent butyrylthiocholine produced by Roche, its blank absorbance could not meet the requirement of the CHE candidate reference method. Impact of butyrylthiocholine reagents from different sources of raw materials for CHE catalytic activity measurements is greater than hexacyanoferrate(III). Merk products CHE catalytic activity measurement value is higher than other manufacturers products.

Standardisation, accreditation and harmonisation

Cod: 1509

STANDARDIZATION OF SAMPLE COLLECTION MATERIAL AND SAMPLE PREPARATION FOR MASS SPECTROMETRY BASED METABOLOMICS ANALYSIS IN CLINICAL RESEARCH

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BACKGROUND: Blood is one of the most used biological fluids for research, diagnostic, health and drug monitoring. Nevertheless up to now its collection is not always performed under standardized conditions. Especially for metabolic phenotyping (targeted quantitative analysis of endogenous metabolites), aspects like anticoagulants or sample storage by dried blood spots (DBS) and dried plasma spots (DPS), become crucial for the comparability of samples e.g. in large-scale metabolomics or biomarker studies in clinical research. The aim of this study was the multiplexed analysis of more than 180 endogenous metabolites (amino acids, acyl carnitines, hexose, biogenic amines, lipids (sphingomyelins, phosphatidylcholines, lyso-phosphatidylcholines)) in blood samples stored under the five most common conditions (heparin plasma, EDTA plasma, citrate plasma, serum, DBS and DPS) in order to determine their comparability in metabolite concentrations and stability. All the samples were collected from the same person on the same time.

METHODS: According to the AbsoluteIDQ® p180 Kit (Biocrates) standard sample preparation procedure 10 µL of each liquid sample and appropriate DBS as well as DPS were processed followed by a standardized UHPLC- MS/MS and FIA-MS/MS method comprised in the kit.

RESULTS: Our study showed that the different methodologies used for blood sampling do not limit the technical performance of the AbsoluteIDQ® p180 Kit procedure and the MS/MS analysis demonstrating the suitability of all tested matrices for metabolomics analysis. However differences in the metabolomic signatures were found between anticoagulated and non anticoagulated samples. From the different metabolite classes measured in the study, concentration differences related to the different sample collection and storage conditions could be identified especially for acylcarnitines and some amino acids.

CONCLUSIONS: This study demonstrates the technical suitability of all five tested sample collection and storage devices and their metabolite specific variations for the metabolome analysis of 180 endogenous metabolites. Particularly DBS and DPS have a high potential to be established as the standard devices in metabolomics analysis.

Standardisation, accreditation and harmonisation

Cod: 1510

DEVELOPMENT OF NOVEL REFERENCE MATERIAL OF WHOLE BLOOD TYPE FOR GLUCOSE MEASUREMENT

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BACKGROUND: Whole blood type reference materials had not been developed for evaluation of SMBG and POCT devices. We succeeded in a whole blood type reference materials identical with fresh whole blood for SMBG devices that use finger-tip blood plasma under ISO 15197, and POCT devices that use venous plasma.

METHODS: Heparinized whole blood samples from healthy person were used for the comparison. Glucose levels of the reference material were prepared about 3.33, 5.55 and 8.33 mmol/L. The comparison was as follows: 1) Three levels of high concentration glucose (0.100±0.001 g) were each transferred to a test tube. 2) Three vials of whole-blood sample were incubated and stored at 4 degree centigrade, and them were left at room temperature for about 10 min. 3) Amounts of 1.00±0.01 g of the incubated whole-blood samples were added to a test tube containing 0.100±0.001 g glucose solution as other 2 levels of glucose concentrations underwent the same manner and mixed. 4) These reference materials were left at room temperature for about 10 min, before the comparison with finger-trip fresh whole blood. 5) The assigned values of the reference materials were measured using the HK-G6PD method after deproteinization, and calibration using JCCRM 511 (ReCCS, Japan) of which uses assigned values were assayed by ID/MS. 6) Evaluations were performed using 12 SMBG devices and 6 POCT devices, the GOD electrode and HK-G6PD methods.

