Analytical technologies and applications

M027

ANALYTICAL ASSESMENT OF INCLIX HBA1C POINT-OF-CARE METOD

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BACKGROUND-AIM

Determination of glycohemoglobin (HbA1c) is a crucial step in diabetes monitoring today. It is routinely measured by various automated methods, but it is an imperativ that the determination of this parameter is readily available, efficient, and cost-effective. The aim of this study was to examine the analytical characteristics of the point of care (POCT) HbA1c method on an INCLIX analyzer (Sugentech Inc.).

METHODS

Analysed patient samples were measured in parallel on INCLIX and Olympus AU 480 analyzers. A patient sample with an initially measured HbA1c level of 5.1% was used to assess imprecision within series. To calculate imprecision between series and reproducibility, two samples with HbA1c levels closest to clinically significant cut-off values for diagnosis and initiation of therapy were used (6.6% and 7.5%). Samples of 44 patients covering a clinically significant measurement range of 5.2% to 11.8% were used for method comparison.

RESULTS

Within series coefficient of variation was 7.58%, and calculated bias was -2.94%. The coefficients of variation for impecision between series were 6.63% and 6.22%, and between days 8.80% and 7.51%. Between method bias and relative bias were 0.3% and 4.5%, respectively. A statistically significant correlation coefficient (two sided test) was 0.871 ($P<0.01$). Using Deming regression analysis, the following equation was obtained: $y = -1.80 + 1.304x$. The 95% confidence intervals for the intercept and slope were -4.90 to 1.29, and 0.827 to 1.781, respectively, indicating that there was no constant nor proportional deviation between the analyzed methods.

CONCLUSIONS

INCLIX POCT HbA1c may be an adequate alternative to the laboratory assesment of HbA1c. The results are available shortly after the blood draw, which enables fast medical decisions and considerably contributes to the efficiency of the health care system.

Analytical technologies and applications

M028

EVALUATION OF BECKMAN COULTER CLIA METHOD FOR PROCALCITONIN DETERMINATION

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BACKGROUND-AIM

The clinical significance of procalcitonin (PCT) as a marker of inflammation is well established. However, it is important to be able to accurately correlate the measured result to the clinical scenario. To ensure a reliable result it is important that the methods used are standardized and that the results are comparable. We aimed to examine the analytical performance of the Beckman Coulter CLIA test for PCT determination, and its agreement with the traditionally used Roche ECLIA method.

METHODS

Two levels of commercial control samples (BioRad, Lyphocheck® Speciality Immunoassay Control) were used to examine the analytical characteristics. Study included 48 patient samples with suspected viral or bacterial infection. PCT serum concentrations were measured using ECLIA test on Roche Elecsys® BRAHMS PCT analyzer and CLIA test on Beckman Coulter Access 2 analyzer.

RESULTS

Coefficients of variation for imprecision in series, between series and day to day measured using commercial control samples were 1.20% and 1.30%, 1.22% and 1.37% and 2.48% and 0.74%, respectively. The extended uncertainties of laboratory measurement results for two clinically relevant decision limits (0.5 ng/mL and 2.0 ng/mL) were 4.8% and 3.0%, respectively. For the entire patient sample (N=48) between methods bias was 0.033 and the relative bias was 0.45%. For PCT positive samples (>0.5 ng/mL) (N=29) between methods bias was 0.519 and the relative bias was 4.19%. Spearman's correlation coefficient was 0.998 ($p<0.001$), for the entire sample, and 0.993 ($p<0.001$), for PCT positive samples. There was no statistically significant difference in PCT values obtained by different methods, for either whole sample ($p=0.409$), or only positive ones ($p=0.888$). Our results presented no constant nor proportional disagreement between the analyzed methods for clinically relevant PCT levels.

CONCLUSIONS

Beckman Coulter CLIA method has the necessary analytical characteristics required for routine laboratory PCT determination and provides reliable and accurate results at different clinically significant decision levels.

Analytical technologies and applications

M029

EVALUATION OF BECKMAN COULTER IMMUNOCHEMICAL METHOD FOR DETERMINATION OF SARS-COV-2 IGG ANTIBODY

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BACKGROUND-AIM

Recently various immunochemical tests have been developped to detect the humoral immunity products to the corona-19 virus. However, methods so far are not yet standardized and are mostly semi-quantitative, which raises concerns about their accuracy in clinical trials. It is important for tests and results to be comparable. We aimed to evaluate the analytical performance of Beckman Coulter CLIA method for SARS-CoV-2 IgG antibody to the RBD part of the S1 viral protein and its agreement with Abbott SARS-CoV-2 IgG CMIA method.

METHODS

Beckman Coulter commercial control samples and 63 serum samples from volunteer subjects were used in this study. Subjects were all tested in a period from November to December 2020, to check for the presence of antibodies due to suspicion of asymptomatic SARS-CoV-2 infection or due to the presence of symptoms. Samples were tested in parallel on Beckman Coulter Access 2 and Abbot Alinity i analyzers.

RESULTS

Coefficients of variation in series and between series for undiluted and diluted control samples (2, 3 and 4 times) were lower than those recommended by the manufacturer ($<$7%). Coefficient of variation between days for diluted higher level control sample was 2.44%, and total intralaboratory uncertainty was 6.34%. Spearman's rho was 0.809 ($P<0.001$). McNemar test showed significant agreement of positive results in 42 of 63 patients (66.7%), while in 9.5% there was agreement of negative results. Fifteen patients tested positive for Abbott and negative for Beckman Coulter test. No patient who tested positive for the Beckman Coulter test was negative for the Abbott test. Total agreement between the Beckman Coulter and Abbott method was 76.2%.

CONCLUSIONS

Beckman Coulter SARS-CoV-2 IgG test can be used for confirmation of a past SARS-CoV-2 infection or to monitor immunity after infection/vaccination. However, negative results cannot rule out an acute SARS-CoV-2 infection, so further testing should be performed. Providing an internal standard for method standardization would substantially reduce method limitations as well as difficulties in interpreting and comparing tests from different manufacturers.

Analytical technologies and applications

M030

RELIABILITY OF DETERMINING LACTATE CONCENTRATION IN CEREBROSPINAL FLUID IN NEWBORNS ON A GAS ANALYZER RADIOMETER ABL 800FLEX

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BACKGROUND-AIM

Lactate concentration in the cerebrospinal fluid represents a very useful parameter for differential diagnosis of bacterial and viral meningitis, a serious health condition in newborns. The aim of this study was to confirm the analytical characteristics of the amperometric method in determining the concentration of lactate on the Radiometer ABL 800FLEX (Brónshój, Denmark) in routine laboratory practice in relation to the analytical characteristics of the spectrophotometric method used on the two spectrophotometric analyzers, Mindray BS-240 and Olympus AU400.

METHODS

We analyzed cerebrospinal fluid taken by lumbar puncture from 60 preterm and term infants, due to suspicion of bacterial or viral meningitis. Samples were tested in parallel on Radiometer ABL 800FLEX gas analyzer, Mindray BS-240 (Mindray Medical International Ltd. Shenzhen, China), and Olympus AU400 (Beckman Coulter, Inc, Brea, USA) biochemical analyzers. Commercial control samples Autocheck 5+ (Radiometer, Brónshój, Denmark) and Biochemistry Control Serum (Human) (BioSystem, Barcelona, Spain) were used for the analytical performance check.

RESULTS

The following coefficients of variation, using commercial control samples, for Radiometer ABL 800FLEX, Mindray BS-240 and Olympus AU400 were obtained: within series 2.12%, 1.26%, and 1.91%, with calculated bias of -0.60%, +1.92%, and -1.20%, for reproducibility 5.06%, 5.53%, and 5.41%, and between series 2.34%, 1.13%, and 2.89%, respectively. Spearman's rho ($P<0.01$) between Radiometer ABL 800FLEX and Olympus AU400 was 0.942, between Radiometer ABL 800FLEX and Mindray BS-240 was 0.935, and between Mindray BS-240 and Olympus AU480 was 0.973. Cusum test revealed no statistically significant deviation from linearity for all three methods comparison ($P>0.05$). Passing-Bablock analysis confirmed that there was no constant and proportional deviation between the analyzed methods when analyzing patients' samples.

CONCLUSIONS

The analyzer Radiometer ABL 800FLEX has satisfactory analytical and technical characteristics for lactate measurement in cerebrospinal fluid. The amperometric measurement on Radiometer ABL800 FLEX analyzer has shown significant agreement with the existing routine methods and could therefore be used as a reliable tool for determining the lactate concentration in the cerebrospinal fluid.

Analytical technologies and applications

M031

SEPSIS MANAGEMENT IMPROVEMENT BY MONOCYTE DISTRIBUTION WIDTH (MDW): UDINE'S EXPERIENCE

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BACKGROUND-AIM

The increasing evidences about the involvement of monocytes in sepsis led to the validation of the new parameter Monocyte Distribution Width (MDW) as Early Sepsis Indicator (ESId).
Aim: this study aimed to evaluate the MDW diagnostic utility to early detect sepsis in adult patients admitted in the Emergency Department (ED) with a complete blood count -Differential (CBC-Diff) as part of the standard clinical practice.

METHODS

The hematology analyzer DxH 900 (Beckman Coulter Inc.) provides the new reportable parameter MDW, included in the Leukocyte 5 Part Differential analysis, cleared by Food and Drug administration (FDA) and European Community In-Vitro-Diagnostic Medical Device (CE IVD) marked as ESId. MDW is calculated as the Standard Deviation of a set of monocyte cell Mean Volume values. A prospective cohort study was conducted in "Santa Maria della Misericordia" University Hospital of Udine (Friuli - Venezia Giulia Region, Italy) on 985 patients aged from 18 to 96. They were classified into four groups based on Sepsis-2 diagnostic criteria: controls, Systemic Inflammatory Response Syndrome (SIRS), infection and sepsis.

RESULTS

MDW was able to differentiate the sepsis group from all other groups with Area under the ROC Curve (AUC) of 0.849, sensitivity of 87.3% and specificity of 71.7% at cut-off of 20.1. MDW in combination with White blood cells (WBC) improves the performance for sepsis detection with a sensitivity increased up to 96.8% when at least one of the two biomarkers are abnormal, and a specificity increased up to 94.6% when both biomarkers are abnormal.

CONCLUSIONS

MDW can predict sepsis and the combination of MDW and WBC significantly increases the clinical value of CBC-Diff results. In the situation of possible diagnostic uncertainty where only two SIRS criteria are met, MDW modulates the probability of sepsis detection, complementing the information of a SIRS score and supporting the clinical decision driven by physician. Moreover, it does not require special order from clinicians, or additional blood draw and is immediately available to report.

Analytical technologies and applications

M032

UIBC MEASUREMENT FOR THE CALCULATION OF TRANSFERRIN IN COMPARISON TO DIRECT TRANSFERRIN DETERMINATION.

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BACKGROUND-AIM

Unsaturated iron binding capacity (UIBC) combined with iron can be used for the calculation of transferrin (TRF) as an alternative to direct measurement methods using the formula UIBC+Iron. The aim of this study was to evaluate the interchangeability between direct measures of TRF (d-TRF) and calculated TRF (c-TRF) through UIBC and iron determination.

METHODS

Precision and bias studies were performed for Iron, d-TRF and UIBC according to CLSI-EP15 A2 using intern quality controls (iQC) for three levels. Each iQC was assayed in triplicated once daily for five days. Within-run and between-run precision, total precision, systematic error and total error were calculated.
754 samples were analyzed and distributed in three groups based on TRF reference ranges: Group 1: d-TRF < 190mg/dL (n=121); Group 2: d-TRF=190-390mg/dL (n=614); Group 3: d-TRF > 390mg/dL (n=19).
d-TRF was analyzed by immunoturbidimetry (AU5800, Beckman Coulter®). UIBC and Iron were measured by spectrophotometry (AU5800, Beckman Coulter®).
Statistical analysis was carried out by MedCalc software to perform Spearman´s coefficient and Passing Bablok regression.

RESULTS

Within-run, between-run and total precision for d-TRF, UIBC and iron were < 4%. Systematic error and total error for d-TRF and iron were < 4% and for UIBC were < 6%.
Spearman's coefficient of rank correlation was 0.985 ($p < 0.0001$) for total values; 0.955 ($p < 0.0001$) for group 1; 0.977 ($p < 0.0001$) for group 2 and 0.769 ($p=0.0001$) for group 3.
d-TRF and c-TRF results showed systematic and proportional differences by Passing Bablock regression for total values ($y=24.58 + 1.11x$), group 1 ($y=-28.53 + 1.41x$), group 2 ($y=32.65 + 1.08x$), group 3 ($y=-89 + 1.38x$). Due to results, a correction factor must be applied to formula.
Formula was corrected using Passing Bablock´s regression equation with an intercept of 32.65 and a slope of 1.08 from the comparison of d-TRF and c-TRF for group 2: TRF= ((UIBC+Iron)–32.65/1.08).

CONCLUSIONS

c-TRF with iron and UIBC is a reliable alternative for the d-TRF determination between 190 and 390 mg/dL which is the one that is in range of normality applying the formula ((UIBC+ Iron)-32.65/1.08). For patients with c-TRF concentration under 190 mg/dL or above 390 mg/dL it will be necessary to measure d-TRF.

Analytical technologies and applications

M033

METHOD COMPARISON OF THYROGLOBULIN ASSAY BETWEEN ATELLICA SOLUTION (SIEMENS HEALTHINEERS) AND INMULITE 2000 (SIEMENS HEALTHINEERS)

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BACKGROUND-AIM

Thyroglobulin (TG) measurement is useful in the diagnosis, follow-up and early detection of relapse in patients with papillary thyroid carcinoma. Normal TG values in healthy population range between 1.36ng/mL and 60ng/mL. In thyroidectomised patients it's a measurement of interest in order to detect a relapse.
In our hospital, TG is analyzed by chemiluminiscent immunoassay using Inmulite 2000 (Siemens Healthineers), obtaining the result after 60 minutes of incubation. Recently, ultrasensitive TG assay (Thyroglobulin Atellica IM assay) was incorporated into the clinical laboratories using Atellica Solution (Siemens Healthineers), allowing to decrease processing time up to 32 minutes and a lower limit of quantification. We show the method comparison results between these two analyzers.

METHODS

We collected blood samples of 67 patients from our hospital. TG blood concentrations ranged from 0.5ng/mL to 984 ng/mL. Thyroglobulin antibodies (TGAb) were also measured in all samples because an interaction between both assays is well known. Precision was evaluated in Atellica Solution using Liquicheck Tumor Marker Control Level 1, 2 and 3 controls (BIO-RAD) (mean concentration: 3.73, 43.2 and 122 ng/mL respectively).

RESULTS

Method comparison between both assays using Passing-Bablok regression analysis resulted in a slope of 0.612 (95% CI: 0.572 to 0.659) and an intercept of -0.209 (95% CI: -0.263 to -0.071) and a correlation coefficient of 0.996.
Between-day precision was studied for each control level, obtaining a coefficient of variation (CV) of 3.64%, 3.32% and 2.14%.
Measurement of thyroglobulin in samples is interfered by TGAb. Regarding to the presence of these anti bodies, no performance difference was detected between both methods and the samples were interference was detected with Inmulite 2000, were the same with Atellica Solution.

CONCLUSIONS

Thyroglobulin Atellica IM assay showed good correlation with Inmulite 2000. Between- day precision showed CV less than 5% for all levels. Moreover this assay reducing analysis time and allows measuring lower concentrations that Inmulite 2000. This can be useful for an earlier diagnostic of relapse.

