Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Technology/Design Development

B-412

Comparison of ARCHITECT 2000 I SR for determination of carbamasepine with VITROS 5600

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Background: Carbamazepine is a commonly prescribed anti-convulsant used mainly in the treatment of seizure disorders, but is also used to treat trigeminal neuralgia. Carbamazepine has also been useful in the treatment of manic depressive patients as an alternative to lithium therapy. Carbamazepine measurements are used to monitor patient compliance and therapy, and to diagnose potential overdose.

Methods: The carbamasepine concentration of 80 serum samples were determined using CMIA (chemilumineseсent microparticle immnoassay) Architect i 2000 and dry chemistry slide method is VITROS 5600. All patients were hospitalized at Department of Neurology at the University Clinics Center of Sarajevo. The reference serum range of carbamaseine in patients is 4-12 mg/L. The quality control, precision and accurancy of Architect i 2000 and VITROS 5600 were assessed.

Results: The quality control was done using quality control serums for low (= 5.37 mg/L), medium (= 9.41 mg/L) and high (= 16.9 mg/L). We have used commercial ABBOTT controls and got CV 1.23 % to 4.50 % for Architect i 2000. We done a quality control using quality control serums for low (= 4.41 mg/mL) and high control (= 9.65 mg/L) VITROS Diagnostic and got CV 1.44 % to 4.14 %). Mean value of carbamasepine serum concentration using ARCHITECT I 2000 (CMIA) was 6.09 +/- 1.54 mg/L and VITROS 5600 (dry chemistry slide method) was 8.47 +/- 2.01 mg/L. It was established that the main difference between Architect i 2000 and VITROS 5600 and it was statistically significant for $p < 0.0001$ according to Paired samples t-test. Correlation coefficient was r = 0.9549 to 0.9863 and regresion line had a slope 1.2741 (1.4433) and a y axis intercept of -0.3644 (0.7748). The ARCHITECT I 2000 carbamasepine assay was performed on the 80 human serum samples in the range 3.84 to 8.59 mg/L and VITROS 5600 5.30-11.7 mg/L. The average mg /L difference bias exhibited by ARCHITECT carbamasepine vs. VITROS carbamasepine assay in this study was -2.38 µg /mL. The 95 % confidence interval of the ng/ml difference bias is -2,1918 to -2,5628 mg/L.

Conclusion: The study shows that it was good agreement in using those methods for detection of carbamasepine. The patients should be monitored on a single method to avoid differences in the results. Different techniques for carbamasepine detection in human serum using different methods which leads to different results.

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Preliminary study on a high-sensitivity hydrogen peroxide detection method using the metal chelating reagent, Chromazurol B (CAB)

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Background:

Quantitation of biological sample components and enzyme activities is indispensable for determining the pathological condition of patients. The rapid determination and return of accurate results is critical to clinical chemical analysis for clinical diagnosis. The sensitivity of analytical techniques is vital to ensuring the accuracy of analyses.

Currently, two detection techniques are used for the majority of clinical enzyme activity measurements. The first of these methods, NAD (P) H, has low sensitivity, but is largely unaffected by reducing compounds. Conversely, the hydrogen peroxide-peroxidase method is highly sensitive, but is adversely affected by the presence of reducing species; especially when measuring urinary constituents.

Therefore, in the present study, we developed a novel high-sensitivity measurement system for hydrogen peroxide (H_2O_2) using a metal chelating reagent, Chromazurol B (CAB).

Methods:

CAB develops an absorption band at 600 nm when chelating Fe^{3+}. In our novel method, Fe^{2+} is oxidized to Fe^{3+} by hydrogen peroxide originating from oxidizing enzymes under acidic conditions. Then, the absorbance of the CAB-Fe^{3+} complex is measured at 600 nm, and the increase in absorbance can be used to determine the quantity of hydrogen peroxide.

In this study, we used a Hitachi Model 7170 and 7600 (P module) automated analyzer to perform a two-point end assay at 37°C. The sample (H_2O_2, 10 µL) was mixed with 200 µL of reagent 1 (117 µmol/L iron (II) sulfate and 0.12 mol/L formic acid buffer (pH 4.0, 25°C)). The mixture was maintained at 37°C for 5 min. After the addition of 24 µL of reagent 2 (975 µmol/L CAB in distilled water), the absorbance was measured at 600/800 (main/sub) nm wavelengths.

Results:

The within-run CVs of the above method using 0.4 and 2.2 µmol/L H_2O_2 solutions were 3.78 and 1.74%, respectively (n = 20). The results exhibited linearity from 0 to 3.0 µmol/L. The detection limit was 0.2 µmol/L. The molar absorption coefficient, indicating the measurement sensitivity, was 202,160 $L \cdot mol^{-1} \cdot cm^{-1}$. This is about seven times or over greater than the sensitivity of the current methods.

Conclusion:

The newly-developed method was highly sensitive to hydrogen peroxide, and may be applicable in a wide range of research and clinical laboratories. In the future, we will investigate the effects of reducing substances in biological samples, such as serum and urine, on the sensitivity of this method.

B-415

Development and Evaluation of the Novel Multi-Test VITROS® XT Chemistry Products TRIG-CHOL Slides

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Background: A new Multi-Test technology is under development that enables more efficient clinical chemistry laboratory diagnostics. The new VITROS® XT MicroSlide technology incorporates two chemistry chips on single dry slide element. This new XT test element enables unique benefits such as higher throughput, reduced sample volume, less reagent storage space, less waste, and ease of use without compromising analytical performance. One example of this technology is a single dry slide with both a triglyceride test and a cholesterol test on a single slide. The VITROS XT Chemistry Products TRIG-CHOL Slides will be developed to quantitatively measure cholesterol concentration and triglycerides concentration in serum and plasma. The VITROS XT TRIG-CHOL Slides contains two multilayered, analytical elements coated on a polyester support. A drop of patient sample is deposited on each chemistry chip (2.5 uL for TRIG and 4.0 uL for CHOL) and is evenly distributed by the spreading layer to the underlying layers. The density of the dye formed through the reaction cascades is proportional to the triglyceride and cholesterol concentrations present in the sample on their respective chemistry chips and is measured by reflectance spectrophotometry.

Methods and Results: We evaluated the accuracy of 90 patient serum samples (TRIG: 21 - 504 mg/dL; CHOL: 69 - 277 mg/dL) on the VITROS XT 7600 Integrated System (in development) compared to the VITROS Chemistry Products CHOL Slides and VITROS Chemistry Products TRIG Slides on a VITROS 5600 Integrated System. The VITROS XT TRIG-CHOL Slides showed excellent correlation with the VITROS CHOL and TRIG Slides. VITROS XT TRIG-CHOL = 0.993 * VITROS TRIG - 1.97; (r) = 1.000 for TRIG; VITROS XT TRIG-CHOL = 1.023 * VITROS CHOL - 1.94; (r) = 0.998 for CHOL. A 50-replicate within day precision study conducted on the VITROS XT 7600 System showed excellent precision. Mean TRIG concentrations of 110.3 mg/dL, 262.0 mg/dL, and 437.1 mg/dL resulted in within-day percent coefficient of variation (%CV) of 0.72%, 0.70%, and 0.50% respectively. Mean CHOL concentrations of 134.8 mg/dL and 236.9 mg/dL resulted in within-day %CV of 0.92% and 0.76% respectively. The linearity for the VITROS XT TRIG-CHOL Slides was determined from an 18-level admixture series. The TRIG test was linear from 11.1 mg/dL to 556.2 mg/dL, and the CHOL test was linear from 24.6 mg/dL to 355.4 mg/dL. The Limit of Detection (LoD) for the VITROS XT TRIG-CHOL Slides was determined based on 240 determinations with 4 low-level samples. The LoD for the TRIG test was 9.7 mg/dL and for the CHOL test was 6.1 mg/dL. The measuring

range for the VITROS XT TRIG-CHOL Slide exceeded 11-525 mg/dL for the TRIG test and 50-325 mg/dL for the CHOL test.

Conclusion: The VITROS XT TRIG-CHOL Slides has exhibited good correlation with serum samples across a broad measuring range with excellent precision, linearity, low end sensitivity, and measuring range on the VITROS XT 7600 Integrated System.

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Comprehensive analysis of CYP2D6 variants and copy numbers using reverse-hybridization and real-time PCR based assays

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Background: The cytochrome P450 2D6 (CYP2D6) is an important liver enzyme involved in the metabolism of up to 25% of clinically used drugs. The CYP2D6 gene is highly polymorphic, with numerous (sub)variants described in the Human Cytochrome P450 Database (www.cypalleles.ki.se). While the most frequent allelic variations are caused by single nucleotide polymorphisms and small insertions/deletions, highly homologous regions in the CYP2D6 gene locus facilitate unequal cross-over leading to large deletions, duplications and gene conversions.

Methods: We developed a reverse-hybridization assay (PGX-CYP2D6 XL StripAssay) for the simultaneous detection of 19 sequence variations in the CYP2D6 gene, which define the most prevalent alleles impacting enzyme activity in Caucasians. For the detection of copy number variations a real-time PCR based assay (CYP2D6 RealFast CNV Assay) was established. The StripAssay and real-time PCR assay were validated on 118 and 98 samples, respectively.