RESULTS: The mixture of a high concentration glucose solution (0.100±0.001 g) and a fresh whole-blood sample (1.00±0.01 g) showed CV values of 0.1~0.3 %. The reference materials stabled after being stored at 4 degree centigrade for about 24 hours. In accuracy evaluation, a relative bias to the assigned values for the GOD electrode was ±5 % and HK-G6PD methods, within ±10 % for POCT devices and ±20 % for SMBG devices.

CONCLUSIONS: We have succeeded in a whole-blood-type RM for glucose measurement. This reference material can be used to evaluate the accuracy and perform calibrations for SMBG and POCT devices.

Standardisation, accreditation and harmonisation

Cod: 1511

REFERENCE MEASUREMENT PROCEDURES AND THEIR APPLICATION IN THE STANDARDISATION OF ENDOCRINE MEASUREMENTS

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BACKGROUND: There have been demonstrable improvements in the quality of clinical analyses with liquid chromatography tandem mass spectrometry (LC-MS/MS) technology being increasingly applied in clinical laboratories for endocrine measurements as it offers analytical specificity superior to that of immunoassays and low limits of quantification. However, the measurement of steroid hormones in complex biological matrices by LC-MS/MS can still show reasonable variability, especially at low concentrations where matrix interferences and matrix effects can be significant. For both immunoassay and LC-MS/MS methodologies the assessment of performance remains a critical issue.

METHODS: The National Measurement Institute of Australia has developed Reference Measurement Procedures (RMPs) based on isotope dilution mass spectrometry for the high-accuracy quantification of progesterone, testosterone, 25-hydroxyvitamin D, and 17#-estradiol in human serum. The RMPs use highly selective two-dimensional LC-MS/MS and gas chromatography with high resolution mass spectrometry (GC-HRMS) to resolve difficult isomeric and isobaric matrix interferences. A comparison of steroid hormone concentrations determined using these orthogonal methods of analysis based on different analytical principles facilitated the rigorous investigation of bias due to matrix effects in these methods.

RESULTS: The RMPs developed form an important part of the global infrastructure required for the standardisation of endocrine measurements and contribute to major international initiatives such as the Asian Pacific Federation for Clinical Biochemistry Testosterone Harmonisation Program and the Vitamin D Standardisation Program. The RMPs were also used to assign metrologically-traceable reference values with low measurement uncertainties of 3-9% (95% level of confidence) for samples used in the Royal College of Pathologists of Australasia Quality Assurance Programs.

CONCLUSIONS: Pathology laboratories using both immunoassays and LC-MS/MS for sample analysis can benchmark their performance against the reference values provided to External Quality Assurance Programs. The use of independent reference values permits a more robust and transparent indication of laboratory performance than consensus-group grading.

Standardisation, accreditation and harmonisation

Cod: 1512

FIRST STEPS IN THE DEVELOPMENT OF A CERTIFIED REFERENCE MATERIAL FOR ANTI-β2GPI IGG MEASUREMENTS

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BACKGROUND: Assays measuring anti-β2-Glycoprotein-I (β2GPI) IgG antibodies are currently the most specific tool to identify patients suffering from the antiphospholipid syndrome. However, there is currently a large variability between method results, mainly arising from different assay characteristics, calibration protocols and the lack of an internationally recognised reference material. The Institute for Reference Materials and Measurements (IRMM) collaborates with the IFCC working group for the harmonisation of autoimmune testing (WG-HAT) in the development of a suitable reference material for the standardisation of anti-β2GPI IgG measurements.

METHODS: The starting serum preparation consisted of plasmapheresis material that was converted into serum by clotting, centrifugation and filtering. For stability reasons, freeze-drying of the material would be desirable. A test batch of lyophilised serum was produced and analysed with 4 different manual ELISA kits that measure anti-β2GPI IgG. Additionally, the liquid serum material and several clinical samples were included in the experiments to assess the impact of lyophilisation, the commutability of the candidate reference materials and parallelism of the dilution curves.