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Analytical technologies and applications

M034

EXPLORATORY ANALYTICAL VALIDATION OF HEMOGLOBIN A0 QUANTIFICATION USING THE BIO-RAD D100 HPLC TO DIAGNOSE DELAYED TRANSFUSION REACTION IN HOMOZYGOUS SICKLE CELL PATIENTS

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BACKGROUND-AIM

Treatment of severe sickle cell anemia, a hemolytic form of anemia caused by polymerization of Hemoglobin S (HbS), comprises frequent transfusion. These patients often develop allo-antibodies; and when presenting with acute anemia, it is therefore difficult to differentiate between a delayed transfusion reaction and a vaso-occlusive crisis. Recently, nomograms of hemoglobin A_0 (HbA_0) fractions in homozygous HbS patients have been published to discriminate both events. However, the analytical feasibility of this strategy on a routine analyzer for hemoglobin A_{1c} (HbA_{1c}) has not yet been investigated.

METHODS

The Bio-Rad D100 is a chromatographic platform for the separation of HbA_{1c} and unglycated HbA_0. Additionally, the anionic exchange mechanism can resolve certain hemoglobin variants, including HbS. Precision of the instrument was determined on different fractions of HbA_0 and HbS according to CLSI-EP15 protocol; a Passing-Bablok regression between the Bio-RAD D100 and Bio-Rad Variant II was performed over a dilution range of 0 to 85 area percent of HbA_0. The obtained characteristics were applied to investigate whether the uncertainty of measurement was compatible with in literature proposed nomograms.

RESULTS

Analysis of variance reported a coefficient of variation between 0.7 and 2 % for fractions of 60 and 20 area percent of HbA_0, respectively. Applying error propagation, the measurement of uncertainty of relative HbA_0 elimination was estimated on 1.8 percent (CV 8 %). There was a very strong correlation between both methods (spearman rho 0.99, $p<0.001$), regression of the HbA_0 area percentage provided a slope of 1.03 (CI 0.99-1.07) and a intercept of -0.01 (CI -0.03 – 0.01). Considering published nomograms, in which a relative drop of the HbA_0 concentration of at least 12 % identified a risk for a delayed type of transfusion reaction, the observed characteristics of the Bio-Rad D100 allow an accurate assessment of the relevant hemoglobin fractions.

CONCLUSIONS

The Bio-Rad D100 instrument for the analysis of HbA_{1c} can be applied off-label for the urgent quantification of HbA_0 fractions. The instrument provides an easy-access platform for a fast differentiation between potential delayed hemolytic transfusion reactions and vaso-occlusive crises.

Analytical technologies and applications

M035

EVALUATION OF THE INTERFERENCE PRODUCED BY HEMOLYSIS, ICTERUS AND LIPEMIA IN THE MEASUREMENT OF DIFFERENT BIOCHEMICAL CONSTITUENTS IN THE ATELLICA SOLUTION® ANALYZER (SIEMENS HEALTHINEERS)

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BACKGROUND-AIM

Clinical laboratories frequently encounter samples showing significant hemolysis, icterus or lipemia (HIL). Interference with biochemistry assays due to HIL is a common problem in clinical laboratory practice and it can be a significant source of laboratory errors with potential to cause serious harm for the patient.
The objective of the study is to evaluate by the HIL index, the interference produced by haemoglobin, bilirubin and turbidity in 32 biochemical constituents using the Atellica Solution® analyzer. This study will be used to detect and quantify such interference, in order to achieve reliable and accurate results.

METHODS

The study was carried out following a protocol of the Spanish Society of Laboratory Medicine (SEQC-ML) called "Procedimiento para el estudio de la interferencia por hemólisis, bilirrubina y turbidez y para la verificación de los índices de hemólisis, ictericia y lipemia".
It was prepared three different samples to test each interference. One blood sample with a haemoglobin concentration of 15100mg/mL, a bilirubin solution achieving a concentration of 67mg/dL and a triglyceride solution with concentration of 2440mg/dL. Additionally, a mix of patients' serum was prepared with concentrations of the constituents close to the reference range.
Through serial dilutions, eight aliquots were prepared with increasing concentration of each interfering agent in order to know from which concentration the constituents were interfered with. Furthermore, HIL index provided by the analyzer in each dilution was measured. The tests were carried out in duplicate, randomly and in the same analytical series.

RESULTS

According to the quality specifications of our own laboratory, the percentage variation of the change was calculated for each dilution respecting to the concentration obtained in the sample without interfering %Variation=100(Ci–C0)/C0.
Ci=result with interfering
C0=result without interfering
To establish the limit of the maximum permissible error for an interference we have chosen the following criteria $Z(CVA^2+CVW^2)^{1/2}$
CVA:analytic variation coefficient of our laboratory
CVW:biological variation coefficient proposed by SEQC-ML
Z:statistical test(1,96 for the 95% of probability)
As a result, significant interference, in increasing order, was obtained for the following constituents:
• Hemolysis interference: Lactate dehydrogenase, Potassium, Aspartate aminotransferase, Folic acid, Alkaline phosphatase, Albumin, Iron, Total proteins, Alanine aminotransferase, Creatine kinase, Gamma-glutamyltransferase, Magnesium, Total Cholesterol, Chloride, Phosphorus, Lipase, Sodium, Triglycerides.
• Lipemia interference: Triglycerides, Phosphate, Uric Acid, Albumin, Aspartate aminotransferase, Total proteins, Total Bilirubin, Amylase, Alanine aminotransferase.
• Icterus interference: Total Bilirubin, Direct Bilirubin Sodium, Triglycerides, Creatinine Total Cholesterol, Gamma-glutamyltransferase.

CONCLUSIONS

Due to the incorporation of new analyzers in the laboratory, it is convenient to study the interference of HIL in the different analytical techniques, to establish the maximum permissible error and verify the reliability of the results issued.
Based on our study, we decided that from the HIL-index that affects each constituent, the result will be removed and a comment will be added to the report, warning of the interference due to HIL and a new sample should be requested, if possible, without the presence of interference.

Analytical technologies and applications

M036

COMPARABILITY OF SELECTED ASSAYS ON COBAS PURE INTEGRATED SOLUTIONS UNDER ROUTINE-LIKE CONDITIONS AT FOUR SITES IN EUROPE AND ASIA

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BACKGROUND-AIM

The novel cobas pure integrated solutions system (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is a serum work area laboratory analyser, comprising two analytical units: a clinical chemistry unit including ion selective electrodes (ISE) (cobas c 303) and an immunochemistry unit (cobas e 402). In this multicentre study, we assessed the comparability of the cobas pure integrated solutions system versus respective routine analysers at four sites under routine-like conditions.

METHODS

The study was conducted at five sites in Switzerland, Germany and the Republic of Korea, from Sep to Dec 2020. At four sites, method comparison experiments using routine leftover samples evaluated comparability of the cobas pure integrated solutions system with respective routine analysers: Beckman Coulter AU5822 Clinical Chemistry Analyzer, Abbott Alinity I, Siemens ADVIA Centaur, and Roche cobas INTEGRA 400 plus, cobas e 411, cobas pro and cobas 8000. In total, 47 selected analytes with 53 applications covering clinical chemistry (ALB, ALP, ALT, APO-A, AST, BIL-D, BIL-T, CA, CHE, CHOL, CK, CREA, CRP, FE, GGT, GLUC, HbA1c, HDL, LDL, LDH, LIP, MG, PHOS, TP, TRIGL, UA, UREA), ISE (Cl, K, Na) and immunochemistry (anti-TSHR, CA 15-3, CEA, E2, Ferr, FOL, FT3, FT4, HCG+Beta, LH, NT-proBNP, PROG, tPSA, TESTO, TnThs, TSH, Vit. B12) were assessed. Passing/Bablok regression analyses were carried out: slopes, intercepts and correlations for method comparisons were calculated. Pre-defined acceptance criteria for each assay were determined before taking measurements for the method comparisons versus Roche methods.

RESULTS

More than 35,000 result pairs were included in the analysis. All 53 applications showed good comparability between cobas pure integrated solutions and the initial results on the respective routine analysers. In total, 218 method comparisons showed a median Passing/Bablok regression slope of 1.00 (67% were between 0.95 and 1.05), a median bias at the medical decision point of −0.1% (87% were ≤5%) and a median Pearson's r coefficient of 0.998.

CONCLUSIONS

The results of this study demonstrate that the cobas pure integrated solutions system delivers comparable and accurate results versus other commercially available analysers across a selection of applications under routine-like conditions.

Analytical technologies and applications

M037

COMPARISON OF TWO LC-MS/MS METHODS FOR THE QUANTIFICATION OF 24,25-DIHYDROXYVITAMIN D3 IN PATIENTS AND EXTERNAL QUALITY ASSURANCE SAMPLE

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BACKGROUND-AIM

In-house developed liquid-chromatography mass spectrometry (LC-MS/MS) methods are used more and more frequently for the simultaneous quantification of vitamin D metabolites. Among these, 24,25-dihydroxyvitamin D3 (24,25(OH)2D3) is of clinical interest. This study assessed the agreement of this metabolite in two validated in-house LC-MS/MS methods.

METHODS

24,25(OH)2D3 was measured in 20 samples from the vitamin D external quality assurance (DEQAS) program and in a mixed cohort of hospital patients samples (n = 195) with the LC-MS/MS method at the Medical University of Graz (LC-MS/MS 1) and at the University of Liège (LC-MS/MS 2).

RESULTS

In DEQAS samples, 24,25(OH)2D3 results with LC-MS/MS 1 had a proportional bias of 1.0 % and a negative systemic difference of -0.05 %. LC-MS/MS 2 also showed a proportional bias of 1.0 % and the negative systemic bias was -0.22 %. Comparing the EQA samples with both methods, no systemic bias was found (0.0 %) and the slope was 1 %. The mean difference of 195 serum sample measurements between the two LC-MS/MS methods was minimal (-0.2 %). Both LC-MS/MS methods showed a constant bias of 0.31 nmol/L and a positive proportional bias of 0.90 %, respectively.

CONCLUSIONS

This study is the first to assess the comparability of 24,25(OH)2D3 concentrations in a mixed cohort of hospitalized patients with two fully validated in-house LC-MS/MS methods. Despite different sample preparation, chromatographic separation and ionization, both methods showed high precision measurements of 24,25(OH)2D3. Furthermore, we demonstrate the improvement of accuracy and precision measurements of 24,25(OH)2D3 in serum samples and in the DEQAS program.

Analytical technologies and applications

M038

ANALYTICAL PERFORMANCE OF THE NEW SENTINEL CALIAGOLD® PIERCE TUBE AND QUANTITATIVE CALPROTECTIN LATEX IMMUNOASSAY ON THE SENTIFIT®270 ANALYZER

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BACKGROUND-AIM

Inflammatory Bowel Disease is a chronic inflammation of the intestines and is diagnosed by a combination of colonoscopy and measurement of fecal calprotectin.
Recently, Sentinel Diagnostics developed a new CALiaGold® pierce Tube in combination with a new latex immunoassay for calprotectin measurement on the SENTiFIT® 270 analyzer. The new tube should have the advantage that patients can store the tube at room temperature instead of 2-8°C prior to faecal sampling.
The aim of the study is to evaluate the analytical performance of the new CALiaGold® pierceTubes and reagents (Sentinel Diagnostics, REF 1151300 and 1151000), compared to the current CALiaGold® pierce Tubes and reagents (Sentinel Diagnostics, REF 1159300 and 1159000).

METHODS

To investigate the preservation of stability of the Sentinel CALiaGold® pierce Tube itself (REF 1151300), tubes without sample were incubated at 2-8°C, 21°C, 28°C and 35°C. After 6 weeks of incubation, tubes were sampled with stool and measured on the same day.
To investigate the conservation of functionality, tubes with sample were incubated at 2-8°C, 21°C, 28°C and 35°C and measured at several time points up to 14 days.
For comparison of the CALiaGold® pierce Tubes, patients received the current (REF1159300) and the new tube (REF 1151300) along with the instruction protocol for stool collection at home.

RESULTS

The new CALiaGold® pierceTube can be stored up to 4 weeks at 21-35°C prior to faecal sampling. After stool collection in the new tube, the stability compared to the current tube is extended to 14 days at 2-8°C and 5 days at room temperature (21-35°C)
Linear regression analysis with 100 home collected stool samples demonstrated a good correlation with the current method (r = 0,928). The results for slope (0.92) and intercept (27) meet the criterion that 95% of the confidence level for slope and intercept must include 1 and 0, respectively.

CONCLUSIONS

The preservation of stability and the conservation of functionality of the new Sentinel CALiaGold® pierce Tube (REF 1151300) in a laboratory setting meet the requirements for faecal sampling by patients at home.
The analytical performance of the new Sentinel CALiaGold® Immunoassay (REF 1151000) on the SENTiFIT®270 analyzer meets the requirements for quantitative determination of calprotectin in human stool.

Analytical technologies and applications

M039

COMPARING THE OPERATIONAL PERFORMANCE OF TWO SERUM WORK AREA SYSTEMS: COBAS® PRO INTEGRATED SOLUTIONS AND ATELLICA® SOLUTION

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BACKGROUND-AIM

A highly efficient serum work area is a key requirement for hospital and commercial laboratories that face increasing pressure to deliver results not only at highest quality, but also increasingly fast. The time to result is an important indicator to evaluate the operational performance. Here, we have determined for two serum work area systems, namely Roche cobas® pro integrated solutions and the Siemens Healthineers Atellica® Solution, the time from sample loading until the last result of the workload is available (time to last result, TLR). Both systems offer analytical units for clinical chemistry (cobas c 503 and Atellica CH 930), immunochemistry (cobas e 801 and Atellica IM 1300) and selective ion measurement (ISE and IMT).

METHODS

Representative workloads were extracted from real use cases, avoiding uniform test patterns. From those workloads, we determined the time to result for the individual analytical units and the entire system. The combined performance of clinical chemistry and immunochemistry was quantified with 300 samples and different fractions of immunochemistry tests of 14% (A) and 18% (B). Additionally, the influence of STAT samples (C) and a doubled number of samples was quantified (D). The clinical chemistry analytical units (cobas c 503 and Atellica CH 930) were characterized individually with 50 samples and a comprehensive metabolic panel of twelve tests (E). The immunochemistry modules (cobas e 801 and Atellica IM 1300) were probed individually with 50 samples each for four panels: a pregnancy panel (F), a hepatitis / HIV panel (G), and two blood screening panels (H and I).

RESULTS

We found the following TLRs for cobas pro integrated solutions and the Atellica Solution, respectively: (A) 1:45 h and 1:36 h, (B) 2:05h and 2:22 h, (C) 1:46 h and 1:58 h, and (D) 3:55 h and 4:12 h. Probing only clinical chemistry, we measured for cobas c 503 and Atellica CH 930, respectively: (E) 0:47 h and 0:44 h. Finally, investigating immunochemistry only, we found as TLR for cobas e 801 and Atellica IM 1300, respectively: (F) 1:39h and 2:02 h, (G) 2:41 h and 3:15 h, (H) 1:36 h and 2:04 h, and (I) 1:09 h and 1:42 h.

CONCLUSIONS

Overall, cobas pro integrated solutions showed the faster TLR for seven out of nine workloads. For two workloads, the Atellica Solution showed the faster TLR.

Analytical technologies and applications

M040

RESULT PRECISION AND ACCURACY ON COBAS PURE INTEGRATED SOLUTIONS DEMONSTRATED AT FOUR SITES IN EUROPE AND ASIA

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BACKGROUND-AIM

The novel cobas pure integrated solutions system (Roche Diagnostics International Ltd, Rotkreuz, Switzerland), a serum work area laboratory analyser, comprises a clinical chemistry unit with ion selective electrodes (ISE; cobas c 303) and an immunochemistry unit (cobas e 402). In this study, analytical performance and overall system functionality of the cobas pure integrated solutions system was evaluated.