Results: The PGX-CYP2D6 XL StripAssay correctly identifies allelic variants with normal (*1, *2, *35, *39), reduced (*9, *10, *17, *29, *41) and no (*3 to *8, *11, *12, *14, *15, *40, *58) enzyme activity, and hence allows the classification of individuals into extensive (EM), intermediate (IM) and poor (PM) metabolizers. In addition, ultrarapid (UM) metabolizers and CYP2D6*5 carriers can be identified by quantifiying their abnormal copy number status using the CYP2D6 RealFast CNV Assay. Both assays demonstrate a test accuracy of >0.99.

Conclusion: The metabolizer phenotype of patients treated with CYP2D6 substrates can be accurately determined by the combined use of both assays.

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Performance of Total Protein, Ultra HDL, Alanine Aminotransferase, Urea Nitrogen and Phosphorus on the Alinity c Analyzer

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Objective: To evaluate analytical performance utilizing photometric technologies for detection of analytes in human plasma/serum and urine on the Alinity c, Abbott's next-generation clinical chemistry analyzer. The Alinity c analyzer is a high throughput instrument testing up to 900 tests per hour. The sample is dispensed into a cuvette followed by reagents. The contents are mixed and incubated allowing for the reaction to occur. If a second reagent is required, the second reagent is added to the cuvette; the contents are mixed again and incubated. Absorbance readings of the sample are taken at regular intervals throughout the process at a primary and if applicable, a secondary wavelength. Data reduction generates a calculated absorbance based on the reaction mode of the assay (rate or end point) and measures the calculated absorbance using a calibration curve to generate a result.

Methods: Key performance testing including precision, linearity, limit of quantitation (LoQ), and method comparison were assessed per CLSI protocols. An assay's measuring interval was defined by the range across which acceptable performance for bias, imprecision and linearity was met.

Results: Total imprecision, LoQ and linearity results along with the defined measuring interval are shown for representative assays in the table below. Results versus the comparator assay demonstrated a slope 0.97 – 1.01 and r = 0.99 - 1.00.

Assay	Total %CV	LoQ	Linearity	Measuring Interval
Total Protein	≤ 3	0.76 g/dL	0.2 - 22.5 g/dL	0.8 – 18.4 g/dL
Ultra HDL	≤ 4	5 mg/dL	5 - 200 mg/dL	5 - 180 mg/dL
ALT	≤ 6	5 U/L	2 - 3899 U/L	5 - 3899 U/L
Phosphorus (Serum)	≤ 4.6	0.62 mg/dL	0.0 - 27.1 mg/dL	0.7 – 25.3 mg/dL
Phosphorus (Urine)	≤ 4.6	4.39 mg/dL	0.3 - 227.2 mg/dL	5.0 – 186.0 mg/dL
Urea Nitrogen (Serum)	≤ 4.5	3 mg/dL	2 - 128 mg/dL	3 - 125 mg/dL
Urea Nitrogen (Urine)	≤ 4.5	40 mg/dL	13 - 2084 mgdL	40 - 1991 mg/dL

Conclusion: Representative clinical chemistry assays utilizing photometric technologies tested on the Alinity c analyzer demonstrated acceptable precision, sensitivity and linearity. Method comparison data showed excellent agreement.

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Total Error Profiles - A New Method for Visualizing Product Performance

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Introduction: The sigma value is a commonly used metric for comparing products and is derived from the TEa, precision, and bias determined at a single critical point. In a clinical laboratory, the sigma value is one simple aid determining whether a product is fit-for-use. Often, however, laboratorians want to understand the performance of a product beyond a single critical point. A Total Error Profile is a valuable tool that can be used to visualize the performance of a product across a range of concentrations.

Methods: A Total Error Profile was created to visualize the performance for three immunoassays. For a given assay, the total error was estimated at multiple concentrations across the assay's measuring interval using the equation: *%total error = 2 x %CV + |%bias|*. To estimate the precision (%CV), a study was conducted at Abbott on each assay using the Alinity i-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, > 70 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity i-series and ARCHITECT $i2000_{SR}$ systems. The mean concentration of the Alinity i-series results were regressed versus the mean ARCHITECT $i2000_{SR}$ results and a weighted Deming regression model was fit. Using the regression model, the %bias was estimated at the same concentrations at which the precision samples were tested. A Total Error Profile was created by plotting the %total error values versus the mean concentration values for the Alinity i-series. Sigma values were calculated using the equation: *sigma = (%TEa - |%bias|) / %CV.* Additionally, a precision profile was created by plotting the within-laboratory %CV values versus the mean concentration values.

Results: The Total Error Profile is a continuous function across multiple concentrations for each assay. The Total Error Profile provides additional rigor beyond the sigma value and precision profiles by leveraging all total error estimates above, below, and near the critical decision points. In the example assay data, the total error decreased as the Total β-hCG concentration increased, whereas the total error increased slightly as the TSH concentration increased.

Conclusion: Total Error Profiles can be useful supplements to sigma metrics and precision profiles by providing a visual assessment of assay precision and bias performance across the measuring interval. Another benefit of the Total Error Profile is that it is not dependent on the TEa goal. This approach is particularly useful for assessing assays with more than one medical decision point and for assays with wide measuring intervals. By providing a detailed analytical understanding of the expected assay performance, these profiles can be useful tools for helping laboratorians understand the full dynamic performance characteristics of each assay and the potential impact to patient results.

B-419

An Aptamer Technology Platform for Cancer Diagnosis

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Background/Objectives: The gold standard in cancer diagnosis is morphological and molecular pathology of a biopsy, which is crucial for timely and effective treatment

of cancers. Molecular diagnoses of cancers using protein biomarkers are mostly antibody-based immunoassays. Development of immunoassays for cancer diagnosis has been hindered by the availability and inherent properties of antibodies. Thus alternative affinity reagents are in need for the development of cancer diagnostics. Aptamers are short (15-100 nt) ssDNAs/RNAs that bind their targets avidly and specifically. Aptamers are often called "chemical antibodies", due to its nature of *in vitro* selection and chemical synthesis. The advantages of aptamers compared to antibodies are broader target range (from small chemicals to macromolecules), better batch consistency, lower cost and they are more amenable to diverse assay formats. Here, we present an aptamer technology platform for cancer diagnostics.

Methods: Systematic Evolution of Ligands by EXponential enrichment (SELEX) was utilized to develop various schemes for *in vitro* selection of both DNA and RNA aptamers. The aptamer technology platform was then applied in a case study of a diagnostic assay for lung cancer.

Results: The variables of aptamer selection schemes were optimized including the library design, the target immobilization methodology, the enzymology for incorporating modified nucleotides into DNA/RNA and the bioinformatics pipeline for aptamer candidate identification. Through the optimization and development, we have established an aptamer selection platform integrating SELEX seamlessly with Next Generation Sequencing (NGS) with high success rate (>80%) of aptamer selection. The aptamer technology platform was applied to develop aptamers for ten biomarkers of lung cancer (e.g. follistatin) with nM affinity (K_d) (Fig 1). Aptasensors for early diagnosis of lung cancer are under development.

Conclusion: We have developed an aptamer technology platform with great potential in cancer diagnosis.

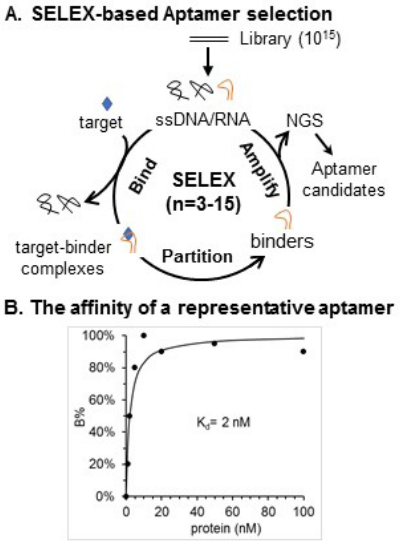

Fig 1. The scheme of aptamer selection based on SELEX (A) and the affinity of a representative aptamer developed (B).

B-420

Routine-like testing of a cobas e 801 module* in an integrated cobas® 8000 configured as an IC/CC consolidator

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Background: The high throughput immunochemistry module from Roche Diagnostics, **cobas e** 801 is the newest member of the **cobas** 8000 modular analyzer series. It is designed to cover almost double the testing capacity of the predecessor **cobas e** modules and offers many features aimed to further enhance lab testing efficiency. During multicenter studies, the new module was tested in dedicated use as well as in combination with all other established **cobas** 8000 analyzer modules. Here we report on the outcome of the routine-like testing experiments processed at Lab Berlin on a platform including ISE, **cobas c** 701 and **cobas e** 801 covering a total of 75 assays, 38 immunochemistry assays and 37 general chemistry and protein assays.

Methods: All experiments were designed to stress the system under full workload conditions for up to eight hours, thereby testing the overall functionality and reliability using simulated routine testing patterns. In addition, while processing these high workloads with > 7000 results, numerous interactions (reagent loading, low sample volume, QC testing, STAT samples added, supplies run low, etc.) were initiated by the operators in order to provoke the system and to check for proper behavior. Aliquoted pooled QC materials were used as samples, the recovery of the respective analyte per aliquot was applied to check the performance throughout the experiment. The time to complete the experiment and the processing time per sample were also analyzed.

Throughout the study, QC's at two concentration levels per assay were measured in order to monitor the system performance and reliability.