RESULTS: Freeze-drying of the serum material did not affect the measurement results of the 4 assays employed ($p=0.0521$). The serum material presented an acceptable degree of commutability with regard to clinical samples, although the commutability assessment was hampered by the poor correlation observed in 4 out of 6 method comparisons. Dilution curves of the serum material and clinical samples showed parallelism with the methods used.

CONCLUSIONS: Feasibility studies on a test batch of serum material showed that the lyophilised form of the material is suitable for a certified reference material intended to be used for calibration of anti-β2GPI assays. It has the same properties as the clinical samples for the methods that were evaluated. Due to these results, a final batch of lyophilised serum material has been processed and it is currently being characterised for stability, homogeneity and anti-β2GPI IgG concentration.

Standardisation, accreditation and harmonisation

Cod: 1514

SIMPLE AND EASY METHOD FOR THE DETERMINATION OF ESTIMATED PLASMA GLUCOSE LEVEL FROM PATIENTS THAT ENTER HOSPITAL: AN EXAMPLE OF MULTICENTRIC DATA MINING

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BACKGROUND: Measurement of glycated hemoglobin (HbA1c) is the gold standard for monitoring glycemic control and assessing diabetes complications. While, HbA1c measures mean glycemic exposure during the past 8-12 weeks, it does not provide information about daily alterations in glucose levels. Therefore, it is recommended that, estimated average glucose (eAG) level calculated from the HbA1c should also be reported as an interpretation of the HbA1c result. The aim of this study was to determine the relation between fasting plasma glucose level and HbA1c at the same time according to different methods of Hba1c measurements in a large population of patients that entered hospital.

METHODS: The HbA1c levels of 162,210 patients that entered different hospitals and laboratories were measured by Bio-Rad Variant II, Tosoh G8, ADAMS A1c, Trinity Boronate Affinity, Chromsystems-HPLC, Roche Tina-quant and Abbott Architect. At the same time, "eAG"s were calculated based on Hba1c levels. Then, the relation between eAG and fasting plasma glucose levels were determined by regression analysis and the concordance correlation coefficient (CCC).

RESULTS: The correlation coefficient (r) between serum plasma glucose and HbA1c levels were found to be 0.809, 0.774, 0.779, 0.817, 0.704, 0.796, 0.747 according to Bio-Rad Variant II, Tosoh G8, ADAMS A1c, Trinity Boronate Affinity, Chromsystems HPLC, Roche Tina-quant and Abbott Architect respectively. The CCC between the eAG that is calculated according to the formulas given in the text and the eAG that is calculated according to NGCP directions (where eAG=(28.7*HbA1c)-46.7), were found to be between 0.9339 and 0.9866.

CONCLUSIONS: Despite the progress made for the standardization of HbA1c measurements, the relation between serum glucose and HbA1c still shows some discrepancies due to the differences in measurement methodologies. For this reason, clinicians and laboratory specialists should be careful throughout the management the diabetes patients. As a conclusion, each laboratory can determine their own eAG according to the data originated by their own analyzer.

Standardisation, accreditation and harmonisation

Cod: 1515

PERFORMANCE ASSESSMENT OF CREATININE METHODS IN GUYANA

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BACKGROUND: It is well known that diabetics are particularly prone to developing renal failure as a complication of their disease process. It therefore comes as no surprise that the incidence of chronic kidney disease (CKD) is increasing in Guyana – a country with a prevalence of diabetes well above the world average. Identifying patients with CKD at an earlier stage will provide opportunities for intervention to slow/prevent progression to end stage disease. The routine reporting of eGFR (estimated glomerular filtration rate) in Guyana will provide this opportunity. Standardized creatinine measurements are required for accurate estimations of GFR.