METHODS

The study was conducted Aug–Dec 2020 at 5 sites (Germany, Switzerland, Poland and the Republic of Korea). At all sites, quality control (QC) materials were measured for applied assays (ISE, clinical chemistry and immunochemistry) at 2 analyte concentration levels; analyte recovery per QC was monitored. At 4 sites, precision studies based on the CLSI EP05-A3 protocol were conducted over 21 days for 34 selected applications (ISE: 3 analytes/6 applications [serum & urine]; clinical chemistry: 10/15 [serum & urine]; immunochemistry: 13/13 [serum]) representing a typical routine panel of assays. Coefficients of variation (CVs) for repeatability, intermediate precision and reproducibility were calculated and compared with pre-defined acceptance criteria. At 4 sites, result accuracy for 48 selected applications (ISE: 3; clinical chemistry: 26; immunochemistry: 19) was validated in a ring trial, including 16 commercial samples and 2 Roche QC samples (IgM anti-HAV), measured in triplicate over 3 consecutive days.

RESULTS

For repeatability and intermediate precision, 152 CVs were calculated. For ISE, clinical chemistry and immunochemistry assays, respectively: 34/48 CVs were ≤1%, 116/120 CVs were ≤2% and 90/104 CVs were ≤2.5% for repeatability; 47/48 CVs were ≤2%, 114/120 CVs were ≤2% and 104/104 CVs were ≤3.5% for intermediate precision; and 11/12 CVs were ≤3%, 27/30 CVs were ≤3% and 20/26 CVs were ≤3.5% for reproducibility. For the 48 applications included in the ring trial analysis of result accuracy, 80% of median recoveries per assay and site were within 2% of the median per assay for all sites.

CONCLUSIONS

The novel cobas pure integrated solutions system provides precise and accurate results for a typical routine panel of assays for measurement of ISE, clinical chemistry and immunochemistry parameters, supporting implementation into routine clinical laboratory practice.

Analytical technologies and applications

M041

SERUM WORK AREA SYSTEM VALIDATION UNDER INTENDED USER CONDITIONS

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BACKGROUND-AIM

As part of the Roche Diagnostics' system design validation concept, external evaluations of the novel cobas pure integrated solutions system (a serum work area laboratory analyser comprising a clinical chemistry unit with ion selective electrodes [ISE; cobas c 303] and an immunochemistry unit [cobas e 402]) were performed at different phases of development; overall system functionality, reliability and user satisfaction were assessed.

METHODS

We conducted a prototype study (1 site: Switzerland), a pilot study (2 sites: Switzerland, Germany) and a comprehensive system performance evaluation (SPE) study (5 sites: Europe, Asia). Selected clinical chemistry, ISE and immunochemistry applications were assessed: prototype (n=22), pilot (n=17), SPE (n=27). At each development phase, routine simulation precision experiments based on the CLSI EP05-A3 protocol evaluated all system components under routine-like user conditions, to identify deficiencies and analyse overall functionality. Coefficients of variation (CVs) were calculated. Precision experiments allowed detection of systematic and random errors for selected assays (representing a typical routine panel of assays). System reliability (measured by percentage of uninterrupted analysis runs) was compared at various phases.

RESULTS

Results from the prototype study led to optimisation of fluid drain management, which was redesigned for the pilot study, and the installation procedure adjusted. In the pilot study, sample pipetting was impaired after a sample with insufficient volume was tested; a software change was introduced during the SPE study and regression testing showed that this led to the system correctly pipetting samples in a repeat run after a sample short alarm occurred. In the SPE study, 18 runs with 604 CV pairs for 27 selected clinical chemistry, ISE and immunochemistry applications were included; an overall system reliability of 99% was achieved, a good relative dispersion (most CVs <2%) was observed and no systematic or random errors occurred.

CONCLUSIONS

Improvement in the functionality of the novel cobas pure integrated solutions system was seen during the development process; good overall system functionality and reliability, which are essential for providing safe and accurate results to operators and patients, were shown.

Analytical technologies and applications

M042

ANALYTICAL EVALUATION OF THE PLASMATIC SUPAR AUTOMATED ASSAY ON ATELLICA CH930 (SIEMENS)

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BACKGROUND-AIM

SuPAR (Soluble urokinase Plasminogen Activator Receptor) is a prognostic and non-specific biomarker of chronic inflammation proposed to evaluate risk stratification in the emergency department (ED) patients. Measurement of SuPar in the triage process may allow identifying patients at high risk in the ED, helping in the decision of discharge or admission of these patients. The aim of this work was to validate the assay with the SuPARnostic TurbiLatex reagent (Virogates) on an Atellica CH930 (Siemens) analyzer.

METHODS

Analytical evaluation was performed on human plasma, both collected on EDTA and lithium heparinate (LH), according to the requirements of the Clinical and Laboratory Standards Institute (CLSI) and the French Valtec protocol for interference studies. This work focused on: limit of blank, limit of detection, limit of quantification, repeatability, intermediate precision, effect of antigen excess and analytical interference. SuPAR results with 105 frozen plasmas provided by Virogates were compared with those obtained in parallel with a Cobas® c111 (Roche). Moreover, a study was carried out to compare results obtained on 52 fresh samples from patients of the St-Antoine hospital drawn on EDTA and LH.

RESULTS

Limit of blank was below 0.7 ng/mL, limit of detection was below 1.4 ng/mL, limit of quantification was found at 2.0 ng/mL, repeatability and intermediate precision evaluated on 3 levels showed CV% below the acceptable limits (10% and 15% respectively). No effect of excess antigen was observed, nor any significant interference with lipemia (up to 5.86 mmol/L triglycerides), hemolysis (up to 3 g/L hemoglobin) and jaundice (up to 446 µmol/L of bilirubin). Finally, the results obtained on Atellica are comparable with those reported on Cobas, whether on samples taken on EDTA or LH. SuPAR concentrations on patients fresh samples are 10% lower when drawn on EDTA compared to samples drawn on LH.

CONCLUSIONS

The method for assaying suPAR in plasma (EDTA or LH) is validated on Atellica CH930. However, for values close to the clinical decision threshold of 4 ng/ml, systematic verification is recommended.

Analytical technologies and applications

M043

ESTABLISHMENT OF ANTIGEN-PRESENTING NANODISCS IN ELISA FORMATS

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BACKGROUND-AIM

Detection of pathophysiological autoantibodies against membrane proteins (MPs) is usually a challenge in clinical diagnostics. This is obvious in the diagnosis of immune thrombocytopenic purpura (ITP). ITP is a bleeding disorder characterized by autoantibody-mediated destruction of platelets and platelet precursor cells. Despite the identification of glycoprotein receptor (GP) complexes (e.g. GPIIb/IIIa) as major immune targets in ITP, diagnostic tests are rare and existing assays are often very laborious and not very sensitive. The use of nanodisc (ND) technology could significantly improve the diagnosis of ITP and other autoimmune diseases targeting MPs. NDs are soluble, discoidal lipid bilayers whose hydrophobic edges are covered by two amphipathic membrane scaffold proteins (MSPs). Incorporation of intact MPs into this artificial membrane system allows their analysis in a natural bilipid environment providing also access to both membrane sides. While NDs are mainly used for structural, biochemical or biophysical studies of MPs, they could also serve as a powerful tool in diagnostic tests in future.
In our study, we analyzed the potential and challenges of NDs as antigen carriers in enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies to MPs in general and to GPIIb/IIIa complexes in particular.

METHODS

We generated HEK293 cell lines stably overexpressing different recombinant MPs (e.g. GPIIb/GPIIIa-Strep or CD147-Strep) and used their lysates as antigen source for intact incorporation into NDs without a complex post-harvest antigen purification step. The composition of ND assembly mixtures was varied and optimized. For this, assembled ND mixtures were analyzed by size exclusion chromatography (SEC) and ELISA.

RESULTS

NDs could be immobilized on ELISA plates either directly or via the Strep-tag of recombinant MPs. SEC chromatograms and ELISA signals depended on the amount and kind of MPs, MSPs, lipids and detergents added in the assemblies. NDs comprising functional GPIIb/IIIa-Strep complexes were restricted to defined fractions after SEC, only partially overlapping with fractions containing CD147.

CONCLUSIONS

There are some aspects to consider when using NDs for diagnostic approaches that are not critical when using NDs in currently typical applications.

Analytical technologies and applications

M044

DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR TRYPTOPHAN METABOLITES QUANTIFICATION IN HUMAN SERUM AND PLASMA

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BACKGROUND-AIM

Tryptophan, an essential amino acid, and its metabolites are involved in many physiological processes including neuronal functions, immune system and gut homeostasis. Three metabolic pathways closely linked the metabolism of tryptophan to the intestinal microbiome. The kynurenine pathway, also called indoleamine-2,3-dioxygenase (IDO) pathway, accounts for about 90 % of tryptophan degradation and leads to kynurenine production and other compounds such as kynurenic, xanthurenic and quinolinic acids. The serotonin pathway accounts for 80 % of serotonin production by the intestine. The aryl hydrocarbon receptor (AhR) pathway leads to the synthesis of indole derivates. The latters can activate the AhR expressed by some immune and intestinal cells. Alterations of tryptophan metabolism are associated with many pathologies such as neurologic and psychiatric disorders, inflammatory bowel diseases (IBD), metabolic disorders and cancer. It is consequently critical to develop reliable quantitative methods for the analysis of tryptophan and its metabolites.

METHODS

In our laboratory, a LC-MS/MS method was set up for the analysis, in a single run, including tryptophan and 20 of its metabolites, corresponding to the three aforementioned metabolic pathways.
A LC-20ADXR Shimadzu chromatographic system in tandem with an ABSciex QTRAP 5500 was used. Chromatographic separation was achieved on a biphenyl column. Analyzes were performed with a run-to-run time of 13 min. After the optimization of chromatographic and detection parameters, the method was validated for serum and plasma.

RESULTS

The repeatability and the intermediate fidelity were performed for the 21 molecules: CV values were ≤ 10 %, and ≤ 20 % for the lowest concentrations. The range of linearity differed from molecules, ranging from 23 nmol/L to 152 µmol/L. Lower detection limits ranged from 0.7 to 65 nmol/L. The comparisons between serum and plasma (collected with different anticoagulants) did not evidence significant differences except for serotonin. The reference values were also established for all components.

CONCLUSIONS

This laboratory-developed method is currently used in research studies for patients affected of IBD. Further applications are also in development for patients suffering from neurodegenerative or neurological disorders.

Analytical technologies and applications

M045

VALIDATION OF A GAS CHROMATOGRAPHY MASS SPECTROMETRY METHOD FOR 2-HYDROXYBUTIRATE MEASUREMENTS IN HUMAN SERUM AS PREDICTOR OF TYPE 2 DIABETES MELLITUS

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BACKGROUND-AIM

Type 2 Diabetes Mellitus(T2DM) is a metabolic disorder that affects millions of individuals worldwide. In the last years metabolomic and clinical studies have identified increased 2-hydroxybutyrate (2HB) serum levels as a risk predictor for the development of T2DM and the deterioration of glycemic control. However, there is a lack of validated methods for the measurement of 2HB. Our aim was to develop and validate a gas chromatography-mass spectrometry(GC-MS) method for the quantification of 2HB in human serum.

METHODS

The method consisted on a liquid-liquid extraction of 300μL of serum with ethyl acetate and, after solvent evaporation, a 2 minutes microwave assisted derivatization with BSTFA:TMCS 1%. 2HB3C13 was used as internal standard. GC–MS analyses were performed on a Shimadzu GCMS QP2010 Ultra instrument operating in SCAN mode and with m/z 205 and m/z 208 as the quantification ions. Linearity was evaluated in calibration curves of six concentrations (5 to 200ng/mL). Intra (n=5) and interassay (n=5) accuracy and imprecision were evaluated in two quality controls(QC), 30 and 125ng/mL, and in serum samples. Stability of 2HB extracts in the autosampler was evaluated at room temperature. Stability of 2HB in serum was evaluated at 4ºC and room temperature, and after three freeze and thaw cycles. Moreover, differences between 2HB levels in plasma EDTA and serum samples (n=10) were evaluated (paired student T-test).

RESULTS

The method was linear for 2HB concentrations ranging from 5 to 200ng/mL, obtaining interassay accuracies values between 97-104% and imprecision values <10% at all six standard levels. Limit of quantification was <5ng/mL. Values of intra- and inter-assay imprecisions of two QC levels and two samples were <10%. Intra and intereassay accuracy of two QC levels and recovery of 2HB in 3 different plasma samples were 93-106%. Extracts in the autosampler at room temperature were stable for at least 96h. Serum levels of 2HB were stable after 24h at 4ºC or at room temperature, and after three freeze and thaw cycles. No differences between 2HB in plasma and serum were found (40.34±28.02 vs 38.76±26.42; p value: 0.8985)

CONCLUSIONS

A sensitive GC–MS method was developed and validated for the quantitative measurement of 2HB in human serum. This method could be used in routine laboratories to evaluate glucose metabolism.

Analytical technologies and applications

M046

DEVELOPMENT OF LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRIC METHOD FOR ESCITALOPRAM

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BACKGROUND-AIM

Escitalopram is a selective serotonin re-uptake inhibitors. These agents prevent reuptake of serotonin to the presynaptic terminals of serotonergic neurons, resulting in increased serotonin levels at neuronal synapses. The recommended therapeutic range for escitalopram is 15-80 ng/ml. Common side effects that occur are insomnia, dry mouth, dizziness, constipation and indigestion. Rare but serious side effects include low sodium blood levels, angle-closure glaucoma, serotonin syndrome, seizure. Therefore, monitoring of plasma escitalopram levels is important for effective and safe treatment. The aim of this study was to develop a simple, fast and accurate tandem mass spectrometric method for the quantification of escitalopram.

METHODS

250 μL of serum was taken into eppendorf tubes and 100 μL of internal standard and 800 μl methanol were added. The mixture was vortexed during 30 seconds and centrifuged at 13000rpm for 10 minutes. The supernatants were taken into clean glass tubes and dried under nitrogen gas at 40°C. The dried residues were dissolved in 200 μl methanol:water (10:90,v/v%) mixture and 40 μL of supernatant was injected.

RESULTS

The linearity range was 5-450 ng/ml (r2>0.998). The limit of quantification (LOQ) was 5 ng/ml. The retention time and total run time were 2.97 and 5 min, respectively. The intra- and inter-assay imprecision ranged under 10%. The mean recovery was 97.4% and matrix effect values were less than 12.5%.

CONCLUSIONS

A rapid, economic, simple, accurate and sensitive method was developed for escitalopram. The method may be used for routine analysis of escitalopram

Analytical technologies and applications

M047

BIOAVAILABLE VITAMIN D AND VITAMIN D METABOLITE RATIO (VMR) OF PREGNANT WOMEN AND ICU PATIENTS BY MULTIPLEX LC-MS/MS FOR SIMULTANEOUS MEASUREMENT OF 25-HYDROXYVITAMIN D, 24,25-DIHYDROXIVITAMIN D, ALBUMIN, AND VITAMIN D-BINDING PROTEIN

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BACKGROUND-AIM

While the total vitamin D concentration has long been used as an indicator of vitamin D deficiency despite of its inadequacy, bioavailable vitamin D (BVD) and vitamin D metabolite ratios (VMR) have emerged as important markers to assess clinical vitamin D reservoir. With recently developed one-step measurement of vitamin D binding protein (VDBP) and vitamin D metabolites by liquid chromatography-tandem mass spectrometry (LC-MS/MS), these two markers not only calculated easily but also can be used for clinical evaluation of vitamin D status for many patients. Thus, we evaluated the usefulness of BVD and VMR in pregnant women, patients in intensive care units (ICU), and healthy control.