Results: In total ~ 93,500 results were generated for 75 different methods over the six week study period. Stable system performance throughout the study was shown for all assays and modules with analyte recovery in QC material well within ± 2sd of the assigned target values.

Proper system functionality and interaction between the used analytical modules was shown during routine simulation precision experiments that included >30 immunochemistry assays from the indication areas thyroid, cardiac, fertility, oncology and infectious disease. Precision CVs calculated for results generated during random testing over >4 hours were mostly <2% and thus only marginally higher than those generated during batch-type testing. Analysis of workflow on our study system showed that **cobas e** 801 in a CC/IC consolidator configuration, can easily process typical workloads of two **cobas e** 602 modules without slowing down the high speed clinical chemistry **cobas c** 701 module (up to 2000 tests per hour). Typical hospital setting type peak workloads of ~900 samples were processed in less than 3 hours. Introduction of STAT samples or other provocations did not hinder the workflow nor lead to system malfunctions, the various workloads were handled efficiently.

Conclusion: The results of this study demonstrate that the **cobas e** 801analyzer met or exceeded laboratory requirements under simulated routine laboratory conditions. It showed excellent analytical performance, high reliability, speed of analysis and ease of use.

*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2017)

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Result consistency of cobas e 801* compared with Roche systems E170, cobas e 601 and cobas e 602 demonstrated in 6 European and US labs

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Background: Manufacturers of in vitro diagnostic products design and develop new analytical systems to meet changing needs of different types of laboratories. When introducing a new technical solution for lab testing in order to, for example enhance testing efficiency, it is of ultimate importance to maintain consistency of analytical results. During a multicenter performance evaluation study in six labs, we compared the results generated on the new immunochemistry analyzer **cobas e** 801 with those generated on the three established Roche high throughput systems, MODULAR E170, **cobas e** 601 (**cobas** 6000) and **cobas e** 602 (**cobas** 8000). Here we report the outcome of our testing on all four analyzers for nine assays in the areas of cardiac, thyroid, oncology and fertility.

Methods: Testing on the **cobas e** 601 module was done using preselected left-over samples and conducted in parallel to that on a **cobas e** 801 module. For comparisons with the E170 and the **cobas e** 602 modules, results generated during routine testing on multiple routine analyzers were compared with those generated under simulated routine conditions on the **cobas e** 801 study systems. Left-over samples were used according to the attained site specific ethic commission or internal review board waiver.

Results

Overview comparisons **cobas e** 801 versus MODULAR E170, **cobas e** 601 and **cobas e** 602

Assay	Unit	Predicate instrument	Type of compare results	Samples (n)	B/P Slope	B/P Intercept	Pearson r
TSH	mIU/L	E170	routine	3815	1.03	0.00	0.998
	mIU/L	e601	study	201	0.96	0.01	0.999
	mIU/L	e602	routine	843	1.05	0.00	0.999
FT4	pmol/L	E170	routine	3255	1.00	0.38	0.983
	pmol/L	e601	study	123	0.98	0.38	0.993
	pmol/L	e602	routine	371	1.01	0.10	0.994
CEA	µg/L	E170	routine	932	1.06	0.01	0.998
	µg/L	e601	study	201	0.95	0.09	0.999
	µg/L	e602	routine	457	1.05	0.03	0.996
CA 15-3	U/mL	E170	routine	207	1.02	0.70	0.994
	U/mL	e601	study	109	0.96	0.60	0.995
	U/mL	e602	routine	207	1.01	0.40	0.972
Estradiol	pmol/L	E170	routine	406	0.99	7.10	0.998
	pmol/L	e601	study	204	0.99	0.60	0.999
	pmol/L	e602	routine	82	0.96	3.40	0.998
Testosterone	ng/mL	E170	routine	652	0.99	0.01	0.996
	ng/mL	e601	study	140	1.01	0.00	0.999
	ng/mL	e602	routine	138	1.02	0.02	0.998
NT proBNP	pmol/L	E170	routine	91	1.02	0.39	0.999
	pmol/L	e601	study	125	1.01	0.38	0.999
	pmol/L	e602	routine	232	0.99	1.89	0.999

Conclusion: The study outcome demonstrates that results generated on the new immunochemistry analyzer **cobas e** 801 are consistent with those generated under different conditions at six labs on other Roche immunochemistry analyzers. All systems use the same reagents based on the Elecsys technology; thereby supporting an easy transition between the systems.

*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2017)

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Abbott Alinity c Sigma Metrics and Precision Profiles for Clinical Chemistry Assays

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Introduction: Assay performance is dependent on the accuracy and precision of a given method. These attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for more than 30 clinical chemistry assays tested on the Alinity c-series. Additionally, precision profile charts were created for a subset of assays to compare the precision performance of the assays tested using the Alinity c-series and the ARCHITECT *c* system.

Methods: A sigma value was estimated for each assay and was plotted on a method decision chart. The sigma value was calculated using the equation: *sigma = (%TEa - |%bias|) / %CV*. A precision study was conducted at Abbott on each assay using the Alinity c-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, 40-100 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity c-series and ARCHITECT c8000 systems. The mean concentration of the Alinity c-series results were regressed versus the mean ARCHITECT c8000 results and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated at a medical decision point. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity c-series and the ARCHITECT c8000 system, where the ARCHITECT c system within-laboratory %CV and mean concentration values were obtained from the assay package insert.

Results: The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near the medical decision point. The precision profile charts of the within-laboratory %CV results for the Alinity c-series overlaid with the ARCHITECT *c* system showed similar performance across the subset of assays evaluated.

Conclusion: Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity c-series clinical chemistry assays had sigma values greater than 5. The precision performance on the Alinity c-series and ARCHITECT *c* systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

B-423

Performance Evaluation of ZS050 in Measurement with SVACs and Wash in Alkaline Detergent

J. Yamaguchi. *Saitama Pref. Saiseikai Kawaguchi General Hospital, Saitama, Japan*

Background: JCA-ZS050 (ZS050) released from JEOL offers distinctive features of micro-volume measurement with the minimum 40µL reaction volume (SVACs: small volume analytical conditions) and wash in alkaline detergent to minimize sample-to-sample carryover. Performances are evaluated for these features.

Methods: SVACs were evaluated by comparing accuracy, precision and correlation with standard analytical conditions (SACs) specified by reagent manufacturers. 31 tests from chemistry, immunoassay and TDM tests were used. The measurement range and the matrix effects are assumed to be the same as the SVACs followed the same sample-reagent ratio with SACs. Accuracy was evaluated using MaCRM, the control serum from Japanese Committee for Clinical Laboratory Standards (JCCLS), for both conditions. Precision was evaluated using 3 levels of samples, 2 levels from controls and 1 level from pooled serum, to check within-run reproducibility. Correlations of test results by ZS050 with SVACs and SACs were evaluated using 100 patient samples to compare the results by AU5800 (from Beckman Coulters, Inc.). Correlation between SVACs and SACs was also evaluated. Sample-to-sample carryover with ZS050 was evaluated using HBs Antigen (HBsAg) which poses grave significance as a test result. The evaluation used concentrated HBsAg recombinant sample of approximately 1,500,000 IU/mL and patient sample with elevated HBsAg levels of 130,000 IU/mL, both measured by ARCHITECT (from Abbott Laboratories) with sensitivity of between 0.15 to 0.25 IU/mL. Carryover of HBsAg to next HBsAg-negative sample by a sample probe after probe wash in water or alkaline detergent was measured by ARCHITECT.

Results: Accuracy with SVACs was within the allowable limit defined by JCCLS in the certificate for MaCRM. Within-run reproducibility as precision demonstrated the equivalence between SACs (0.00 to 3.74%CV among 31 tests) and SVACs (0.00 to 3.92%CV among 29 tests). 2 out of 31 tests presented higher CV while some indicated better within-run reproducibility with SVACs. Correlation coefficient among the test results were calculated as follows: 0.959 to 0.999 with slope of 0.852 to 1.125 and intercept of -5.094 to 6.292 between AU5800 and SACs, 0.960 to 0.999 with slope of 0.876 to 1.134 and intercept of -6.514 to 3.764 between AU5800 and SVACs, and 0.965 to 0.999 with slope of 0.952 to 1.028 and intercept of -7.916 to 0.778 between SACs and SVACs. Sample-to-sample carryover was measured positive (0.68 IU/mL) after water wash and below the sensitivity after alkaline wash for 1,500,000 IU/mL sample, and below the sensitivity after water wash and alkaline wash for 130,000 IU/mL sample.

Conclusion: The results demonstrated high accuracy with SVACs. Good precision was obtained for 29 tests while parameters in SVACs require re-examination for 2 tests with higher CV. The correlation was found good for all three relationships. These results suggest that reagent volume reduction of 24 to 65% can be expected against SACs with 52.3 to 115.6µL reaction volume. Further discussion remains for optimal analytical conditions to maximize the performance of both ZS050 and reagents. For sample-to-sample carryover, alkaline wash is suggested from the result that carryover from 1,500,000 IU/mL sample was below the sensitivity.

B-424

Precision profiles for 20 assays on the cobas e 801* immunochemistry analyzer

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Objectives: When launching a new analytical system, there are various requirements on data generation to fulfill the regulatory needs for registration and launch around the globe.