METHODS: A total of sixteen private and public Guyanese laboratories were recruited to the study. Each laboratory received 3 sets of human serum samples having clinically relevant concentrations of creatinine. The creatinine values (RV) were assigned to these samples by a credentialed creatinine reference method (ID-GCMS). Each test sample was analyzed three times on each of three days for a total of twenty-seven measurements (nine measurements for each reference sample). From these data, within-sample imprecision was calculated. The mean of the nine sample results was taken as the lab's reported value for that sample. These data were plotted relative to the assigned RVs. The resulting linear regression equation was used for post-analytical correction of the lab's calibration bias. In addition, each laboratory provided 100 recently reported patient creatinine test results (age, gender, result). These data were used to estimate the potential impact of creatinine standardization on the reporting of eGFR in Guyana.

RESULTS: The mean imprecision (CV) across the three test samples was 9% and the mean bias (relative to RV) was 11%. Left uncorrected, current creatinine measurements in Guyana would cause 36% of all adults in the country to be misclassified on the basis of their lab reported eGFR.

CONCLUSIONS: The performance of creatinine methods in Guyana show a high degree of imprecision and bias compared to IDMS reference values. These data emphasize the importance of creatinine standardization as a pre-requisite for implementing the routine reporting of the eGFR in Guyana.

Standardisation, accreditation and harmonisation

Cod: 1516

COMMUTABILITY STUDY ON CANDIDATE MATERIALS FOR THREE NEW ENZYME CERTIFIED REFERENCE MATERIALS

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BACKGROUND: The Institute for Reference Materials and Measurements (IRMM) of the European Commission is developing three new Certified Reference Materials (CRMs) for the standardisation of the serum enzymatic activity concentration measurements of lactate dehydrogenase (LD), creatine kinase (CK) and alanine aminotransferase (ALT). Their main intended use is for verifying the performances of the corresponding Measurement Reference Procedure developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) but they could also be used as calibrators by IVD manufacturers providing their commutability is proven. Therefore this project started with a commutability study on possible candidate materials. The IFCC Committee for Reference Systems for Enzymes and its collaborating international laboratories participated to the realisation of this study.

METHODS: The study included 14 pools of human serum, the currently available CRMs and candidate recombinant materials for LD, CK and ALT. Samples were analysed with nine commercial systems in eight laboratories and results were compared to those of the IFCC Reference Procedure.

RESULTS: Different statistical tools such as bias plots, standard error of least square regression, Correspondence Analysis, Bland-Altman plots and Passing-Bablok regression were used to compare commercial assays and to identify the most commutable materials. The commutability of the new candidate material for LD is similar to the current CRM (ERM-AD453/IFCC). For CK, the present material (ERM-AD455/IFCC), constituted by the MB isoenzyme purified from human heart tissue removed in the course of autopsy, appears to be more commutable but for ethical reasons, the use of the new proposed recombinant material would be an alternative. Finally, the new candidate materials for ALT show relatively similar commutability properties and were compared in terms of their potential impact on the re-calibration of commercial assays.

CONCLUSIONS: The commutability of five recombinant candidate materials for LD, CK and ALT was assessed using nine commercial systems. The results will be used to select the most suitable materials for the production of new CRMs for LD, CK and ALT at IRMM.

Standardisation, accreditation and harmonisation

Cod: 1517

COMPARISON OF ADVIA CENTAUR, ARCHITECT AND COBAS INTEGRA 800 SYSTEMS ON HOMOCYSTEINE ASSAY

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BACKGROUND: Homocysteine (Hcy) is a amino-acid, with thiol, which is produced by the intracellular demethylation of methionine. Elevated levels of total Homocysteine (tHcy) have been considered as a major risk factor for cardiovascular assessment. Elevated levels of tHcy are caused by: genetically determined enzymatic disturbance in homocysteine metabolism, folic acid and B12 vitamin deficiency, renal failure, aging, medications. This is the total Homocysteine (tHcy), which is determined, with normal ranges 5-15 μmol/L. Increased levels of tHcy, 15-30 μmol/L, have been found to be related with mild hyperhomocysteinemia, levels of tHcy 30-100 μmol/L with moderate hyperhomocysteinemia and levels of tHcy above 100 μmol/L with severe hyperhomocysteinemia. The aim of this study is to compare the systems: ADVIA Centaur (direct chemiluminescence immunoassay), Architect (direct chemiluminescence immunoassay) and Cobas Integra 800 (enzymatic assay), on serum Homocysteine assay.