METHODS

Serum samples were collected from 60 healthy individuals, 50 pregnant women, and 50 ICU patients. Total 25-$(OH)D_3$, 25-$(OH)D_2$, and 24,25-$(OH)_2D_3$ levels were quantified by LC-MS/MS. Trypsin digestion methods for quantification of VDBP and determination of VDBP isoforms were used. The free vitamin D, BVD and VMR were calculated by using total 25(OH)D, VDBP, 24,25-$(OH)_2D_3$, and albumin concentrations. We compared the results of VDBP concentration from monoclonal ELISA kit to those from LC-MS/MS.

RESULTS

In comparison with healthy controls, calculated biomarkers from LC-MS/MS were significantly lower in ICU patients compared to healthy controls (median 1.20 ng/dL vs 2.62 ng/dL, $P < 0.0001$ in BVD, 5.55 vs 6.50, $P = 0.0366$ in VMR, and 4.76 pg/mL vs 6.96 pg/mL, $P = 0.0015$ in free vitamin D), while free vitamin D which calculated using VDBP from monoclonal ELISA kit was not significantly different (7.47 pg/mL vs 7.60 pg/mL , $P = 0.9330$). Vitamin D markers were also significantly low in pregnant women in all quantifying methods, which show significant difference and lower P-values in using LC-MS/MS ($P = 0.0030$ vs $P < 0.0001$ in BVD, $P = 0.0384$ vs $P < 0.0001$ in free vitamin D).

CONCLUSIONS

BVD and VMR which calculated from the results by one-step measurement using LC-MS/MS more accurately reflect the vitamin D reservoir of patients than those from the results using older monoclonal ELISA kits. To evaluate clinically useful and reliable vitamin D conditions in patients and pregnant women, the one-step LC-MS/MS measurement of vitamin D and its metabolites may be used as efficient and accurate method.

Analytical technologies and applications

M048

STUDY OF POTENTIAL INTERFERENCES IN THE MEASUREMENT OF VITAMIN D BY THERMO SCIENTIFICTM CASCADIONTM SM CLINICAL ANALYZER COMPARING TO LIAISON XL FROM DIASORIN®

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BACKGROUND-AIM

Vitamin D status is assessed by measuring the major storage forms 25-OH-vitamin D3 and D2, which can be epimerized at the C3 position, with higher amounts in children under 1 year.
Our aim was to evaluate the analytical performance of the measurement of 25-OH-vitamin D in the Cascadion SM Analyzer, a fully automated LC/MS-MS, in comparison to DiaSorin® Liaison XL Immunoassay.

METHODS

735 serum samples (564 adults and 92 children under 1 year) were analyzed in parallel by both analyzers. The results comparison was done based on Passing-Bablok non-parametric regression and Bland-Altman bias estimation. Accuracy was assessed using material from an External Quality Assurance Scheme (DEQAS,n=19), and estimated using Pearson correlation. MedCalc® and GraphPad® were used for statistical analysis.
An interference from lipemia (>589 mg/dL) was observed for the immunoassay. Therefore, the study was extended with 79 samples from patients with elevated triglyceride serum levels.

RESULTS

The Cascadion SM 25-Hydroxy Vitamin D Assay did not exhibit a significant bias when compared on average with the immunoassay (<5% for both adults and children). Bland-Altman comparison of the Cascadion SM assay showed a mean absolute bias of 1 ng/mL to Liaison XL (95% limits of agreement (LoA) −8.5–10.5) for adults and 0.3 ng/mL (95%LoA −14.3–14.8) for children.
Results obtained on both analyzers demonstrated an agreement with assigned values by EQA of r=0.954 (95% confidence interval (CI) 0.885–0.982) for Liaison XL and r=0.995 (95%CI 0.988–0.998) for Cascadion SM.
Liaison XL analyzer showed an average -33.1% negative bias at high lipemia samples compared to the Cascadion SM analyzer. There was no direct relationship noticeable between degree of lipemia and bias.

CONCLUSIONS

Majority of results from both analyzers were similar and considered as comparable. No cross-reactivity with the C-3 epimer of 25-OH-vitamin D was observed at any concentration or for low age patient's during the study. However, the significant negative bias in high lipemia samples shown by Liaison XL was thoroughly studied and reported to the manufacturer.
Results obtained on both analyzers demonstrated good agreement with assigned values by EQA (although slightly better for the Cascadion SM Analyzer).

Analytical technologies and applications

M049

EFFICIENCY STUDY INTELLIGENT LABORATORY AUTOMATION SYSTEMS

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BACKGROUND-AIM

The new intelligent laboratory automation systems (LAS) change the concept of the old conveyors (TRACK), improving the management system and flow of samples, and allowing for the optimization of technical and human resources in our laboratories, as well as an improvement in response times, and a stability of these times independent of the load on the LAS. Our first organizational objective was the integration of all laboratory disciplines (haematology, biochemistry, immunochemistry, including serology and coagulation) into the LAS, while at the same time, not negatively affecting the response time for emergencies. This is complex, but the system must prioritize these samples over routine samples, allowing us to obtain optimal response times.
To analyze the turnaround times of emergency samples processed on the Beckman- Coulter DxA 5000 system under low- and high-routine workloads.

METHODS

Our laboratory is equipped with the DxA 5000 system, a high-performance automated sample handling system, capable of prioritizing emergency samples regardless of workload. For this system, we will process 100 emergency samples during low-workload hours (<500 tubes/hour) and during the peak workload hours of the DxA 5000 system (>1000 tubes/hour), comparing the response times with each other.

RESULTS

The Haematology response time with a low workload was 9 minutes, and with a high workload, it was 13 minutes; for biochemistry it was 35 and 37 respectively; and for coagulation it was 27 and 28. The complete analysis turnaround times are 35 and 37 minutes, with a difference of only 2 minutes.

CONCLUSIONS

The incorporation of emergencies into the DxA5000 system allows us to optimize resources and homogenize response times, as there are no major differences in response times irrespective of the workload, and the system efficiently prioritizes emergency samples over routine samples.

Analytical technologies and applications

M050

PREDICTIVE VALUE OF MOTILITY AND KINETIC PARAMETERS IN PREGNANCY OUTCOMES BY DONOR SPERM INTRAUTERINE INSEMINATION

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BACKGROUND-AIM

In semen analysis, a risk of bias can occur if motility is determined by the manual method. Sperm Class Analyser (SCA®) software is able to categorize spermatozoa according to the WHO 4 classification: Type A (rapid and progressive), B (medium progressive), C (non progressive) and D (static). Furthermore, this method includes some kinetic parameters that are not possible to determine by the subject: curvilinear velocity (VCL, m/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity (LIN), straightness (STR), wobbley (WOB) and amplitude of lateral head displacement (ALH, µm). Some of these parameters have been described as pregnancy predictors in conjugal artificial insemination. Therefore, a male factor related to the couple infertility occurs in many cases. So, it is adequate to evaluate the prognostic ability in donor sperm samples, which have been previously diagnosed as normozoospermic.

METHODS

It is a retrospective study of results of artificial insemination by donor (AID) cycles in a Human Assisted Reproduction Hospital Area between 2017 and 2019. Seminal samples were processed by gradient selection. The SCA® v4.2 software was used to assess the sperm motility. The study variables were: Type A, Type B, Type A+B, VCL, VSL, VAP, LIN, STR, WOB and ALH. The result variable was clinical pregnancy evaluated by gestational sac finding 3 weeks after insemination. Two groups were defined: group a (clinical pregnancy) and group b (absence of pregnancy). SPSS v21.0 program was used to carry out the statistical analysis. Kolmogorov-Smirnov test was employed to evaluate normality. Student's T-test and Kruskal- Wallis test were used for normal and non-normal distribution variables, respectively.

RESULTS

Of the 53 AID cycles included, 14 concluded with pregnancy (group a). We did not obtain significant differences in age between groups. Normality test only reflected a normal distribution of the variable Type A+B (progressive motility). We did not find any significant difference between groups in any of the study variables.

CONCLUSIONS

Current results do not offer evidence of pregnancy prognostic value of kinetic and motility parameters evaluated by SCA® on donor sperm samples.

Analytical technologies and applications

M051

ANALYTICAL PERFORMANCE OF THE NOVEL C-REACTIVE PROTEIN AND D-DIMER TEST ON THE EXDIA TRF PLUS ANALYZER

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BACKGROUND-AIM

The Exdia TRF analyzer (Axon Lab, Zaltbommel, The Netherlands) is a multiparameter portable immunoassay analyzer equipped with innovative imaging-based Time Resolved Fluorescence technology. The analyzer can be used in the general practice or in a point-of-care environment to exclude e.g. infection or deep vein thrombosis by measuring C-Reactive protein (CRP) or D-Dimers (DDI), respectively.
The aim of the study is to evaluate the analytical performance of the novel Exdia CRP and DDI immunoassays on the Exdia TRF analyzer.

METHODS

Reproducibility of the CRP and DDI immunoassays was measured in 5-7 fold in low and high control samples (Seronorm^{TM} Cardiac Acute Liquid L-1B and L-2) and patient samples with low, medium and high levels of the analytes. Li-heparin plasma samples (n=37) in the range of CRP 1.5 – 149 mg/L were used for comparison between the Exdia CRP assay and the CRP wide range assay on the Siemens Atellica IM 1300 analyzer. Citrate anticoagulated plasma samples (n=50) in the range of DDI 135 – 3650 ng/mL were used for comparison between the Exdia DDI assay and the HemosIL D-Dimer HS 500 assay on the ACL TOP350 analyzer (Werfen, Breda, The Netherlands). All tests were performed within 4 hours after venipuncture.

RESULTS

Within day precision results of the Exdia CRP ranged from 7.4 to 11.7% and those of the Exdia DDI from 4.3 to 12.3%. Day-to day precisions ranged from 7.8 to 9.3% for CRP and from 7.6 to 11.1% for DDI. All reproducibility results were within the specifications of the manufacturer.
The Exdia CRP linear regression analysis demonstrated a good correlation with the CRP wide range method on the Atellica IM analyzer (r = 0.968). The results for slope (0.97) and intercept (4.5) meet the criterion that 95% of the confidence interval for slope and intercept must include 1 and 0, respectively.
The Exdia DDI regression analysis demonstrated a good correlation with the HemosIL D-Dimer HS 500 assay (r = 0.982). The slope of the Exdia DDI (1.17) deviates from 1; the intercept (-7.8) did not deviate from 0.

CONCLUSIONS

The analytical performance of Exdia CRP as well as Exdia DDI demonstrate results that meet the requirements for quantitative determination of CRP and DDI in a general practice or point-of-care setting.

Analytical technologies and applications

M052

DEVELOPMENT OF A LIQUID CHROMATOGRAPHY MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF VITAMIN K1, MENAQUINONE-4, MENAQUINONE-7 AND VITAMIN K1-2,3 EPOXIDE IN SERUM OF PATIENTS WITHOUT SUPPLEMENTS

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BACKGROUND-AIM

Vitamin K refers to a structural group of strong lipophilic naphthoquinone-derivatives with beneficial role in blood coagulation, bone metabolism and growth. The determination of vitamin K concentrations in the blood, which comes only from food sources that are not artificially enriched, might be of clinical relevance in different patient groups, however, only a few laborious methods are available yet

METHODS

We developed and validated a simple liquid chromatography–tandem mass spectrometric (LCMSMS) method for the determination of vitamin K1, menaquinone-4 (MK-4), menaquinone-7 (MK-7) and vitamin K1-2,3 epoxide in human serum and proved the method in a study cohort with carbohydrate malabsorption

RESULTS

For measurement of vitamin K analytes, we used a QTRAP 6500 LCMS device (SCIEX, Foster City, CA, USA) with atmospheric pressure chemical ionisation. After liquid/liquid extraction for sample cleanup and separation on a on a Kinetex ® 5µm F5 column (100 Å, 150 x 4.6 mm, Phenomenex, USA), detection was performed in the multiple reaction mode. By using 250 µL serum, the lower limits of quantification (LOQ) were 0.06 nmol/L for all analytes. Mean recoveries were between 60% (MK-7) and 85% (VK-1), intra- and inter-assay CVs were all < 15% in the specified working range (0.06-6.0 nmol/L). The method was applied to 162 patients with carbohydrate malabsorption testing. Ninety-seven patients with identified fructose malabsorption showed a trend to higher MK-4 and MK-7 concentrations (median, IQR) compared to 65 individuals without fructose malabsorption (0.18, 0.27-0.41 vs. 0.13, 0.18-0.24 nmol/L, p =0.09) and (0.74, 0.42-1.47 vs. 0.59, 0.42-0.87 nmol/L, p = 0.064), but the differences did not reach statistical significance. Regarding vitamin K1 serum concentrations there were obviously no significant differences (0.37, 0.17-0.63 vs. 0.38, 0.23-0.55 nmol/L, p=0.536) between these subgroups

CONCLUSIONS

The described method is suitable to investigate large clinical trials, which may have the clinical potential to elucidate the function of vitamin K and its relationship to different diseases patterns and may be implemented in clinical laboratory routine

Analytical technologies and applications

M053

A NEW METHOD FOR MULTIPLEX ROUTINE ANALYSIS OF STEROID HORMONES IN PLASMA USING NEW 2D-LC/MS/MS TECHNIQUE

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BACKGROUND-AIM

The aim of the study was to introduce a new heart-cut 2D-LC/MS/MS method with easy preparation of any sample for determination of 15 steroid hormones (11-deoxycortisol, 11-deoxycorticosterone, 17α-hydroxyprogesterone, 21-deoxycortisol, aldosterone, androstenedione, corticosterone, cortisol, cortisone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), dihydrotestosterone, estradiole, progesterone, and testosterone) in human plasma, suitable in routine practice.

METHODS

The determination was performed on 2D-LC/MS/MS system. The plasma sample is deproteinized with acetonitrile, followed by phase separation with brine and magnesium sulphate, centrifugation. The organic phase is then injected into the system. After injection, the required analytes are separated from the matrix on an extraction column (YMC-Triart Diol-HILIC), collected on a TRAP column (InfinityLab Poroshell 120 EC¬C18 column guard), and then eluted on an analytical one (InfinityLab Poroshell 120 EC) where separation takes place. An Agilent 6470 Triple Quadrupole LC / MS mass spectrometer with electrospray as the ionization source was chosen as the detector. The whole preparation process takes only 10 minutes per sample, then an hour for 40 samples in series. The analysis of one sample for all 15 steroid hormones takes a total of 18 minutes.

RESULTS

The method detection limit (MDL) for 15 steroid hormones were in the range between 0.008 nmol/L (2.88 pg/mL) for aldosterone, and 0.873 nmol/L (0.252 ng/mL) for DHEA. Only DHEAS has the MDL at a higher concentration of 12.7 nmol/L (4.68 ng/mL). For all the analytes, the lowest calibration point relative standard deviation was lower than 10.8%, so we can measure the lowest concentration of interest with good precision

CONCLUSIONS

We introduced an easy and fast method for routine diagnosing and differential diagnosis of disorders of steroid secretion. Our new 2D-LC/MS/MS multiplex method needed only 10 minutes of preparation and 18 minutes of analysis for all analytes. This is in contrast with the standard methods with the extraction step, which takes 60 minutes or more of sample preparation. Another benefit of our method is directly analyzing a plasma sample after simple protein precipitation, followed by phase partition, without sample derivatization or evaporation of the organic solvent. Thanks to optimized valve settings and the HILIC column we are able to remove the lipids from the sample during analysis, and thus extend the lifetime of the RP column.