Here we report on the outcome of a study performed at three sites to generate precision profiles according to CLSI EP05-A3 (5 days) using the 3x5x5 model for 20 assays on the **cobas e** 801 analyzer.

Methods: Aliquots of quality control material and multiple human specimen pools with analyte concentrations across the measuring range of the respective assay, were prepared and distributed to all study sites. Testing was done on five days in 5-fold determinations per sample pool at three sites. The 20 assays included in this study covered the indication areas Cardiac (NT proBNP II, Troponin T, CK-MB and Myoglobin), Oncology (CEA, CA 15-3 II, CA 19-9, CA 72-4, CYFRA, HE4, AFP and HCG+beta), and Infectious disease (Anti HBc IgM, HBsAg Quant, CMV IgG, CMV IgG Avidity, CMV IgM, Toxo IgG, Toxo IgG Avidity and Toxo IgM). CVs were calculated per site as within- lab precision and across the sites as reproducibility including the components repeatability, between-day and between-lab.

Results: The analyte concentrations ranges covered per assay are shown in the table below:

Assay	Unit	Concentration range	Assay	Unit	Concentration range
NT proBNP II	pg/mL	~13 - 30000	AFP	IU/mL	~2 - 900
Troponin T	pg/mL	~9 - 9500	HCG+beta	IU/mL	~5 - 8000
CK-MB	ng/mL	~1 - 300	Anti HBc IgM	COI	~0.1 - 2
Myoglobin	ng/mL	~30 - 2000	HBsAg Quant	IU/mL	~3 - 4500
CEA	ng/mL	~1- 950	CMV IgG	U/mL	~0.5 - 470
CA 15-3 II	U/mL	~4 - 290	CMV IgG Avidity	%	~20 - 100
CA 19-9	U/mL	~10 - 900	CMV IgM	COI	~0.2 - 4
CA 72-4	U/mL	~2 - 200	Toxo IgG	IU/mL	~0.5 - 600
CYFRA	ng/mL	~1 - 70	Toxo IgG Avidity	%	~40 - 90
HE4	pmol/L	~15 - 1300	Toxo IgM	COI	~0.1 - 4

The calculated within-lab CVs for all 3 sites were mostly < 2 % over all assays across the tested concentrations. Similar repeatability, between-day and between-lab variation resulted in reproducibility CVs of < 3 % for most samples, indicating the stable and consistent performance over all assays and sites.

Conclusion: The precision CVs for the 20 assays tested on the **cobas e** 801 demonstrate the good result consistency of the new high-throughput immunochemistry analyzer within and across labs.

*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2017)

B-425

Fundamental evaluation of a novel reagent for Interleukin 2 receptor measurement using general clinical chemistry analyzers

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Background: Soluble interleukin 2 receptor (IL-2R) is generally used for the diagnosis and disease activity monitoring for malignant lymphoma and any other lymphoproliferative disorders in routine laboratories. Recently, a novel reagent based on the latex enhanced immunoturbidimetric method for use on fully automated general clinical chemistry analyzers has been developed. Here we evaluated the analytical performance of this novel reagent, Nanopia IL-2R (Sekisui Medical Co., Japan). We also examined the distribution of serum IL-2R concentration in our routine laboratory. **Methods:** Serum samples collected from our inpatients/outpatients were used to evaluate the analytical performance of a novel reagent, Nanopia IL-2R on the 7180 Clinical Analyzer (Hitachi High-Technologies, Japan), and compared with IMMULITE 2000 XPi Immunoassay System and its dedicated reagents (Siemens Healthcare Diagnostics, USA) using correlation analysis. We also investigated the distribution of inpatient/outpatient serum IL-2R concentrations falling above the upper reference limit (582 U/mL) from December in 2003 to November in 2015 (12 years) using the clinical research database system. Moreover, patients with serum IL-2R concentration above 10000 U/mL were separately assessed for their pathological conditions. These studies have been approved by the ethical committee in Hamamatsu University School of Medicine. **Results:** Serum IL-2R was measured in 10646 specimens for 12 years and concentrations above the upper reference limit were observed in 2258 patients. Approximately half of the specimens were ordered by hematologists. Significant elevation above 10000 U/mL was observed in 1.6% of the total specimens. The highest value of 131000 U/mL was observed in a patient with malignant lymphoma. Other patients with significantly elevated levels of IL-2R carried malignant lymphoma, leukemia, malignant diseases, autoimmune diseases and infections. The within-run precision (CV) examined using control specimens was 2.14, 1.22 and 1.32% at approximately 500, 2000 and 5000 U/mL, respectively (n=20). The dynamic range was from 50 to 10000 U/mL. No significant interferences were observed with coexisting materials when analyzed with Interference Check A Plus and Interference Check RF Plus (Sysmex Co., Japan). The relationship between Nanopia IL-2R and Immulite 2000 examined using patient specimen was 0.992 for the correlation efficient and y=0.979x - 5.40 for the regression line (n=168). **Discussion:** The basic performance of Nanopia IL-2R was acceptable. This latex enhanced immunoturbidimetric assay reagent can be applied on any general clinical chemistry analyzer and does not require specific immunoassay analyzers. The dynamic range is acceptably wide and reasonable relative to the distribution of serum IL-2R concentrations in our hospital. In conclusion, this reagent would be useful for the diagnosis and monitoring of lymphoma and other lymphoproliferative disorders.

B-426

Bio-Rad BioPlex® 2200 and DiaSorin LIAISON® XL Throughput Comparison Study

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Background: Multiplex technology that combines multiple assays into one test bead is assumed to have a higher throughput and faster TAT than other immunoassay instruments. As laboratories are expected to do more with less, it is important to ensure the automation selected meets the needs of the laboratory. This study was conducted to compare the throughput of Bio-Rad BioPlex® 2200 (multiplex technology) with DiaSorin LIAISON® XL (conventional technology) in a real life scenario at a reference laboratory. **Methods:** Three sample scripts were developed based on real-life laboratory ordering patterns for infectious disease assays that are common to the BioPlex 2200 and the LIAISON XL. Each script was comprised of 350 samples, each sample having from 1-7 different tests ordered. Samples were tested on the LIAISON XL first, followed by the BioPlex 2200 for the same length of time. Data was collected to capture the length of time to pipette all assays, the time to first result, and the number of assays completed within the LIAISON XL run time. **Results:** Table below shows the results of the study.

	Script 1		Script 2		Script 3	
Test Case Description	MMRV, Treponema, EBV Panel, HSV-1,2, Toxo IgG, Rubella IgG & IgM, CMV IgG & IgM, plus Vitamin D on LIAISON XL		MMRV, Treponema, EBV Panel, HSV-1,2, Toxo IgG, Rubella IgG & IgM, CMV IgG & IgM, plus HAV IgM on XL, ANA on BioPlex 2200		MMRV, Treponema, EBV Panel, HSV-1,2, Toxo IgG, Rubella IgG & IgM, CMV IgG & IgM	
Measured Variable	LIAISON XL	BioPlex 2200	LIAISON XL	BioPlex 2200	LIAISON XL	BioPlex 2200
Total Assays Completed	729	631	900	895	839	818
Total Samples Processed	350	324	350	348	348	338
Total Test Time	6 h 23 min	6 h 23 min	8 h 05 min	8 h 05 min	7 h 29 min	7 h 29 min
Time to First Result	32 min	44 min	34 min	44 min	33 min	44 min
Time to Pipette 100 Assays	48 min	63 min	47 min	48 min	48 min	60 min
Time to First 100 Results	1 h 17 min	1 h 47 min	1 h 25 min	1 h 33 min	1 h 19 min	1 h 32 min
Time to First 200 Results	2 h 02 min	2 h 37 min	2 h 17 min	2 h 29 min	2 h 17 min	2 h 36 min

Conclusion: The LIAISON XL illustrated a faster time to first result and a higher throughput; regardless of the test menu it was presented with. Multiplex technology has the potential to have a very high throughput of tests, if tests ordered on patient samples utilize all of the tests on each bead. In a real life laboratory environment, tests are not ordered in the multiplex bead configuration and therefore, the BioPlex 2200 is not able to maximize the technology. The LIAISON XL is not inhibited by reagent configuration, as each assay has its own individual reagent; therefore, allowing for consistent time to pipette assays, time to first result, and time to complete testing.

B-427

Using Dried Tissue Homogenates to Preserve Solid Biospecimen for Molecular Analysis

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Background: Solid biospecimens from excised tissue represent a great source of molecular information that enables the discovery and development of personalized medicine and molecular diagnostics. Conventional methods for preservation of solid biospecimens such as FFPE or flash freezing have numerous limitations including, sample integrity, cost, infrastructure, and can be hazardous to the end user. We propose an innovative and universally accessible method to streamline biospecimen preservation, leveraging the advantages and successes from dried blood spot technology. The objective of this study is to assess the feasibility of preserving solid biospecimen as dried homogenates for molecular analysis. The recovery and quality of molecular analytes, RNA and DNA, from dried tissue homogenates was investigated.