METHODS: The serum homocysteine levels of 30 patients (17 males, 13 females, mean aged 50,3 years) were measured using direct chemiluminescence immunoassay (ADVIA Centaur; Siemens Healthcare Diagnostics and Architect;Abbott Diagnostics) and enzymatic method (Cobas Integra 800;Roche Diagnostics). The statistical analysis was performed using SPSS version 13.0.

RESULTS: Linear regression analysis of the correlation yielded the following statistics:
i) tHcy(Architect) = 0.875 tHcy(ADVIA Centaur) + 0.071 μmol/L
(r=0.993, r2=0.988, p<0.001)
ii) tHcy(ADVIA Centaur) = 0.847 tHcy(Cobas Integra 800) + 5.860 μmol/L
(r=0.964, r2=0.930, p<0.001)
iii) tHcy(Architect) = 1.040 tHcy(Cobas Integra 800) - 0.457 μmol/L
(r=0.998, r2=0.997, p<0.001).

CONCLUSIONS: In summary, our results demonstrate the significant correlation (r2=0.997) between Architect and Cobas Integra 800 systems (different methods). The lower correlation of ADVIA Centaur System with Cobas Integra 800 and Architect could suggest a correction of serum homocysteine concentration in this analyzer.

Standardisation, accreditation and harmonisation

Cod: 1518

VALUE ASSIGNMENTS BY IFCC PRIMARY REFERENCE PROCEDURES ON CRM-001C, A 7TH LOT OF JAPANESE REFERENCE STANDARD FOR ENZYMES

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BACKGROUND: CRM-001c, Japan Committee for Clinical Laboratory Standards (JCCLS) certified reference standard for enzymes of Japan Society of Clinical Chemistry (JSCC) method, which is a composition of 7 kind enzymes of human recombinant, was certified on July, 2013 as 7th lot. The form was lyophilized and its shelflife was set for 6 years when stored at less than or equal to -20 centigrade, based on the performance of the former lots. The value assignments on CRM-001c by IFCC primary reference procedures (PRP) were attempted besides JSCC consensus methods.

METHODS: The IFCC value assignments were conducted by an accredited laboratory in Germany.

RESULTS: The IFCC values of AST and ALT, in which the catalytic activities are measured in the presence of pyridoxal phosphate, on the contrary in the absence in the JSCC method, were four to five percent higher than those by the JSCC. As for LD, in the different condition of pH (9.4 vs. 8.8) and buffer (NMG vs. DEA), the IFCC value was approximately 8% higher. The IFCC values of AMY, CK and γ-GT, in which the methods are the same (AMY) or nearly the same (CK and γ-GT), were equivalent with the JSCC. Approximately three times higher value than the JSCC was shown on ALP, in which the buffer (AMP vs. EAE) and pH (10.2 vs. 9.9) were different from each other. In all enzymes, the loss of the catalytic activities during storage by JSCC based methods, was not observed for at least six years in the previous lot under the given condition.

CONCLUSIONS: On a major EQA program in Japan, the CVs on the enzymes were well converged to approximately 3%, in which more than 3,000 laboratories participate. The good results were largely owing to the prevalence of the CRM-001. As the material is stable for long-term storage and commutable with many kinds of commercial reagents, the contribution to the international standardization for enzymes is expected with the values assigned by IFCC PRP on CRM-001c.