Analytical technologies and applications

M054

DETERMINATION OF DEXAMETHASONE BY 2D-LC/MS/MS TECHNIQUE

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BACKGROUND-AIM

Our goal was to introduce a method for the determination of dexamethasone (DXM). Knowledge of its concentration and cut-off value will significantly help to refine the results of the dexamethasone suppression test and thus rule out false-positive results.

METHODS

The determination was performed on 2D-LC/MS/MS system. The plasma sample containing DXM is deproteinized with acetonitrile, followed by phase separation with brine and magnesium sulfate, centrifugation. The organic phase is then injected into the system. After injection, the required analytes are separated from the matrix on an extraction column (YMC-Triart Diol-HILIC), collected on a TRAP column (InfinityLab Poroshell 120 EC¬C18 column guard), and then eluted on an analytical one (InfinityLab Poroshell 120 EC) where separation takes place. An Agilent 6470 Triple Quadrupole LC / MS mass spectrometer with electrospray as the ionization source was chosen as the detector. The whole preparation process takes only 10 minutes per sample, then an hour for 40 samples in series. The analysis of one sample takes a total of 18 minutes.

RESULTS

This procedure reaches the detection limit of the method of 0.09 nmol/L. The repeatability and intermediate precision were then at three levels in the concentration range of the calibration curve of 1.504 - 60.15 nmol/L (R2 = 0.9999) to 2.3% RSD. The conformity of the method with the reference material reached three different concentration levels in the range of 98.2 - 104.2 %.

CONCLUSIONS

Immunomethods commonly used for steroid determination are not available for dexamethasone. In addition, the other steroids interfere during immunoassays and often do not have the required assay sensitivity. Using the 2D-LC/MS/MS method, we are able to determine dexamethasone at a sufficiently low level to verify its cut-off value (~ 3.3 nmol/L). We are currently using this method to determine the entire steroid spectrum along with DXM, which is used to refine the dexamethasone suppression test.

Analytical technologies and applications

M055

INTERFERENCE OF NATURAL METABOLITES AND DRUGS IN ENZYMATIC DETERMINATION OF CREATININE AND URIC ACID

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BACKGROUND-AIM

Determination of substrates by enzymatic methods often terminates with production of hydrogen peroxide and its detection in Trinder reaction, catalyzed by peroxidase. Interferences by reducing agents present in blood plasma, leading to falsely lower results have been repeatedly reported. The aim of the study was to assess the influence of common metabolites and drugs on determination of creatinine and uric acid; these analytes occur at the lowest concentration from commonly measured substrates and are therefore most susceptible to interference.

METHODS

Creatinine and uric acid were determined in pooled plasma samples by enzymatic methods (UA2 a CREP2, Roche, on Cobas c702 analyzer, Roche) ending with a Trinder reaction. The effect of potentially interfering substances, both natural metabolites and drugs in real concentrations, has been investigated, both in plasma and by mixing with a blood sample when extracted from an intravenous cannula. For creatinine determination the influence of the interfering substance at different analyte concentrations was assessed on the metamizole interference model.

RESULTS

The largest negative biases were provided by the following drugs: dobesilate, etamsylate, N-acetylcysteine and metamizole. Bilirubin interference has been demonstrated but was clinically insignificant. Ascorbate oxidase activity present in the reagent was sufficient to eliminate interference of physiological concentrations of ascorbic acid, but the result was already affected with high dose therapy. Adrenaline, noradrenaline and dobutamine are administered diluted as infusions, therefore their effect was low or even negligible. Lactate and 3-hydroxybutyrate did not affect Trinder reaction.

CONCLUSIONS

Influencing the enzymatic determination of creatinine and uric acid by certain metabolites and drugs should be considered even at their commonly occurring concentrations.
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Analytical technologies and applications

M056

MULTICENTER PERFORMANCE EVALUATION OF THE NEW ELECSYS VITAMIN D TOTAL III ASSAY

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BACKGROUND-AIM

The performance of the Elecsys® Vitamin D total III assay (Roche Diagnostics International Ltd), including intermediate precision and repeatability, was characterised by conducting method comparisons versus commercially available comparators, examining serum and plasma matrices and calculating diagnostic accuracy versus reference LC/MS-MS values.

METHODS

The Elecsys Vitamin D total III assay (cobas e 601 analyser) was examined under routine conditions at three laboratories (Heidelberg, Germany; Habach, Germany; Baltimore, Maryland, USA). Intermediate precision and repeatability were calculated using five human serum pools (HSP1–5) and two PreciControl materials (PC1 and PC2; 5-day model, one reagent lot in accordance with CLSI-EP05-A3 criteria) and compared against prespecified acceptance criteria. Method comparisons were conducted using a serum sample verification panel with predefined, reference LC/MS-MS values (Centers for Disease Control and Prevention; CDC). Characterisation of the Elecsys Vitamin D total III assay was performed at all three sites. Comparator assay studies were performed at Heidelberg and Baltimore. Between-method differences were assessed using unweighted Deming regression. A separate serum/plasma comparison analysis with the Elecsys Vitamin D total III assay was conducted at a single site (St. Louis, Missouri, USA), using samples from apparently healthy adults and assessed using Passing-Bablok regression (PBR).

RESULTS

Repeatability (HSP1 [16.8–18.4 ng/mL], SD 0.87–1.07; HSP5 [94.5–98.0 ng/mL], CV 1.58–2.76%) and intermediate precision (HSP1, SD 1.14–1.77; HSP5, CV 2.00–4.13%) met the prespecified acceptance criteria across all three sites. Good agreement was observed between the Elecsys Vitamin D total III assay and the comparator assays (Pearson's r, 0.958–0.982; slope, 0.921–1.15; intercept, -5.26–0.907) and also between the Elecsys Vitamin D total III assay and the CDC verification panel target values (Pearson's r, 0.960–0.986) across sites, and in serum and plasma samples (n=462; Pearson's r, 0.972; PBR, y=0.103+0.984x).

CONCLUSIONS

The Elecsys Vitamin D total III assay demonstrated good analytical performance and compared favorably with other commercially available assays, supporting its use as an aid in determining vitamin D sufficiency.

Analytical technologies and applications

M057

INTERCHANGEABILITY STUDY OF THE RESULTS BETWEEN A BECKMAN COULTER® AU5800 CHEMISTRY ANALYSER AND A GEM PREMIER® 5000 ANALYSER.

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BACKGROUND-AIM

The existence in clinical laboratories of different measurement procedures for the same magnitude makes the comparability of the results between them necessary for monitoring patients during their clinical process. The aim of this study was to verify the interchangeably of the results between a Beckman Coulter® AU5800 analyser and a GEM Premier® 5000 point of care system (POCT).

METHODS

The study was performed in 63 serum samples from patients whom glucose, potassium, sodium and chloride had been requested. All the sera were free of endogenous interferences (haemoglobin, bilirubin and triglycerides). They were processed with a maximum difference of 2 hours by both analysers during ten days. Quantities of magnitudes covered the medical decision limits. The results were analysed using the Passing-Bablok regression, considering the POCT as the measurement procedure under study. The statistical analysis was performed by MedCalc® (version.20.010).

RESULTS

The Passing-Bablok regression showed:
Glucose y= -8.09+0.98x, y-intercept confidence interval (CI) at 95% (-10.00 to -5.09) and slope CI 95% (0.95 to 1.00).
Sodium y= 11.21+0.93x, y-intercept CI 95% (2.00 to 29.40) and slope CI95% (0.80 to 1.00).
Chloride y= 4.00+1.00x, y-intercept CI 95% (4.00 to 17.13) and slope CI95% (0.88 to 1.00).
Potassium y= 0.00+1.00x, y-intercept CI 95% (-0.31 to 0.00) and slope CI95% (1.00 to 1.07).

CONCLUSIONS

The regression study found that there were no constant and proportional systematic differences observed in the measurement of potassium. However, constant systematic differences were observed in glucose, sodium and chloride between both measurement procedures.
In conclusion, POCT results for the measurement of glucose, sodium and chloride were not interchangeably with the chemistry analyser; therefore patients should be always monitoring by the same assay. The results obtained for potassium in both systems were interchangeable, so they could be considered as single virtual equipment.

Analytical technologies and applications

M058

COMPARISON OF RED BLOOD CELLS PARAMETERS IN SYSMEX XN-2000, YUMIZEN H2500 AND BECKMAN COULTER DXH 900.

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BACKGROUND-AIM

The complete blood count is a group of tests that provide information about the cells that circulate in blood, including red blood cells (RBC), white blood cells, and platelets. RBC are flexible and oval biconcave disks which are responsible for delivering oxygen to the body tissues. They inform about the patient condition in relation to the number or the quality of these cells. Numerous automated analyzers are used in clinical laboratories to perform complete blood cell analysis. The aim of this study is to compare some of the RBC parameters in Sysmex XN20, Yumizen H2500 and Beckman Coulter DxH 900 analyzers to know if there are significant differences between them.

METHODS

A total of 674 samples were processed in the were processed in the in Sysmex XN20, Yumizen H2500 and Beckman Coulter DxH 900 analyzers following the ISCH recommendations. Passing-Bablok(PB) regression method and Bland-Altman (BA) analysis were performed for the comparisons. Spearman's test was used to obtain the correlation coefficient.

RESULTS

We compared the results obtained from the 3 analyzers in the following parameters: erythrocytes, hemoglobin (Hb), mean corpuscular volume (MCV) and Red Blood Cell Distribution Width (RDW). We observed a high correlation in all the parameters studied.
Erythrocyte and Hb showed the best correlation results among the 3 analyzers. (Rho spearman of 0,99 and 0,98 respectively).
BA studies of MCV showed consistently lower values in Beckman, followed by Sysmex and Horiba values. (Mean= 89;90;91fl, respectively).
BA study of RDW presented consistently lower values in Horiba followed by Beckman and Sysmex. (Mean=14,30; 14,50; 14,68%, respectively)
PB regression showed 95% confidence interval (CI) for intercept did not include value 0 and the 95% CI for slope did not include value 1 in the comparative results for all the parameters, therefore, the three analyzers were not interchangeable.

CONCLUSIONS

New analytical analyzers must be verified by the Clinical Laboratory to ensure that there are no major differences when changing methodology.
Red Blood Cells parameters of Sysmex, Horiba and Beckman equipment have shown that the methods are not interchangeable using a PB regression. However, it does not interfere with the clinical decisions.

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Analytical technologies and applications

M059

MEASURING GLYCOSYLATED FERRITIN WITH CONCANAVALIN-A: ASSAY DESIGN AND VALIDATION

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BACKGROUND-AIM

Ferritin is the major iron-storage glycoprotein. Ferritin glycosylation can be assessed by the differential affinities of ferritin glycoforms for Concanavalin A (ConA) as described by Worwood et al. The fraction of serum ferritin bound to ConA is called "glycosylated ferritin" (GF). Low GF reflects macrophagic activation and is a biomarker used in adult-onset Still's disease (AOSD), macrophage activation syndrome (MAS), and Gaucher disease diagnosis and therapeutic management. More recently, it has also been proposed as a prognostic marker in COVID-19. We aimed to become a reference laboratory for GF measurement in AOSD, MAS, and Gaucher, which must be accredited according to the ISO 15189 standard. Here, we describe and validate the method used in our laboratory to determine GF.

METHODS

Ferritin was determined by a Siemens immunoassay on Dimension Vista 1500® analyzer after separation of ferritin glycoforms according to their affinity for ConA. Patient sera or quality controls were used. Our laboratory's database, comprising 16843 GF measured from 2000-2021, was analyzed to describe GF values.

RESULTS

A 15 min incubation time in TRIS-barbital buffer pH 8 of serum with 0.4 or 0.5 mL ConA-Sepharose in a final volume of 1 mL allowed the optimal separation of ferritin glycoforms. The ferritin assay's performance in TRIS-barbital buffer was excellent (CV intraassay<1%, interassay<4%, recovery 78.8 to 115.5% from 3.2 to 407 µg/L). GF assay after separation of glycoforms with ConA-Sepharose evidenced good reproducibility for most samples and tolerable imprecision for strongly pathological samples. Inter-operator CVs ranged from 6.2% to 21.6%. The inter-laboratory comparison survey did not report any discordant clinical interpretation according to the 20% AOSD decision level; in our laboratory's database, <20% GF levels were scarce, compatible with the low incidences of AOSD, MAS, and Gaucher disease. In most patients, GF levels ranged between 26 and 58%, slightly lower than values described in the literature for healthy individuals.

CONCLUSIONS

Using only easy-to-procure, commercially available reagents, GF assay was fast and straightforward to perform, offering good performance for laboratories servicing clinicians treating AOSD, SAM, Gaucher disease, and COVID-19 patients.

Analytical technologies and applications

M060

ANALYSIS OF VOLATILE ORGANIC COMPOUNDS (VOCS) IN THE BREATH OF COLORECTAL CANCER (CRC) SUBJECTS BY CYRANOSE 'ELECTRONIC NOSE'.

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BACKGROUND-AIM

The analysis of volatile organic compounds (VOCs) represents an interesting field of analysis to characterize many diseases. The studies performed in the last ten years are showing the existence of a real 'fingerprint' of VOCs as gas component in biological district. A footprint destined to vary in composition and concentration in the course of transmissible and non-transmissible diseases.
Although the number of compounds find in the different districts and the complexity of the pre-analytical variables make difficult to standardize tests and sampling, the VOC analysis on urinary and faecal samples is already in the application phase to investigate colorectal cancer (CRC) and pilot screening programmes were started.
The purpose of the study performed at Careggi University Hospital (Ethics Committee no. 16770-20) is the analysis and the characterization of the VOCs in breath samples able to discriminate subjects with different risk to develop CRC.
This report presents the preliminary information about the training of Cyranose 'electronic nose' on the expired material to discriminate normal and hill subjects to obtain an easy and fast clinical assessment.

METHODS

The breath of 13 subjects with diagnosis of CRC and 9 subjects negative to the fecal occult blood test, aged 55-70 years, was collected in Teflon bags (Cyranose AS Kit, Sensigent USA) and analysed by Cyranose (Sensigent, US), an electronic system based on specific algorithm investigating the response supported by 32 sensors.
To train the electronic system, the two groups were classified according to clinical information as negative subjects with FIT-Hb test and positive subjects with CRC diagnosis.

RESULTS

Training of the electronic nose with this group of subjects obtain a discrimination capacity of 90% and an Euclidean distance of 5 between healthy and ill subjects.

CONCLUSIONS

Used electronic nose is an analitycal sistem easy to use, able to support fast informatiosn on volatile compounds in biological matrix. Although not able to characterise specific chemical compounds represents an useful solution to discriminate class of chemicals and many clinical application can be supposed for this techniques.
Collected data confirms the existence of a digital “fingerprint” of the VOCs in breath of CRC patients. The small number of samples available still does not allow the achievement of the specific requirements (discrimination capacity 95% and Euclidean distance> 5) indicate by manufacturer for the use of instruments in clinical setting .

Analytical technologies and applications

M061

ANALYSIS BY GC-MS OF VOLATILE ORGANIC COMPOUNDS IN BREATH OF PATIENTS WITH COLORECTAL CANCER (CRC).