Methods: 30 - 50 mg of rat tissue (liver, kidney, lung, heart, and spleen) was excised from a frozen section and homogenized with PBS in a Dounce homogenizer. The homogenate was then added to a HemaSpot -HF device by pipette and allowed to dry at room temperature. Total RNA was extracted using the E.Z.N.A. mini column (Omega BioTek) and quantified by NanoVue and or a RiboGreen assay. RNA quality was determined by a Bioanalyzer. Reverse transcription was carried out by SuperScript IV (Applied Biosystems) and p53 expression was measured by Taqman (ABI). Genomic DNA was isolated from dried homogenates by a white blood cell lysis method. DNA recovery was measured by PicoGreen assay.

Results: : RNA and DNA was found to be stable in dried homogenates stored at ambient temperatures up to six months. The criteria used for acceptable RNA was an absorbance values (A260/A280) of ≥ 1.8. The p53 gene was detected by Taqman analysis in all tissue types studied (liver, kidney, and spleen) with greatest expression observed in rat liver tissue. The highest content of genomic DNA was observed in the dried liver samples.

Conclusion: The ability to detect molecular analytes from dried solid biospecimens demonstrates feasibility for preserving tissue biopsies as dried homogenate for molecular analysis. This method provides a simplified, low cost tissue specimen preparation and storage method with minimal processing and refrigeration while maintaining sample integrity for analysis of critical molecular analytes.

B-428

Basic performance of the new sepsis marker; presepsin immunoassay on STACIA®

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Background/Purpose: Presepsin is a useful sepsis biomarker found in Japan. It is known to be produced by cleavage of CD14 with some proteases in a phagocytosis process. Therefore, it is said that presepsin value is less susceptible to the effect of trauma, burns and surgery than PCT and also increases rapidly at an early stage of sepsis. As a result, presepsin is also said to reflect the clinical course and severity of sepsis patients well. Recently, presepsin received higher recommendation than PCT as an auxiliary diagnostic test in Japanese version of sepsis guideline 2016.

We evaluated the assay performance of a new presepsin immunoassay on STACIA® and examined its usefulness, reliability and continuity with a current PATHFAST® Presepsin data.

Principles/Methods: STACIA® is an all-in-one instrument which consolidated major clinical assay principles such as chemiluminescence enzyme immunoassay (CLEIA), coagulation time, chromogenic substrate, latex agglutination, immunoturbidimetry, biochemistry. All results are available within 19 minutes and throughput is 270 tests per hour with fully random access.

The test method of presepsin assay on STACIA® is fully automated one-step sandwich immunoassay based upon CLEIA. Alkaline phosphatase (ALP)-labeled anti-presepsin monoclonal antibody reacts with presepsin in sample. After that, anti-presepsin monoclonal antibody-coated magnetic latexes (MG-LTX) specifically react with presepsin. Finally, after B/F separation, ALP on MG-LTX decomposes CDP-Star® substrate to an excited intermediate, which produces luminescent signal. Presepsin concentration is determined by comparison with the calibrator signal.

Results: The presepsin assay has an analytical assay range from 50 to 20,000 pg/mL. Between day repeatability showed that a within-run and a total imprecision were less than 5%. Dilution recovery was excellent with mean recoveries within ±10% for all samples. Method comparison against PATHFAST® Presepsin showed a good correlation: y = 1.06x - 43.24, r = 0.994, n = 142 (y: STACIA® CLEIA Presepsin, x: PATHFAST® Presepsin). Further, it was shown a good correlation between plasma and serum samples: y = 0.99x + 31.07, r = 0.998, n = 98 (y: serum, x: EDTA plasma). The reference interval for normal donors was 59.0 -249.6 pg/mL, n = 198 (EDTA plasma).

Conclusions: The newly developed presepsin immunoassay was rapid and precise assay. In addition, it was highly correlated with the current method and further it could test large numbers of specimens at central laboratories. These results suggest that STACIA® CLEIA Presepsin is useful for the daily monitoring of sepsis patients. Furthermore, that may lead to the rapid medical care, e. g. drug administration for treatment of sepsis.

B-429

Evaluation of the Roche immunochemistry platform cobas e 801 module

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Objectives: The **cobas e** 801 module was evaluated by Cleveland Clinic Laboratories with selected applications of assays that represent the entire assay menu and challenge all functionalities of the **cobas e** 801 module. The **cobas e** 801 module is the newest member of the cobas 8000 modular analyzer series. The **cobas** 8000 modular analyzer series system is a fully automated, random-access, software controlled system for immunoassays and photometric analysis. The evaluation configuration consisted of **cobas** 8000 core and a **cobas e** 801 module.

Methods: The system and selected assays were evaluated for within-run-precision (1 run x 21 replicates on one measuring cell), repeatability CLSI (EP05-A3) (21 days x 2 runs/day x 2 replicates), performance of daily QC as a measure of calibration stability, routine simulation precision, in which a test CV based on randomized testing is compared to a CV based on within-run batch testing.

Results: Within-run-precision was well within acceptance criteria of CV ≤5% for all assays except folate, which had acceptance criteria of SD ≤0.35. Repeatability precision met the acceptance criteria of CV <5%.

Test, Unit	Within-Run-Precision %CV		Repeatability (21 day) %CV						
	mean	CV	mean	CV	n	mean	CV	mean	CV
A-HCV II, COI	0.07	1.1	3.70	0.6	84[2]	0.07	1.1	3.70	0.8
CA 15-3, U/mL	22.1	1.7	91.1	1.5	168	22.3	1.7	91.4	1.6
CEA ng/mL	4.86	1.1	47.1	0.8	168	4.87	1.4	47.4	1.2
E2, pg/mL	87.6	0.9	415	1.3	168	79.7	2.5	391	1.4
FOL, ng/mL	2.80	5.5[1]	10.9	2.2	84[2]	2.92	4.2	11.7	2.7
FT4, ng/dL	1.19	1.5	3.14	2.0	168	1.21	2.1	3.11	2.5
Pro BNP(STAT) pg/mL	148	1.1	4832	1.1	84[2]	149	1.9	4874	1.3
Pro BNP, pg/mL	132	1.3	4304	0.9	168	135	2.0	4363	1.6
TESTO, ng/dL	551	1.4	235	1.5	168	534	1.7	226	1.5
TSH, µU/mL	1.49	0.8	8.74	0.6	168	1.48	1.4	8.60	1.3
[1] SD is 0.15 (criteria SD ≤0.35) [2] Performed on only one measuring cell									

Precision of daily QC was evaluated on 8 tests over a period of days (shortest 65 days to longest 95 days), with multiple runs and reagent e packs between calibrations. Good precision for two control levels demonstrated acceptable calibration stability (CV's 1.7% to 3.7%). Routine simulation precision testing with 627 requests analyzed in 2 hours 19 minutes had reference CV's of 0.7 to 2.0 and random CV's ranging 0.8 to 1.6.

Conclusion: Analytical performance on the **cobas e** 801 module met acceptance criteria for within-run-precision, repeatability, QC precision over many days, runs and reagent e packs demonstrating calibration stability, and routine simulation precision.

B-431

A Novel High Speed and High Performance Calprotectin PETIA for Serum and Plasma Samples

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BACKGROUND

Human calprotectin (MRP8/MRP14) in serum and plasma has proven in several publications to be a promising inflammation bio-marker in several inflammatory conditions. Currently only ELISAs exist for the quantification of calprotectin in serum and plasma.

A fast, high performance Calprotectin PETIA (Particle Enhanced Tubidimetric Immunoassay) is under development by Gentian Diagnostic As. It is believed to be cheaper and faster (due to random access options) than Calprotectin ELISAs.

The assay uses polyclonal avian antibodies, raised to detect human MRP8/MRP14 complexes. Avian antibodies have the advantage of not reacting with rheumatoid factors, human anti-mouse IgG antibodies (HAMA) or the human complement system.

The assay calibrators contain highly pure Calprotectin antigen from human granulocytes, value assigned by UV_{280} and Biuret.

The purpose of this study was to demonstrate high speed and high performance of the Gentian Calprotectin PETIA on Abbott Architect c4000 Clinical Chemistry Analyzer.

METHODS

Calprotectin was measured in human serum and plasma samples using the Gentian Calprotectin PETIA on Architect c4000. The assay consists of reaction buffer (R1) and immunoparticles (R2), calibrators (6 levels) and controls (2 levels).

The following studies were performed and assessed according to CLSI guidelines, where applicable.

- Precision (within run and total within lab)
- Detection capability (LoB, LoD and LoQ)
- Linearity
- Security zone
- Interference and cross-reactivity
- Between instrument variations and lot variations
- Method comparison (vs IDK® Calprotectin ELISA)
- Assay stability

The assay was designed to have a calibration range of approx. 0-20 mg/L, with QC controls of ~1.0 and ~10.0 mg/L.

RESULTS

Gentian Calprotectin PETIA demonstrated

- Detection capability: LoB (0.05 mg/L); LoD (0.07 mg/L); LoQ (0.30 mg/L)
- Security zone up to 100 mg/L
- Within run precision: CV from 0.24 % to 3.45 % for samples in the range (0.90-16.0 mg/L); total within lab precision (20 days, 2 runs per day, 2 replicates per run): CV < 6 % for samples in the range (0.90-16.0 mg/L)
- Linear range: 0.39-18.19 mg/L
- Method comparison (vs IDK® Calprotectin ELISA): correlation ($R^2 > 0.98$) when measuring serum samples (n >100; n: number of samples) spanning from approx. 0.50-18.0 mg/L
- Lot variations: average % bias (Bland Altman) between two lots was 4.20 % when measuring serum samples (n >100) spanning from approx. 0.50-18.0 mg/L- Instrument variations (Architect c4000 vs Mindray BS400): average Passing Bablok slope 1.02 and average intercept 0.03 mg/L when measuring serum samples (n >100) spanning from approx. 0.50-18.0 mg/L
- Interference: no significant interference was detected by testing 8.0 g/L hemoglobin, 600 mg/L bilirubin and 10.0 g/L intralipid.
- Cross reactivity: no significant cross reactivity was observed with monomer MRP8, MRP9 and MRP6
- Assay stability: on board stability of reagents (> 8 weeks); calibration curve stability (2 weeks)
- Total test time: approx.10 minutes

CONCLUSIONS

The Gentian Calprotectin PETIA demonstrates high speed and high performance in these evaluation studies. The assay could be used as tool for professional lab users in order to measure calprotectin in serum and plasma precisely, accurately and rapidly.

B-433

Development of a Novel Assay for the Simultaneous Identification of Deinococcus radiodurans and Determination of Susceptibility to a Selected Antibiotic

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Objective: To examine the ability of a novel diagnostic ELISA to identify *Deinococcus radiodurans* in a reduced time when compared to culture. *D. radiodurans* is a relatively new bacteria, first discovered in spoiled food which had first been irradiated. Although slow growing, the bacterium has been shown to be very resistant to radiation, extremes in temperature, and dehydration. As culture of this organism may take up to two weeks, food and beverage handlers, as well as medical device manufactures, would benefit greatly from an assay that allows for more rapid detection of *D. radiodurans*.

Methods: Microtiter wells were coated with polyclonal IgG rabbit antibody directed against *D. radiodurans* at a dilution of 1:50 in coating buffer. After 120 minutes, wells were emptied and then blocked with StartingBock™. After blocking for 30 min, wells were emptied, and then bacterial isolates were added. For the 30-minute test, a series of dilutions of *D. radiodurans* were prepared in PBS, starting at 10^7 bacteria/ml, and diluting out ten-fold to 10^0 bacteria/ml. Samples were either run with *D. radiodurans* alone, or in the presence of other selected bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, Gordonia, *Streptococcus agalactieae*, Kocuria, Roseamonas) all at 10^7 bacteria/ml. In order to increase the limit of detection, *D. radiodurans* dilutions were prepared in FastidiousBroth™, and cultured for predetermined lengths of time. After this culture period, samples were added to microtiter wells diluted 1:1 in phosphate buffered saline (PBS), and allowed to stand at room temperature for 30 minutes. Following this incubation step, wells were washed, and then bound bacteria was detected with HRP-conjugated anti-*D. radiodurans* antibody at 1:50 dilution for 20 minutes at room temperature. Wells were then washed, and signal was generated with TMB solution. Optical density was read at 450 nm.

In order to determine antibiotic susceptibility, *D. radiodurans* dilutions were incubated for 72 hours in increasing concentrations of Cefazolin, starting at 0.08 ug/ml, and increasing up to 32 µg/ml. Cefazolin was selected from a panel of antibiotics which were shown to promote inhibition of growth by in-house turbidity assays.

Results: Following a 30-minute incubation in PBS, *D. radiodurans* was detected at a limit of 10^6 bacteria/ml. No interference was observed with any of the other bacteria tested. By increasing the incubation time in broth, the limit of detection increased to 10^5 bacteria/ml at 24 hours, 10^4 bacteria/ml at 48 hours, and 10^1 bacteria/ml following 72 hours. When dilutions of *D. radiodurans* were prepared in the presence of Cefazolin, the limit of detection (LoD) decreased as the antibiotic concentration

increased: with 0.08 µg/ml Cefazolin, the LoD was 10^2 bacteria/ml; with 0.8 µg/ml Cefazolin, the LoD was 10^4 bacteria/ml, and with 8 µg/ml Cefazolin, the LoD was 10^6 bacteria/ml; following 72-hour incubation.

Conclusion: This novel assay allows for the simultaneous identification *D. radiodurans* and determination of antibiotic susceptibility in as little as 72 hours.

B-435

Evaluation of the Abbott Alinity Clinical Chemistry and Immunoassay Systems

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Background. To evaluate the performance of the newly developed Alinity clinical chemistry (CC) and immunoassay (IA) systems in an independent laboratory and compare it to the performance of the respective Architect systems.

Methods. For CC performance AST, ALT, calcium, and total protein were analyzed. For IA qualitative and quantitative HBsAg-Assays, anti-HCV, combined HIV antigen and antibody (HIV-Combo), HTLV-I/II, and syphilis were analyzed. For all linearity and precision tests control material was used.

Results. For all four CC assays linearity of the Alinity results was excellent with coefficients > 0.999 over a broad concentration range. Slopes of the regression lines were between 1.00 and 1.04. Within day, between day, and total %CV was always <1.8 % with the exception of the low level of ALT which had a between day %CV of 2.6% and a total %CV of 3.56%. Analytical precision of the IA are listed in the table.

Throughput of the Alinity CC system was compared to Architect c8000 module and was approx. 10% higher. The IA system performed approx. 160 tests per hour. Finally, correlations of the Alinity results with the Architect system were analyzed with serum or plasma samples. For CC all correlation coefficients were > 0.99 over a broad concentration range. Agreement between Alinity and Architect IA was between 95 and 100% for all assays.

Conclusions. Several CC and IA tests for the novel Alinity systems have been evaluated under conditions of routine laboratory testing. Linearity, precision, and correlation to the current Architect systems have been fully satisfying. Sample throughput of the Alinity systems is moderately higher than with the Architect systems, but with a much smaller footprint.

Immunoassay precision			
Assay	**Level**	**Within day %CV**	**Total %CV (95% CI)**
HBsAg (qual)	neg	8.32	10.56 (7.48-17.95)
	pos	1.84	1.98 (1.51-2.89)
HBsAg (qual – confirm)	pos	2.40	2.68 (2.04-3.93)
HBsAg (quant)	neg	0.00	0.00
	pos*	2.69	2.77 (2.15-3.88)
Anti-HCV	neg	5.35	5.92 (4.52-8.62)
	pos	3.04	3.71 (2.73-5.78)
HIV-Combo	neg	9.70	11.19 (8.41-16.70)
	pos*	2.55	2.85 (2.17-4.18)
rHTLV I/II	neg	8.02	8.12 (6.33-11.34)
	pos	7.53	8.24 (6.31-11.90)
Syphilis	neg	0.00	0.00
	pos	1.25	1.42 (1.07-2.10)

*) If more than one positive level was tested, the lowest positive level is shown

B-436

Biologic drug monitoring assays

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Background: Monoclonal antibodies (mAbs) are among the most rapidly growing class of pharmaceuticals and the most expensive. Pharmacokinetic (PK) variability among patients treated with these drugs is significant, typically varying by over two orders of magnitude, and blood drug levels correlate with clinical efficacy in most cases. This variation can be compounded in combination therapies or by formation of anti-drug antibodies during prolonged treatment. Nonetheless, therapeutic dose monitoring is not routine practice partly to due to the lack of robust clinical laboratory or point-of-care (POC) assay solutions.

Methods: Peptide mimetic ligands are an attractive option for immunoassay reagents because they are stable, highly selective, and easier to develop and manufacture than natural ligands or anti-idiotype antibodies. We have developed mimetope peptides, termed Veritopes™, against a broad range of therapeutic mAbs, and these peptides are ideal for capture and quantification of free and active mAbs in biological samples such as human serum. We have implemented these peptides in ELISA format, where they are used as a surrogate ligand to capture the drug. We have developed Veritopes for several widely used mAb drugs, including natalizumab, vedolizumab, rituximab, trastuzumab, ipilimumab, and pembrolizumab. Veritope ELISAs can be commercialized as laboratory developed tests (LDTs). Alternatively, Veritopes can be integrated into lateral flow assays for POC dose monitoring applications such as patient stratification during clinical trials or personalized dosing of marketed drugs.

Validation: When used as a capture reagent in ELISA, these mimetope peptides display sufficient sensitivity, specificity, and linearity across the requisite concentration ranges relevant for most mAb PK studies. In all cases, the selected peptides effectively bind only the intended target in the presence of circulating human IgG and do not crossreact with other mAbs. The natalizumab ELISA was analytically validated in a CLIA setting in preparation for future marketing as an LDT. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for natalizumab in undiluted serum were determined to be 2.0ug/mL and 16.0ug/mL, respectively. These data were obtained from 5 independent runs, where each sample was run in triplicate. The Limit of Blank (LoB) and Limit of Detection (LoD) were 0.6ug/mL and 0.8ug/mL, respectively. Intra- and inter-assay accuracy and precision were determined using spike and recovery experiments with three concentrations of natalizumab covering the dynamic range and analyzed in five independent runs either in triplicate or quintuplicate. Analyte recovery was calculated for each concentration as a measure of accuracy and was consistently between 80% and 120% of nominal concentrations (Calibrated value/Nominal Value*100). Intra- and inter-assay precision were calculated using the same samples, and the coefficients of variation (%CV) were below 15% for all concentrations tested (SD/mean*100).

Results and Conclusion: Veritopes are robust reagents adaptable to a variety of immunoassay formats suitable for both laboratory and POC measurements of mAb levels in biological samples such as human serum. Direct monitoring of mAb drug levels in patients will enable precise, personalized dosing that can improve outcomes, minimize side effects, and reduce treatment costs.