S. Rapi [1], S. Dugheri [4], G. Cappelli [2], N. Mucci [2], L. Trevisani [2], A. Bonari [1], F. Staderini [3], G. Arcangeli [2], F. Cianchi [3], a. Fanelli [1]

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BACKGROUND-AIM

The analysis of Volatile Organic Compounds (VOCs) represents an interesting application for the characterization of many pathologies. Studies report the presence of VOCs 'fingerprint', linked to communicable and non-communicable diseases, characterized by composition and concentration of volatile compounds in different biological matrices. High number of compounds and the pre-analytical variables make difficult to perform and to standardize this kind of VOCs analysis on different biological samples (urine, stool and breath); however, they are now in the application phase to use in colorectal cancer screening (CRC). The purpose of the study, carried out by AOU Careggi of Florence (Ethics Committee no. 16770) is to investigate VOCs in the breath to discriminate subjects with increased risk for CRC. This report contains information on the VOCs characterizing subjects affected by CRC.

METHODS

The breath of 13 subjects (age 55-70) suffering from CRC (P) and 9 subjects negative for the occult blood test (N) was collected in a breath sampling bag and analysed by gas chromatography-mass spectrometry (GC-MS Shimadzu GC2010. Japan) with a 5% phenyl separative column to investigate differences in VOCs compositions. The peaks resulting from the analysis were integrated by instrumental software to quantify their variation between studied groups.

RESULTS

Three volatile compounds, with retention times of 12.4, 16.4, 16.8 minutes respectively, report the main differences between the two groups. The mass spectra of the investigated compounds are compatible with 4 Me-Octane (C8), 1 I-Nonane (C9) and 2.2 Me-decane (C10). The average expression of the three compounds in the 2 groups are: C8-N = 120.94 ± 85.39-C8-P = 142.76 ± 86.02 (n.s.); C9- N = 3371.62 ± 1468.65-C9-P = 16060.28 ± 846.20 ($p < 0.01$); C10-N = 4186.38 ± 1739.53-C10-P = 2016.63 ± 1136.28 ($p < 0.01$).

CONCLUSIONS

Collected data confirm the presence of a VOCs fingerprint in subjects affected by CRC. A significant difference in the mean expression of C9 and C10 in subjects affected by CRC was previously reported in literature and confirmed from the present data. Instead, in our group the difference in C8 compound do not result significant for the high variance due to the small number of samples.

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Analytical technologies and applications

M062

EVALUATION OF A NEW BENCHTOP CLINICAL CHEMISTRY ANALYSER INCORPORATING NOVEL LED PHOTOMETRIC CARTRIDGES

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BACKGROUND-AIM

The ELITechGroup Selectra Mach5® is a fully automated random access benchtop clinical chemistry system, for medium sized laboratories or to provide capacity for special tests.
The Mach5 incorporates an innovative optical system, which is a fully enclosed photometric cartridge (PhC). Each PhC is comprised of a wavelength specific LED, glass optical filter, beam splitter, reference detector, detector, and programmable Printed Circuit Board.
The aim of this study was to compare the analytical performance of the Mach5 incorporating the new PhC (12 used) with a benchtop clinical chemistry system utilising a conventional optical system, typically Halogen or Tungsten Lamp combined with a series of optical glass filters.

METHODS

The assay capability of the Mach5 and reference system were assessed using Sigma metrics, calculated from patient comparison studies (bias) and precision data, obtained using approved CLSI protocols EP5 and EP9. Data for a total of 21 routine clinical chemistry parameters, using various wavelengths and assay modes was collected and analysed. Sigma metrics for each analyte were calculated and assessed against the CLIA 2019 Routine Chemistry Performance Specifications (allowable total analytical error, TEa), except for DBili, where the RCPA allowable limit of performance was used.

RESULTS

On the Mach5 13 of the 21 (62%) routine assays achieved a sigma metric of ≥5 (ALT, ALP, DBili, Iron, LDH, Mg, Phos, Urea, TBili, Ca, CK, Creat & GGT), with the last 5 achieving a sigma value of ≥6, compared with the reference system where 9 of the 21 assays (42%) achieved a sigma metric ≥5 (TP, Gluc, LDH, Trig, Creat, Ca, TBili, ALP & CK), the last 6 with a sigma value of ≥6.
7 assays returned a sigma metric value of between 3 and <5 (Amy, AST, Chol, Gluc, TP, Trig & UA), compared with 6 for the reference system (AST, ALT, ALB, DBili, Urea & UA). 1 Mach5 assay (Alb) had a sigma metric of 2, compared with the reference system, where 6 assays recovered a sigma metric ≤2 (Chol, Amy, Iron, GGT, Mg & Phos).

CONCLUSIONS

This study has shown that the Mach5, with its uniquely designed photometric cartridge-based optical system, is very capable of performing to the required standards of a routine diagnostic testing laboratory.

Analytical technologies and applications

M063

COMPARISON OF THREE DIFFERENT METHODS FOR THE QUANTIFICATION OF SERUM AND PLASMA N-TERMINAL PROPEPTIDE OF TYPE III PROCOLLAGEN (PIIINP)

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BACKGROUND-AIM

The amino terminal propeptide of type III procollagen (PIIINP) is a promising biomarker for sarcopenia and an indicator of liver fibrosis and cirrhosis. The first available method for its quantification was a radioimmunoassay (RIA) produced by Orion but non-radioactive methods are now available. No comparison of these methods has so far been performed.

METHODS

We compared the competitive Orion (Aydan Oy) RIA UniQ PIIINP assay with 2 non-competitive methods, Siemens ADVIA Centaur and Cisbio ELISA to evaluate the harmonization of the assays. We also evaluated the commutability of the assays on EDTA plasma and serum. Serum and EDTA plasma samples from sarcopenic patients were collected (n=25), between May and June 2021. Both EDTA plasma and serum PIIINP concentrations were measured with the 3 methods. Bland-Altman plots, Passing Bablok regressions and correlation analyses were used to compare the results (μg/L).

RESULTS

There was a poor agreement between RIA and other methods: Centaur = 3,33 [95%CI: 2,33; 4,41] × RIA – 6,44 [95%CI: -13,55 to -4,47] in serum (r= 0,20) and Centaur = 3,89 [3,36; 5,05] × RIA – 7,31 [-11,80; -4,85] in plasma (r= 0,20). ELISA = 4,36 [2,63; 10,70] × RIA - 12,36 [-43,46; -4,40] in serum (r= 0,15) and ELISA = 4,30 [2,92; 6,68] × RIA – 8,18 [-16,50; -3,48] in plasma (r= 0,17). A much better concordance was observed between Centaur and ELISA : Centaur = 0.82 [0,48; 1,40] × ELISA + 1.76 [-3,15; 4,74] in serum (r= 0,62) and Centaur = 1,00 [0,59; 1,44] × ELISA + 0.23 [-3,21; 2,59] in plasma (r= 0,69). Finally, the regression equations between serum and plasma were Centaur (plasma) = 0,88 [0,80; 0,96] × Centaur(serum) – 0,63 [-1,59; -0,006] (r= 0,88) and ELISA (plasma) = 0,83 [0,65; 1,01] × ELISA (serum) + 0,15 [-2,09; 2,04] (r= 0,88).

CONCLUSIONS

Orion (Aidan Oy) UniQ PIIINP RIA assay presented significantly discrepant results compared to Siemens ADVIA Centaur and Cisbio ELISA. The reason could be linked to a lack of specificity of the RIA which probably cross-reacts with pro-PIIINP. ADVIA Centaur and ELISA methods are correlated and suitable for the PIIINP detection. However, serum and plasma present significantly different results with ADVIA Centaur and serum should be preferred.

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Analytical technologies and applications

M064

HIGHLY FLUORESCENT 360 NM EXCITABLE EUROPIUM(III) LABEL FOR TIME-RESOLVED-FLUORESCENCE IMMUNOASSAYS

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BACKGROUND-AIM

In many clinical applications, especially with point-of-care testing, smaller analysis setups and better analytical sensitivity are often desired. Time-resolved-fluorescence (TRF) technology with lanthanide and especially Eu3+ chelate labels have been successfully used as a platform for many clinical tests that require high analytical sensitivity. The clear drawback of TRF equipment is the need to use large and expensive equipment with high intensity excitation source like xenon lamp or lasers. To reduce equipment cost and to enable smaller TRF equipment, it would be desirable to use high powered light emitting diodes (LED) as an excitation source. Commonly available high power ultraviolet (UV) LEDs have an emission wavelength of 360-365 nm or higher, whereas commonly used lanthanide labels have an optimal excitation wavelength below 340 nm. We now report a new Eu3+ chelate with excellent analytical properties and proven good analytical performance in cardiac troponin I (cTnI) assay.

METHODS

The novel label based on 2-(pyridin-4-ylethynyl)pyrazine chromophore was synthesized, conjugated to glycine, purified by HPLC and characterized for luminescence properties. It was also tested as an antibody conjugated label in a clinical cTnI immunoassay and compared against a previously reported reference label 9d-α-galactose Eu(III) which has seen wide use in clinical applications.

RESULTS

The new label was excitable at 360 nm with an excitation maximum at 361 nm. The new label also exhibited very good fluorescence decay profile with decay time at 20 degrees Celsius being 1,01 ms. The decay profile of the new label also exhibited higher temperature dependence as compared to the reference chelate when measured in buffer solution at 40 and 60 degrees Celsius. The new label showed enhanced analytical sensitivity when tested in cTnI assay, both at 360 nm and 340 nm. At 360 nm with the new chelate over 12-fold improvement could be achieved as compared to the reference chelate (6,2 pg/ml vs. 77 pg/ml).

CONCLUSIONS

The developed chelate has potential to enable development of simple and small LED-based TRF measuring equipment for rapid and highly sensitive analysis of cTnI and other immunoassay-relying analytes.

Analytical technologies and applications

M065

A COMPARISON BETWEEN THE EXENT® MASS SPECTROMETRY ASSAYS AND STANDARD SEROLOGICAL TECHNIQUES FOR THE FOLLOW-UP OF A PATIENT WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE WHO DEVELOPED MULTIPLE MONOCLONAL PROTEINS

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BACKGROUND-AIM

Monoclonal gammopathy of undetermined significance (MGUS) has an annual risk of progression to multiple myeloma of 0.5-1.0% per year, so patients require long-term monitoring. We describe the results from a patient who initially presented with IgA kappa MGUS, which evolved into multiclonal MGUS. Sera from this patient were retrospectively analysed with the EXENT assays to investigate if mass spectrometry (MS) could detect the additional paraproteins earlier.

METHODS

EXENT (The Binding Site Group Ltd.) is the commercial name for the technology previously described as QIP-MS. MS was performed by: incubating diluted serum with antisera specific for IgG/A/M, total kappa, total lambda, free kappa and free lambda conjugated to paramagnetic beads; reducing and eluting purified immunoglobulins; spotting eluates onto MALDI target plates and acquiring by MS. Serum free light chains (sFLC) were measured using Freelite™ assays (The Binding Site, UK) on a Cobas c501 turbidimeter (Roche, Switzerland). Capillary zone electrophoresis (CZE) and immunofixation (IFE) were performed on CAPILLARYS 2 and HYDRAYS 2 SCAN instruments (Sebia, France).

RESULTS

In samples from 2007 and 2008, immunofixation identified a single IgA kappa paraprotein. This was detectable by MS at m/z 11657 along with additional abnormalities at m/z 11765 in IgG kappa, two further in IgA at m/z 11931 and 12037 and monoclonal kappa FLC at m/z 11931 for the light chains in their 2+ charge state. These abnormalities persisted in MS spectra and by 2014 an IgG kappa monoclonal protein and a second small IgA band were also detectable by IFE. All 4 paraproteins remained detectable by EXENT; m/z values were consistent across all follow-up samples within +/- m/z 3. sFLC levels increased as renal function deteriorated but the sFLC ratio remained within the renal reference range during follow-up.

CONCLUSIONS

EXENT detected the evolving paraproteins earlier than standard electrophoretic techniques. In addition, monoclonal kappa FLC were detectable by MS that were not detectable by turbidimetry. The ability to track monoclonal proteins using their unique m/z enables unequivocal identification and tracking of low-level monoclonal proteins and differentiation of emerging monoclonal FLC from an increasing polyclonal FLC background.

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Analytical technologies and applications

M066

THE OPPORTUNITIES OF PAP-TECHNOLOGY OF CERVICAL SMEARS IN THE DIAGNOSTICS OF HORMONAL STATUS (PILOT STUDY)

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BACKGROUND-AIM

Recent data suggest a relation between hormonal status and the development of cervical dysplasia by expression of estrogen/progesterone receptors of cervical epithelium in women of reproductive age. The aim of our study was to reveal acceptable opportunities in the diagnostics of hormonal status by PAP-technology.

METHODS

Cervical smears of 10 691 patients of reproductive age (17-40 years) have been studied by PAP-technology. Smears were collected in Gagua Clinic (Tbilisi, Georgia) during 2017-2021. Estrogen ensures that the uppermost layers of the epithelium are mature. They are consisting of large polygonal flat, eosinophilic cells with a pyknotic nucleus. Progesterone impedes the normal maturation of the squamous epithelium resulting in a predominance of intermediate cells on the smear.

RESULTS

Monohormonal changes were observed in 2446 cases (22.87%). The prolonged effect of Progesterone was expressed in 939 cases (38.38%). Among of them cervical dysplasia was observed in 42 cases (1.72%), atypical squamous cells of undetermined significance (ASC-US) – in 24 cases (0.98%), atypical squamous cells - cannot exclude high-grade squamous intraepithelial lesion - ASC-H – in 2 cases (0.8%). Hypoestrogenic background inconsistent with the age norm was expressed in 153 cases (6.25%). Among of them cervical dysplasia was observed in 28 cases (1.14%), ASC-US – 56 cases (2.29%), ASC-H – 4 cases (1.6%).

CONCLUSIONS

PAP-technology has potential to give two important information within one study. It can be considered as a cheap and affordable alternative for determining the local hormonal status, which improves the prediction and management of the cervical precancerous conditions; at the same time obtained information is valuable in the context of reproductive health.

Analytical technologies and applications

M067

CYTOLOGY OF BUCCAL SMEARS VS. COLPOCYTOLOGY IN THE DIAGNOSTICS OF HORMONAL STATUS DURING PREGNANCY (PILOT STUDY)

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BACKGROUND-AIM

Recent studies showed that buccal smears carried out the information about hormonal status in females. The aim of our study was to reveal new opportunities in the diagnostics of this condition during pregnancy using cytological study of buccal smears.

METHODS

Buccal and vaginal smears of 1575 pregnant patients (age range - 17-40 years; gestational age – 1-12 weeks) were collected in Gagua Clinic (Tbilisi, Georgia) during 2017-2021 and have been studied cytologically. Unlike obtaining the vaginal smears, buccal smear was taken with a brush, was placed in saline solution, centrifuged at 1500 rpm for 5 minutes, the saline solution was removed, the precipitate was deposited and dissolved on the subject glass by a glass beaker, dried at room temperature in a horizontal position, and stained with Methylene Blue like as in the case of vaginal smears. It was determined the amount of superficial, intermediate, parabasal and basal cells, maturation index, and assessed the marked cytolysis related to the abundance of lactobacilli. The hormonal status was defined by complex reconciling the data obtained.

RESULTS

A normal cytological picture of buccal smears was shown in 1245 patients (79.0%). Hormonal changes were observed in 330 cases (20.9%). False positive results (buccal vs. vaginal smears) were found in 29 cases, and false negative – in 25 cases. The sensitivity was 90.8% (95%CI - 87.1%-93.7%), specificity - 97.3% (95%CI - 96.2%-98.1%), and diagnostic accuracy - 95.9% (95%CI - 94.8%-96.9%). Among of the hormonal changes estrogen deficiency was expressed in 133 cases (8.4%). The progesterone deficiency was observed in 197 cases (12.5%).

CONCLUSIONS

1) Cytology results of buccal smears did not significantly differ from vaginal smears. 2) The expression of hormonal changes by the cytological study of buccal smears will be useful to make the precise diagnosis, to choose the adequate management. 3) This technique is comfortable, safe and acceptable for patients. These findings require the further investigations to make evidence-based conclusions.

Analytical technologies and applications

M068

DETERMINATION OF METHYLMALONIC ACID IN SERUM BY LC-MSMS WITHOUT DERIVATISATION STEP

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BACKGROUND-AIM

Methylmalonic acid (MMA) is a biochemical marker for functional vitamin B12 deficiency. MMA is a small and very polar dicarboxylic acid. The major challenge for MMA analysis in biological fluids is its separation from succinic acid (SA), which is derived from the citric acid cycle and usually present in much higher amounts as compared to MMA. Interference with SA is difficult to overcome because the chromatographic characteristics of SA are very similar to those of MMA and both share the same relative molecular mass. Thus most LC-MS/MS methods for determination of MMA require a complicated and time consuming derivatization step to achieve separation of MMA and SA.

METHODS

We developed a simple LC-MS/MS method for quantitative determination of MMA in human serum. For sample preparation were used Vivaspin 500 - centrifugal concentrators with a molecular cut-off of 10kD. Ultrafiltrate was applied directly to chromatography. Separation was achieved on Luna Omega C18 column by gradient elution using 0.1% (v/v) formic acid in water (mobile phase A) and 0.5% (v/v) formic acid in acetonitrile (mobile phase B) with run time of 4.5 min. Electrospray ionization and multiple reaction monitoring (116.9 -> 55.0 mz) was used.

RESULTS

Detection limit was 13,6 nmol/. Lower limit of quantification (LLOQ) was 40 nmol/l and quantitation was linear up to 4200 nmol/l. Intra- and interassay precision ranges from 2.2 – 6.8 % and 6.4 – 8.5%, respectively. Intraassay accuracy was 88.8 – 100.0% and interassay accuracy was 92.2 – 102.5%.

CONCLUSIONS

Sample preparation by ultrafiltration proved feasible as MMA is not protein bound. Use of the protein free ultrafiltrate avoided protein precipitation by acids (which degrades MMA) or organic solvents (which degrades chromatographic separation). Renouncement of derivatization makes this method more versatile and beneficial for smaller sample series.

Analytical technologies and applications

M069

IMMUNOASSAY EQUIVALENCY BETWEEN THE ATELLICA® CI 1900* AND ATELLICA® IM 1600 ANALYZERS

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BACKGROUND-AIM

The Atellica® CI 1900 Analyzer* is an integrated chemistry and immunoassay analyzer employing both Atellica® CH and Atellica® IM assays. This investigation was designed to evaluate the analytical performance of the Atellica IM Alpha Fetoprotein (AFP), Hepatitis B Core IgM (aHBcM), High-Sensitivity Troponin I (TnIH), Total Human Chorionic Gonadotropin (ThCG), and Thyroid Stimulating Hormone 3-Ultra™ (TSH3UL) assays on the Atellica CI 1900 Analyzer.

METHODS

Precision and method comparison were used as performance indicators for the Atellica CI 1900 Analyzer.

RESULTS

The Atellica IM AFP assay's within-lab precision ranged from 2.4 to 7.1% CV. AFP method comparison (n = 44) for the Atellica CI 1900 Analyzer versus the Atellica IM 1600 Analyzer using weighted Deming regression yielded a slope of 1.05, y-intercept of −1.07 ng/mL, and r of 1.00. The Atellica IM aHBcM assay's within-lab precision ranged from 2.9 to 6.8% CV. aHBcM method comparison (n = 45) for the Atellica CI 1900 Analyzer versus the Atellica IM 1600 Analyzer yielded 100% positive agreement and 100% negative agreement. The Atellica IM TNIH assay's within-lab precision ranged from 1.5 to 3.5% CV. TNIH method comparison (n = 50) for the Atellica CI 1900 Analyzer versus the Atellica IM 1600 Analyzer using weighted Deming regression yielded a slope of 0.96, y-intercept of 0.6 pg/mL, and r of 1.00. The Atellica IM ThCG assay's within-lab precision ranged from 1.7 to 4.0% CV. ThCG method comparison (n = 50) for the Atellica CI 1900 Analyzer versus the Atellica IM 1600 Analyzer using Deming regression yielded a slope of 1.02, y-intercept of −5.54 mIU/mL, and r of 0.99. The Atellica IM TSH3UL assay's within-lab precision ranged from 2.7 to 4.8% CV. TSH3UL method comparison (n = 51) for the Atellica CI 1900 Analyzer versus the Atellica IM 1600 Analyzer using Deming regression yielded a slope of 1.03, y-intercept of −0.007 uIU/mL, and r of 1.00.

CONCLUSIONS

When evaluated on the Atellica CI 1900 Analyzer, the AFP, aHBcM, TNIH, ThCG, and TSH3UL assays demonstrated performance equivalent to that of the same assays on the Atellica IM 1600 Analyzer. These assays demonstrated acceptable precision and method comparison.
*Product under development. Not available for sale. Future availability cannot be guaranteed.

Analytical technologies and applications

M070

A NOVEL UPLC-MS/MS METHOD FOR PLASMA ANTIBIOTICS FOR CLINICAL RESEARCH

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BACKGROUND-AIM

A clinical research method for a large panel of antibiotic drugs in plasma was developed; azithromycin (5-500 ng/mL); ciprofloxacin and clindamycin (0.1-10 µg/mL); ampicillin, cefotaxime, chloramphenicol and linezolid (0.5-50 µg/mL); cefazolin, cefepime, ceftazidime, cefuroxime, flucloxacillin, meropenem and sulbactam (1-100 µg/mL); daptomycin and piperacillin (2-200 µg/mL).

METHODS

Matrix matched calibrators and QCs were prepared using in-house stocks and pooled plasma. Samples (50 µL) were treated with internal standard in methanol. A water/methanol/ammonia gradient was used with a Waters ACQUITY UPLC BEH C18 2.1 x 1.7 µm, 100mm column on a Waters ACQUITY UPLC I-Class FTN and Xevo TQD mass spectrometer utilizing polarity switching in a 5-minute run.

RESULTS

No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators. Analytical sensitivity investigations indicated precise quantification (≤20% CV, ≤15% bias) at concentrations equal to or lower than the lowest concentration calibrator. Total precision and repeatability were assessed (3 pools, 5 replicates, 5 days; n=25) and determined to be ≤12.5% RSD. Linearity experiments determined the method provided first or second order polynomial fits over the ranges analyzed; additionally, each run met acceptance criteria (coefficient of correlation ≥ 0.995, determined concentrations of calibrators ±15% of nominal, ±20% in the case of the lowest calibrator). Post-column infusion experiments demonstrated analytes eluted in regions free of major ion suppression or enhancement. Evaluation of matrix effects at low and high concentrations indicated compensation by the internal standard. Addition of high concentrations of several endogenous and exogenous materials did not affect quantification.

CONCLUSIONS

This quantitative method for clinical research demonstrates very good precision with minimal matrix effects and allows for the multiplexing of a large panel of antibiotics in plasma in a short run time.
For Research Use Only. Not for use in diagnostic procedures.

Analytical technologies and applications

M071

CASCADIONTM SM CLINICAL ANALYZER: HARMONIZATION OF THE IMMUNOSUPPRESSIVE DRUGS AND 25-OH-VITAMIN D LC-MS/MS ASSAYS: A STEP FORWARD IN CLINICAL MONITORING

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BACKGROUND-AIM

The Thermo Scientific™ Cascadion™ SM Clinical Analyzer is an automated system designed as a fully integrated solution for routine and specialized clinical testing, applying the specificity of mass spectrometry to the automated clinical laboratory. As a recent development, there are initial data on the field performance of this equipment.
We aim to assess a between-analyser comparison of Cascadion systems in two clinical laboratories of high complexity hospitals, as well as to study their robustness and performance in a clinical laboratory setting.

METHODS

EDTA-whole blood (immunosuppressants) and serum (vitamin D) samples were collected in both clinical laboratories and divided into two aliquots, one for the testing in the same centre and the second transported on dry ice to the other laboratory for the duplicate testing.
Far-out values were discarded by Tukey test. Data was analysed using Passing-Bablok regression analysis and Pearson r correlation.

RESULTS

Cascadion Immunosuppressants TDM Assay (ISD), ng/mL
Tacrolimus (n=70): r>0.99 (95% confidence interval (CI):0.9836–0.9937); slope=1.06 (95%CI: 1.01–1.08), intercept=-0.06 (95%CI: -0.27–0.19).
Ciclosporin A (n=55): r>0.99 (95%CI: 0.9780–0.9925); slope=1.05 (95%CI: 1.03–1.07), intercept=0.29 (95%CI: -2.28–2.37).
Sirolimus (n=39): r>0.94 (95%CI: 0.8932–0.9699); slope=0.94 (95%CI: 0.86–1.04), intercept=0.03 (IC95%: -0.36–0.46).
Everolimus (n=38): r>0.99 (95%CI: 0.9829–0.9954); slope=0.95 (IC95%: 0.90–0.99), intercept=0.18 (95%CI: -0.08–0.45).
Cascadion 25-Hydroxy Vitamin D Assay, ng/mL
25-OH-Vit D total (n=79): r>0.99 (95%CI: 0.9929–0.9971); slope=1.04 (95%CI: -3.65–3.65), intercept=0.53 (95%CI: 0.01–1.66).

CONCLUSIONS

The correlation study for both the ISD and the Vitamin D panels demonstrated excellent concordance between laboratories (r>0.94) that was maintained over the time. There was no constant or proportional bias.
We conclude that both Cascadion analyzers are fully standardized and harmonized, offering consistency and comparability of results. This facilitates the accurate patient diagnosis and treatment, regardless of where testing takes place.

Analytical technologies and applications

M072

ANALYTICAL PRECISION PERFORMANCE EVALUATION OF SELECTED IMMUNOASSAYS ON THE IMMUNOCHEMISTRY ANALYTICAL UNIT OF COBAS PURE INTEGRATED SOLUTIONS

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BACKGROUND-AIM

cobas® pure integrated solutions (Roche Diagnostics International Ltd) is a serum work area laboratory analyser consolidating clinical chemistry/ion selective electrolyte (cobas c 303) and immunochemistry (cobas e 402) testing in plasma, serum and urine on a single, compact platform. We conducted a multicentre study to evaluate the analytical performance of selected immunoassays on cobas pure integrated solutions versus cobas legacy analysers.

METHODS

Precision experiments based on the CLSI EP05-A3 protocol were conducted at three sites (Ludwigsburg/Heidelberg, Germany; Wroclaw, Poland) during May–June 2021, with two runs/day for ≥5 days per instrument. For each sample and run, a single determination of three separate aliquots was performed. Nine routine immunoassays were studied: anti-HBs II, HBsAg II, anti-HBc IgM, anti-HBc II, anti-HBe, HBeAg, anti-HCV II, and free and total PSA. Samples included Roche-produced Elecsys® quality control materials (low/high concentration) and anonymised human sample pools covering the respective assay measuring ranges (negative/positive); identical samples and reagents were used at each site. Comparator legacy instruments included cobas e 601, e 602 and e 801 analysers. Coefficients of variation (CVs) for repeatability and intermediate precision (per site), and reproducibility (across all sites) were calculated and compared with pre-defined acceptance criteria.

RESULTS

For repeatability, 16/17 CVs were <5% for assays on both cobas pure and the legacy systems; for intermediate precision, 17/17 versus 15/17 CVs, respectively, were <8%; and for reproducibility, 17/17 versus 15/17 CVs, respectively, were <10%. CVs for repeatability around the medical decision point or cut-off value for cobas pure ranged from 0.9% (HBeAg) to 4.7% (HBsAg II); those for intermediate precision ranged from 1.3% (anti-HBc II) to 6.5% (anti-HBs II), and for reproducibility they ranged from 2.3% (total PSA) to 8.9% (anti-HBc IgM).

CONCLUSIONS

The analytical precision performance of cobas pure integrated solutions for a selected panel of immunoassays was comparable to, or better than, the legacy systems. These observations support the implementation of the immunochemistry analytical unit of cobas pure into routine clinical laboratory practice.

Analytical technologies and applications

M073

EVALUATION OF COMPARABILITY BETWEEN CASCADION SM CLINICAL ANALYZER AND COBAS E801 IN THE DETERMINATION OF 25-HYDROXY-VITAMIN D: OVERCOMING THE LIPEMIA INTERFERENCE

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BACKGROUND-AIM

In recent decades, few molecules have been the subject of as much debate as vitamin D. This micronutrient, in addition to carrying out the regulation of phosphocalcic metabolism, has pleiotropic effects as an immunomodulatory, antimicrobial and cardiovascular protective hormone. High quality 25-hydroxyvitamin D (25-OHVitD) results enable a more effective evaluation of a patient's nutritional status, in which deficiency (<12.5ng/mL) or compliance within therapeutic target (30 ng/mL) are more accurately delimited. 25-OHVitD has a very high number of metabolites, molecular modifications and even epimers that have historically resulted in problems of unspecificity. Unlike the immunoassay (IA), the automated LCMS/MS based Cascadion system is able to distinguish between these alternative forms. Here we summarize the results from a method comparison study.

METHODS

This study included a total of 449 serum patient samples with results across the measuring range of total 25-OHVitD. The levels of this prohormone were measured by IA in the Cobas e801 (Roche Diagnostics®) and by LC-MS/MS with the CascadionTM SM Clinical Analyzer (Thermo ScientificTM). A subset of 42 lipemic samples was also determined in both analyzers in order to assess interference. Statistical analysis was carried out by MedCalc software. Far-out values were discarded by Tukey test.

RESULTS

Method comparison (Cobas vs. Cascadion) (n=449):
25-OHVitD total: r>0.97 (95%CI: 0.9691–0.9786); slope=0.98 (95%CI: 0.95-1.00), intercept=0.83 (95%CI: -0.00–1.41). Non-parametric Wilcoxon test, p: 0.6493.
Interference study (Cobas vs. Cascadion) (n=42) :
25-OHVitD total: r>0.90 (95%CI: 0.83–0.95); slope=0.96 (95%CI: 0.83-1.14), intercept=-1.78 (95%CI: -0.04–1.32). Paired t-test, p: 0.0259.

CONCLUSIONS

The correlation study provided excellent concordance and regression results, with no proportional or constant systemic bias between both methods. Accordingly, Wilcoxon's p-value demonstrated no significant difference (p>0.05). However, about 20% of samples showed deviations of a non-systematic bias of >25%. We could infer that those discrepancies could be caused by lipemic interferences (p=0.0259). In conclusion, even if both methods are interchangeable, Cascadion gives more precise results due to its LC-MS/MS technology.

Analytical technologies and applications

M074

EXPANSION OF THE MEASURING RANGE OF SUPAR IN PLASMA AND SERUM FROM PATIENTS IN HEMODIALYSIS

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BACKGROUND-AIM

suPAR is a plasma marker of chronic inflammation, and an elevated suPAR is consistently associated with a worse outcome in a variety of clinical conditions. The upper limit of the measuring range is 16 µg/L (suPARnostic® TurbiLatex assay), and patients in hemodialysis frequently have suPAR levels exceeding that level. Our aim was to investigate performance of the suPAR assay above the recommended limit of 16 µg/L in plasma (EDTA and citrate) and serum samples.

METHODS

Plasma (EDTA and citrate) and serum were sampled from 20 patients at the initiation and at the end of a hemodialysis treatment day. Samples were analyzed by rapid latex particle-enhanced turbidimetric immunoassay (ViroGates A/S, Denmark) on the c702 Roche Cobas® 8000-measurement system. We measured 32-34 plasma and serum samples in duplicates (for repeatability).