B-437

Report on a European and two Korean population clinical trials for multiplex detection of HIV and HCV

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Background:

A novel, multiplex detection system for infectious diseases is based upon standard ELISA protocols, with the main difference that the process is conducted in an actual 3-D environment. The sol-gel nanoporous capturing technology represents a powerful approach where the sol-gel matrix constrains the motion of the encapsulated biomolecules (proteins, peptides, chemicals, antibodies, oligonucleotides, etc.) without physical adsorption or any modification. This technology can be applied to multiplex immunoassay platform because several disease biomarkers can be immobilized and tested concomitantly in a single well.

Methods:

The Hi3-1 Multiplex HIV1/2 and HCV antibody detection kit, a two-step fluorescence-based immunoassay, is designed to detect antibodies against HCV protein (Core, NS3, NS4, NS5) and HIV 1/2/O type protein, respectively, in human serum or plasma. Sol-gel spots are arrayed on the bottom of a microtiter plate wells, and antigens from HIV1/2/O and HCV are encapsulated within two sets of spots in each well. HIV and HCV antibodies in serum or plasma bind to antigens in the sol-gel spots and form antigen-antibody-fluorescently labeled secondary antibody complexes. Following a wash cycle, fluorophore-labeled secondary antibodies against human IgG and IgM are added to the wells. After washing to remove samples and unbound fluorescently labeled antibodies, the plate is scanned in a fluorescence scanner. In the absence of HIV and HCV antibodies, no fluorescence is detected. The clinical trial for this system is performed according to CTS guideline (Guidance on the In Vitro Diagnostic Medical Devices Directive 98/79/EC; Commission Decision of 3 February 2009) and KFDA guidelines (Release No.B1-2012-5-005).

Results:

In the Clinical trial at the Korea University Guro Hospital in Korea, the results suggest that the sensitivity of both the HIV-Ab and HCV-Ab assays using Hi3-1 kit was 100.00% [100%, n=353: including 102 HIV Korean positive specimens, 150 HIV 1, 100 HIV 2 and 1 HIV 1 O subtypes, and n=431 HCV Korean positive] and the concordance of the corresponding HIV Ab and HCV Ab assays between the Hi3-1 system and the Architect systems for negative specimens was 99.96% (n=4,479 negative specimens for HIV) and 99.76% (n=4,150 negative specimens for HCV), respectively.

In the Clinical trial at the Seoul St. Mary's Hospital in Korea, the result showed a highest sensitivity (100%, n = 500 HIV-positive specimens and n = 400 HCV-positive) and specificity (100% for HIV 1/2 and 99.84% for HCV, n = 4,306 negative specimens) by using the kit, which simultaneously screens for the presence of HIV1/2 and HCV antibodies.

In the clinical trial at CERBA in France, a total of 3400 clinical negative samples (collected from the CERBA Specimen Service of France) were tested for HIV1/2 and HCV using the Hi3-1 kit. Concordance of the corresponding HIV Ab and HCV-Ab assays between the Hi3-1 and Architect systems for panel 1 was 99.97% and 99.82%, respectively.

Conclusion:

Given that the new technology has sensitivity and specificity equivalent to the commercially available CLIA tests, the sol-gel based microarray has the potential to be used as a high-throughput screening tool for simultaneous detection of HIV and HCV in blood banks.

B-438

Process Qualification for Production and Purification of OC125 Antibody in Cell Culture

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Background: Production of quality monoclonal antibody (mAb)suitable for serological diagnosis and compliant with ISO standards and Regulatory Regulations, is key to *IVD* industry. Ascites generation and bioreactor systems represent *in vivo* and *in vitro* approaches for mAb production, respectively. Bioreactor systems have some advantages over ascites generation in preventing the introduction of endogenous contaminating protein and reducing variability in antibody generation (Marx 1995; Jackson 1996; Bruce 2002). This study was to qualify the production and purification of OC125 antibody, a mAb against cancer biomarker OC125 defined antigen, produced in a bioreactor system. **Methods:** Three lots of an OC125 proprietary mouse hybridoma cell line were cultured in the Applikon EZ-Control bioreactor containing serum-free media for at least 70 days, with at least 14 harvests. The supernatants were purified on a mAb SelectSuRe column, concentrated with a Pellicon unit and polished with SP Sepharose HP column. Samples from each lot were assessed for functionality by incorporating the antibody on a mainstay *IVD* immunoassay platform, and characterized with the HPLC, isoelectric focusing electrophoresis (IEF), Immunoelectrophoresis (IEP) and SDS-PAGE electrophoresis. Moreover, the hybridoma cells were collected prior to culture and at the end of 90 day culture to undergo cDNA sequencing of the antibody. **Results:** The collected total volumes of three lots of culture supernatants were 533, 893 and 1301 L, and the final yields of purified antibodies were 6.3, 45.7 and 59.3 g, respectively. The purified OC125 antibody samples were coated on the solid phase of a mainstay *IVD* automated immunoassay platform. Functional testing of a reagent set with the OC125 antibody-coated on solid phase led to an acceptable Calibrator B/A ratio >7; Calibrator B, C, D, E, and F within the limit of 12.4 - 25.5, 40.7 - 108.2, 119.3 - 322.1, 272.3 - 718.2 and 624.9 - 1363.6 U/mL, respectively; and Control L, M and H within the limit of 32.0 - 48.0, 240.0 - 360.0 and 520.0 - 780.0 U/mL, respectively. SDS-PAGE of the antibody showed a single primary band of ~200 kDa in non-reduced gels, and two primary bands of ~50 kDa and ~30 kDa, respectively. IEF gel running of the antibody showed three bands at pI of 6.5 - 7.0 consistently. IEP gel running denoted the OC125 mAb as IgG1 type. HPLC-SEC analysis demonstrated the purity of all three lots of the purified mAb at 100% with a clear uniform peak at the retention time of ~7.5 minutes consistently. All of the gels were comparable to or better than the reference. The sequencing results denoted 100% alignment match between the cells prior to culture and that at the end of the 90-day culture, indicating the cell line was stable during the 90-day production cycle. **Conclusion:** The functionality and characterization of three lots of OC125 antibodies derived and purified from the bioreactor-based cell culture system met all of the acceptance criteria for release and bulk functional testing. The methods and techniques utilized to produce and purify the antibody are validated to be a robust process for production and purification of OC125 Antibody.

B-439

Advanced Centrifugal Microfluidic Platform for the Automation of Clinical Assays

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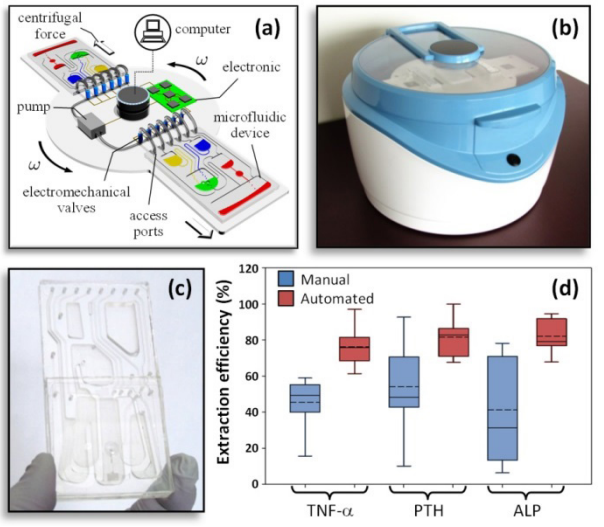
Background: Centrifugal microfluidics offers the interesting prospect to automate the liquid handling steps required by clinical assays. Unfortunately, traditional centrifugal microfluidics offers only limited liquid control capabilities, which is problematic for the integration of complex assays. Herein, we report the development of an automated protein extraction assay from whole blood using a novel centrifugal microfluidic platform where advanced liquid control is achieved through a combination of centrifugal forces and active pneumatic pumping.

Methods: We fabricated a centrifugal microfluidic platform capable of applying air pressure pulses (0-5 psi) to the ports of microfluidic devices while the platform is rotating at high speed (1000 rpm), providing precise control to automate on-chip liquid handling steps (Fig. 1a and b). Microfluidic devices were fabricated from low-cost thermoplastic materials and contained no active components such as valves or electrodes (Fig 1c). Capture of target proteins is performed on 100 µm silica beads functionalized through carbodiimide chemistry and conjugated with antibodies for TNF-α, PTH or ALP.

Results: Extraction of target proteins from whole blood is shown here as an example of an assay that can be automated with the developed technology. For this assay, the following steps were successfully automated: metering and transfer of a density gradient medium, transfer and metering of a blood sample from an external tube (600 µl), blood fractionation, plasma extraction, active back-and-forth displacement of plasma through a bead-bed containing functionalized silica beads, three washes, elution, and transfer of eluted sample to an external vial. The automated assay demonstrated significantly higher protein extraction efficiency (about 80%) and lower variance compared with assays performed manually using standard protocols (Fig 1d).

Conclusion: Using the developed centrifugal microfluidics platform, a multistep protein extraction assay was successfully automated using passive thermoplastic microfluidic devices, which highlights the potential of the technology for clinical applications.