RESULTS

suPAR in citrate plasma are as expected -14.8 % (95% Limit of Agreements: -22.9 % to -6.6 %) lower than suPAR in EDTA plasma.
In serum matrices, we found a linear constantly decreasing relation between serum and EDTA plasma: the relative difference decreases (p=0.0005) with a constant slope of -0.33 % and with an intercept of 10.6 % (higher) results in serum compared to EDTA plasma. Repeatability (coefficient of variation) only increases by 1-2 % points, if the measuring range is extended from 16 to 25 µg/L.

CONCLUSIONS

suPAR can be reproducibly quantified in a range up to 25 µg/L, hence above the recommended 16 µg/L using the new turbidimetric assay. Both EDTA and citrate plasma can be applied at levels up to 25 µg/L.

Analytical technologies and applications

M075

DETERMINATION OF FREE CORTISOL BEFORE AND AFTER EXTRACTION IN 24-H URINE SAMPLES

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BACKGROUND-AIM

Measuring urine free cortisol level is an important step in Cushing's syndrome diagnosis. In our laboratory, routine analysis of cortisol in 24-hour urine is performed on an automated immunoassay Beckman Coulter DxI 600 analyzer (CLIA), preceded by cortisol extraction with ethyl acetate. With this step, water-soluble metabolites are removed. On the other hand, this procedure is time-consuming and prone to preanalytical errors due to manual work. Patient samples are collected, stored frozen, and analyzed once a week. Furthermore, ethyl acetate is an irritant and a flammable chemical with a special disposal process. The aim pf this summary was to examine whether analyzing urine directly without extraction could be an alternative.

METHODS

For that purpose 26 patient samples were analyzed immediately after the reception, with and without extraction. In addition, urine cortisol without extraction was measured for 52 patient samples and compared with the results without extraction obtained on Abbott Architect i1000SR (CMIA) whose calibrators are traceable to the LC-MS/ MS method, and the LC-MS/MS calibration is verified by certified reference materials.

RESULTS

A Passing-Bablock regression analysis of results with and without extraction reveled a regression equation: y = -257.60+ 4.61 x (95% CI for intercept: -1356.58 to 27.32; 95% CI for slope: 2.27 to 13.57), from which is obvious that the methods are not comparable. These differences were expected and further comparisons were made.
The obtained Passing-Bablock equation for CMIA-CLIA comparison was: y= 87.34+3.22x (95% CI for intercept: 38.31 to 134.23; 95% CI for slope: 2.73 to 3.63), which indicates a statistically significant constant and proportional deviation in the CMIA (Abbott) and CLIA (Beckman Coulter) methods. Given the differences between the reference ranges of these methods, the results can only be compared at the clinical decision level. In this context, the obtained results fulfilled the acceptance criteria for the kappa coefficient, with a percentage of agreement of 100% (kappa = 1.0).

CONCLUSIONS

In order to reduce toxic laboratory waste and the danger of poisoning or fire, we decided from now on to determine free urinary cortisol without prior extraction on Abbott Architect i1000SR due its traceability to LC-MS/MS.

Analytical technologies and applications

M076

EVALUATION OF THE NEW ASSAY ACCESS SARS-COV-IGG (1ST IS): COMPARISON WITH ACCESS SARS-COV-IGG II ASSAY ON ROUTINE SAMPLES.

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BACKGROUND-AIM

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic led to market introduction of many antibodies' determination assays. The first International Standard (IS) for anti-SARS-CoV-2 immunoglobulin (Ig) G was recently introduced with NIBSC code 20/136, aiming antibody assays harmonization. Aim of this study was to compare the new Access SARS-CoV-IgG (1st IS) to the current assay in routine in Cupra Marittima's BIOS laboratory.

METHODS

123 consecutive serum samples collected in Vacutest KIMA 5 mL tubes with gel and clot activator during three days of routine (vaccinated and no vaccinated subjects) were tested with both assays. Routine assay: Access SARS-CoV-IgG II (IgG II); new: Access SARS-CoV-IgG. Both are chemiluminescent paramagnetic particle non-competitive immunoassays with paramagnetic particles coated with recombinant protein specific for S1 receptor binding domain RBD and monoclonal antibody anti human IgG conjugated with alkaline phosphatase, performed on UniCel DxI800 (Beckman Coulter Inc.). IgG II semi-quantitative/qualitative six-points calibration at 0; 5; 25; 100; 200; 450 AU/mL. 1st IS quantitative/qualitative six-points calibration at 0; 20; 100; 400; 800; 1800 IU/mL (BAU). Limit of Quantitation 20% Coefficient of Variation: IgG II ≤ 2 AU/mL, 1st IS 8 IU/mL. Cut-offs: IgG II <10 AU/mL, 1st IS < 30 IU/mL.
Statistical analysis by MedCalc® Statistical Software version 20.009 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

RESULTS

IgG II values from 2 to 487, median 45.12; 1st IS values from 0.75 to 1908, median 167.84.
Correlation coefficient r = 0.9948, 95% IC 0.9926 to 0.9964, significant P< 0.0001. Cohen Weighted Kappa 0.9282 (95% Confidence Interval CI 0.82975 to 1.00000). There were two discrepant samples: 7.94 IgG II / 38.63 1st IS and 9.23 IgG II / 39.9 1st IS.

CONCLUSIONS

Comparison between assays calibrated without and with 1st IS, even if with same virus target and architecture, is not suitable for regression analysis. Lacking common standard, patient must be followed with the same method.
The new Access SARS-CoV-IgG (1st IS) assay represents a considerable improvement for routine determination of SARS CoV 2 IgG. Changing from the previous IgG II assays is guaranteed by very good strength of k Cohen agreement and optimal r.

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Analytical technologies and applications

M077

FERRITIN TECHNIQUES COMPARISON. DIFFERENCES BETWEEN TWO ANALYTIC METHODS

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BACKGROUND-AIM

Ferritin is a very requested analiytic parameter. Its main purpose is to evaluate the total amount of iron available in our organism, in order to produce haemoglobin which is vital for red blood cells optimal function. In addition, ferritin is a acute phase reactant that suffers a high elevation in its value in the context of some viral and bacterial infections. For this reason, and in the current situation due to the pandemic of SARS Cov-2, the request of this parameter has been increased in the last two years. The current inmunoassay method used in our laboratory slows down the sample's flow, esentially because of the lack of capacity to stock all the the required reagents in Cobas e702 module. We are going to evaluate if the new spectophotometric method in e602 module is more efficient than the old one, in order to satisfy the requests of ferritin and give faster and more accurate results.
The main aim of this study is to evaluate if the results of ferritin levels detected by particles enhanced inmunoturbidimetry in Cobas e602 analyzer (Roche diagnostics GmbH, Manheim) are similar to the results obtained by electrochemiluminiscense by inmunoassay in the current Cobas e702 analyzer (Roche diagnostics GmHb, Mannheim).

METHODS

In order to compare and evaluate the results of both techniques, reagents were installed in the two different analyzers. Same quality controls as in daily routine were used and a total amount of 41 values were registered of routine samples by both techiques. These results were processed with the program Method Validator ver. 1.19, and a Passing-Bablock non lineal non parametric regression stadistic analysis was perform. Pearson's correlation coefficient (r) was also calculated.

RESULTS

Both techniques are similar in all the linearity range of the technique (5-1000 mg/dL). However, it is necessary to apply a correction ecuation shown below:
Ferritin (e 602) = 1,13 x Ferritin (e 702) + 4,58. Pearson's coefficient=0.998. Confidence interval 95% is from 1,11 to 1,16 and y-intercept is from 1,99 to 5,87.

CONCLUSIONS

There is a good correlation between both techniques, despite the fact that because of the existance of a systematic error results are not completely interchangeable and we have to apply a correction factor.
In addition we obtain faster results with the new technique thanks to the e602 module which has more capacity of samples and reagents.

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Analytical technologies and applications

M078

EVALUATION OF ATELLICA CHEMISTRY CH930 ASSAY PERFORMANCE FOR HBA1C MEASUREMENT

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BACKGROUND-AIM

Glycated hemoglobin (HbA1C) is formed through non-enzymatic glycation of the adult haemoglobin, HbA. The measurement of HbA1c is crucial to monitor the glycaemic index in patients with diabetes mellitus to try and prevent its chronic complications such as nephropathy, retinopathy and cardiovascular diseases. There are four methods commonly used to measure HbA1C namely immunoassay, ion-exchange high-performance liquid chromatography (HPLC), boronate affinity HPLC and enzymatic assays. Each of these methods has varying performances in HbA1c measurement, hence, thorough evaluation of each method is mandatory prior to its clinical use.We aim to evaluate the Atellica Chemistry (Atellica CH930) assay performance for measuring HbA1c as well as analyse the correlation of the Atellica CH930 with Biorad Variant II Turbo.

METHODS

This is a prospective study analysing only the existing HbA1c samples of selected UMMC in-patients on the Atellica CH903 analyser with the current analyser, Variant II Turbo Biorad Ion Exchange HPLC. The HbA1C assay on Atellica CH930 involved pretreatment of the whole blood sample followed by latex agglutination measurement of specific HbA1C. While the HbA1C assay in Biorad Variant II Turbo is using cation-ion exchange high performance liquid chromatography. Precision study was done using protocol from CLSI EP15-A3. Correlation study was done using 69 samples analysed concurrently in both Atellica CH930 and Biorad Variant II Turbo. Bland-Altman plot and Passing-Bablok were used to analyse the data.

RESULTS

We were able to verify the within and between day precisions of Atellica CH930 as claimed by the manufacturer. The correlation study showed that the Atellica CH930 has negative bias when compared to Biorad Variant II Turbo.

CONCLUSIONS

In the presence of carbamylated haemoglobin, HPLC method showed higher results of HbA1 between compared to immunoassay method. However, immunoassay showed better performance in the analysis of blood samples with carbamylated Hb whereas the HPLC was better for samples with variant Hb. This suggests that the choice of HbA1c methodology should depend on the need that is suitable for that specific population.

Analytical technologies and applications

M079

SIMPLE LIQUID-LIQUID EXTRACTION FOLLOWED BY LC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF LYSO GB1 AND LYSO GB3 LEVELS IN PLASMA

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BACKGROUND-AIM

While globotriaosylsphingosine (Lyso GB3) are a biomarker of Fabry disease (FD), glucosylsphingosine (Lyso GB1) is a highyl sensitive and spesific biomarker of Gaucher Disease (GD). The aim of this study is to develop a simple dispersive liquid-liquid extraction (DLLE) method for the co-detection of Lyso GB3 and Lyso GB1.

METHODS

The chromatographic separation was operated on an Acquity UPLC C18 (50 mm × 2.1 mm, 1.7 μm) with gradient elution using %98 %2 Water/Methanol containing 0.05% formic acid and methanol as the mobile phase at the flow rate of 0.4 ml/min and total run time was only 5 minutes. Development of DLLE procedure includes optimization of some important parameters such as type and volume of extraction and disperser solvent and volume and concentration of salt solution.

RESULTS

The dichloromethane-methanol couple was found to be a suitable extraction-disperser solvent couple. As a result of optimization studies, the best results were obtained with the following configuration: extraction solvent volume: 0.5 mL, dispersion solvent volume: 1 mL, NaCl concentration and volume: 10%, 3 mL.The detection range of the method was 0.02-100 ng/mL for Lyso GB3 and 0.005-100 ng/mL for Lyso GB1. The intra-day and inter-day reproducibility studies are below 10% for both analytes. The method offered high accuracy and a clean matrix for both analytes.

CONCLUSIONS

Finally, the validated method was succesfully performed in FD, GD and healthy individuals.

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Analytical technologies and applications

M080

A MACHINE LEARNING APPROACH FOR THE IDENTIFICATION OF MILD COGNITIVE IMPAIRMENT USING DNA METHYLATION

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BACKGROUND-AIM

Age related mild cognitive impairment is likely to become an increasingly important issue as the population in most western countries ages. Treatments to delay cognitive impairment have had mixed results but they usually tend to be more efficient when started in the early stages of the disease. Hence, early detection might play a significant role. Mild cognitive impairment might be difficult to diagnose using only clinical evidence as the social, economic and educational background of the patient can have a substantial impact on the outcome of standard cognitive tests. Hence, having a quantitative indicator that points to the presence of the illness might we useful. In this regard DNA CpG methylation might be a suitable approach. DNA CpG methylation levels have been analyzed in the context of several neurodegenerative disease such as Alzheimer, Parkinson and Huntington disease. These disease change the methylation profile of the individual. This approach has also been used to create accurate biological clocks, able to rather precisely estimate the chronological age of an individual by analyzing the methylation data.
DNA methylation is an epigenetic change that can be used as a biomarker and has been studied in several types of different tissues ranging from brain matter to blood. Blood is a convenient source of DNA methylation data as it can be easily obtained through a simple blood test. Technological advancements have made possible in recent years to analyze hundreds of thousands of CpG per patient in a rapid and inexpensive way. One of the most frequently used machines for DNA methylation analyzes approximately 450,000 CpGs but there are machines that can analyze up to 800,000 CpGs, which is the one used in this research.

METHODS

DNA methylation data was obtained from the GEO database (accession code GSE 190540). This dataset is publically available. The dataset contained the CpG DNA methylation data for 45 individuals of which 25 suffer from mild cognitive impairment and the rest are control individuals. The DNA methylation data was obtained from peripheral blood leucocytes. In practice, blood is an ideal candidate for this type of analysis as it is easy to obtain without the need to do for instance a brain biopsy or other highly invasive procedures. Approximately 800,000 DNA CpG methylation data were obtained from each patient (both control and cognitively impaired). The data was divided into two datasets (training and testing) of roughly the same size. A support vector machine (SVM) algorithm was used for classification purposes. One of the common problems when using this type of machine learning approach for classification purposes is the issue of local minima. This issue is particularly relevant when, as in this case, the problem has dimensionality issues. In this type of dataset there tends to be a limited amount of cases (45) and a very large amount on inputs (800,000 per patient) which is the above mentioned dimensionality issue. Hence, the first step is to reduce the dimensionality of the input data. This was accomplished using a Lasso regression approach. This algorithm makes some of the coefficients of the regression equal to zero. In other words, it selects a suitable subset of inputs from the initial input set. Through this method the dimensionality on the input data was reduce from 800,000 inputs (CpGs) per patient to only 12. The SMV model was trained with the training dataset (with roughly half of the cases) and tested with the testing dataset with the other half of the cases. The analysis control for the age, gender and race of the patients.

RESULTS

The model using SVM without dimensionality reduction had no classification value with error rates close to 50%. After the dimensionality reduction there was a statistically significant improvement in the classification accuracy, with error rates of approximately 18.7% in the testing dataset. As expected the error rates obtained with the training dataset were smaller but these are not good representations of the actual accuracy of the proposed approach. The 18.7% error rate, i.e., number of patients misclassified, is a likely a more accurate representation of the accuracy of the approach when applied to a new dataset.

CONCLUSIONS

Algorithms such as support vector machines (SVM) using DNA methylation data as an input are a relatively accurate options for the identification of patients suffering from mild cognitive impairment. This type of approach requires the reduction of the dimensionality of the input data in order to generate meaningful classifications. This dimensionality reduction was accomplished using a Lasso algorithm. The results are less accurate than the ones obtained analyzing other illnesses such as Alzheimer's disease (AD). This might be related to illnesses like AD potentially having a larger impact on DNA methylation levels compared to afflictions such as mild cognitive impairment.