B-440

A Comparison of the New Beckman Coulter DxC 700 AU Clinical Chemistry System to the UniCel DxC 800 Synchron Clinical System

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Background: The Beckman Coulter DxC 700 AU analyser is the latest clinical chemistry system from Beckman Coulter. It is a fully automated, random access analyzer, designed for medium to high throughput laboratories, with a throughput of 1200 tests/hour including ion selective electrodes. The purpose of this study was to compare the recovery of patient samples on the new DxC 700 AU with the UniCel DxC 800 Synchron Clinical Systems for a selection of routine assays.

Methods: To compare the Beckman Coulter DxC 700 AU and the UniCel DxC 800 Synchron Clinical Systems, several Beckman Coulter assays were selected for evaluation that covered serum and urine sample types and a range of assay methodologies. These systems were compared using patient serum or urine samples. Samples were run in duplicate and the sample means compared using Deming regression.

Results: All DxC 700 AU assays showed excellent correlation with the UniCel DxC 800 Synchron Clinical System. The Deming regression statistics parameters for selected assays are summarised in the table below.

Assay	Units	N	Slope (95% CI)	Intercept (95% CI)	R	Range
Glucose	mg/dL	130	1.030 (1.028 to 1.033)	-2.7 (-3.2 to -2.3)	1.000	12.5 to 760.4
Creatinine	mg/dL	122	0.966 (0.962 to 0.969)	0.05 (0.04 to 0.07)	1.000	0.38 to 23.54
BUN	mg/dL	98	0.990 (0.987 to 0.993)	-0.4 (-0.5 to -0.3)	1.000	11.0 to 123.8
Albumin	g/dL	121	0.935 (0.916 to 0.954)	0.36 (0.28 to 0.44)	0.988	1.56 to 5.22
Total Protein	g/dL	134	1.022 (1.010 to 1.033)	0.1 (0.0 to 0.1)	0.996	3.4 to 10.2
ALP	U/L	110	1.065 (1.059 to 1.071)	0.50 (-0.2 to 1.2)	0.999	24.8 to 718.6
AST	U/L	118	0.913 (0.908 to 0.918)	-3.0 (-3.2 to -2.8)	0.999	8.2 to 360.2
GGT	U/L	73	1.181 (1.175 to 1.187)	-1.2 (-1.6 to -0.8)	1.000	6.2 to 459.9
IgG	mg/dL	120	0.895 (0.886 to 0.904)	30.4 (20.1 to 40.7)	0.997	174.9 to 2845.3
Urinary Albumin	mg/dL	77	1.023 (1.010 to 1.036)	-0.2 (-0.4 to 0.1)	0.997	1.6 to 29.4

Conclusion: The results of the study demonstrate that the new Beckman Coulter DxC 700 AU analyzer has comparable performance to the UniCel DxC 800 Synchron Clinical System.

B-441

Quality Control Algorithm for Protein Determination Using Coomassie Brilliant Blue

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Background: Accurate protein determination is essential in biochemical laboratories and for the manufacture of immunoassays. Interfering substances can lead to erroneous results. Quality control (QC) testing helps to identify issues. When using dyes such as Coomassie brilliant blue (CBB), bromocresol green, and bromocresol purple, spectral analysis represents an elaborate, but laborious QC. Reference measurements at single wavelengths, typically at wavelengths far higher than the actual measurement, are more practical. However, subtle changes caused by interfering substances can be missed. We set out to assess the use of reference measurements closer to the actual measurements as a QC.

Methods: Using the CBB assay in a microplate format as a model, we determined the spectra of various proteins at different concentrations ranging from 10 to 100 µg/mL using the SpectraMax Plus Microplate Reader (Molecular Devices). Focusing on the determination of bovine serum albumin with and without interfering substances, the utility of applying absorption values at various reference wavelengths around the actual measurement wavelengths was assessed.

Results: The use of the isosbestic point of the CBB protein reaction at ~530 nm proved to be a convenient reference measurement. The interference of substances such as glycine, SDS, and HEPES could be determined by an upper and lower limit independent of the absorption at the actual measurement wavelength. When using other reference wavelengths, e.g., 440 nm, a function between the absorption at the actual and reference measurement wavelengths had to be applied. Adequate upper and lower confidence limits of polynomial or linear regression could be used as thresholds to identify the interference.

Conclusion: Reference measurements and respective algorithms can aid in the identification of erroneous results of protein determination by CBB and potentially other assays for protein determination using respective dyes.

B-442

Evaluation of Immunoglobulin G_2 and Immunoglobulin M_2 Assays* on the Atellica CH Analyzer**

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Background: This study evaluated the performance of the Immunoglobulin G_2 (IgG_2) and Immunoglobulin M_2 (IgM_2) assays,* clinical chemistry PEG-enhanced immunoturbidimetric assays*, being developed for use on the Atellica™ Chemistry (CH) Analyzer** (Siemens Healthcare Diagnostics Inc.).

Methods: Performance testing was conducted using Clinical and Laboratory Standards Institute (CLSI) guidelines and included precision (CLSI EP05-A3); method comparison (CLSI EP09-A3); limit of blank (LoB), detection (LoD), and quantitation (LoQ) (CLSI EP17-A2); interference (CLSI EP07-A2); and serum/plasma equivalence (CLSI EP09-A3) studies.

Results: Assay range for the IgG_2 assay was 140–3400 mg/dL (1.40–34.00 g/L) and for the IgM_2 assay was 21.0–330.0 mg/dL (0.21–3.30 g/L). Observed agreement in patient sample method comparison studies using Deming regression: Atellica CH IgG_2 assay = 1.00 × ADVIA® Chemistry IgG_2 assay + 6 mg/dL (+ 0.06 g/L) (r = 0.999, n = 113, range: 148–3593 mg/dL [1.48–35.93 g/L]); Atellica CH IgM_2 assay = 1.01 × ADVIA Chemistry IgM_2 + 2.5 mg/dL (+ 0.02 g/L) (r = 0.999, n = 105, range: 21.7–324.7 mg/dL (0.22–3.25 g/L). Precision was evaluated across the assay range using serum/plasma pools and commercial quality control materials. Each sample was assayed in duplicate twice a day for 20 days. IgG_2 assay repeatability and within-lab precision were ≤1.6% CV and ≤1.8% CV, and IgM_2 assay repeatability and within-lab precision were ≤1.1% CV and ≤2.5% CV, respectively. LoB and LoD were observed to be 7 mg/dL (0.07 g/L) and 21 mg/dL (0.21 g/L) for the IgG_2 assay and 1.9 mg/dL (0.02 g/L) and 3.9 mg/dL (0.04 g/L) for the IgM_2 assay, respectively. Interference ≤10% was observed for the IgG_2 assay with hemolysate (1000 mg/dL), bilirubin (50 mg/dL), and lipemia (1000 mg/dL) and for the IgM_2 assay with hemolysate (1000 mg/dL), bilirubin (60 mg/dL), and lipemia (1000 mg/dL). Agreement of serum and plasma in the IgG_2 assay is represented by lithium heparin plasma = 1.01 × serum − 26 mg/dL (− 0.26 g/L) (r = 0.998, n = 54, range: 252–2498 mg/dL [2.52–24.98 g/L]) and in the IgM_2 assay is represented by lithium heparin plasma = 1.01 × serum − 2.1 mg/dL (− 0.02 g/L) (r = 0.999, n = 54, range: 25.8–319.5 mg/dL [0.26 – 3.20 g/L]) and K EDTA plasma = 0.99 × serum − 0.1 mg/dL (− 0.00 g/L) (r = 0.999, n = 54, range: 25.8–319.5 mg/dL [0.26–3.20 g/L]).

Conclusions: The Immunoglobulin G_2 and Immunoglobulin M_2 assays tested on the Atellica CH Analyzer demonstrated acceptable performance in all tested areas.

*All assays under development and not available for sale. Future availability cannot be guaranteed.

** Not available for sale. Not CE marked. Future availability cannot be guaranteed.

B-443

Evaluation of Cell-Free DNA Recovery During Extraction Using A Next-generation Sequencing Assay

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Background: In solid organ transplant recipients, circulating cell-free DNA from the donor in the background of the recipient's DNA measured as percent donor-derived cfDNA (dd-cfDNA) is an important marker for allograft rejection. We have developed a non-invasive next-generation sequencing (NGS) assay that utilizes SNPs located throughout the genome to quantify the dd-cfDNA (AlloSure®). Prior knowledge of donor and recipient genotypes are not required to measure the proportion of cfDNA in the recipient's plasma that is released from the donated organ. The levels of cfDNA found in plasma are low which challenges the recovery of cfDNA during sample extraction. In order to accurately quantify the low amounts of cfDNA, the extraction method must be robust and reproducible. Extraction methods must be tested to identify the best method for each specific assay employing cfDNA.

Objectives: The objective of this study was to determine the reproducibility and linearity of cfDNA extraction in our lab. Reproducibility was analyzed using multiple operators, replicate extractions and extractions on multiple days. Linearity was assessed by performing dilutions of plasma made before extracting.

Methods: Blood was collected from normal healthy volunteers into Streck Cell-Free DNA BCT® collection tubes. Panels were created by mixing plasma from one individual (donor) into the plasma of another (recipient) in proportions that are consistent to those found in transplant patients. Three panels were created to test

reproducibility representing 3 different spike-in levels. To assess linearity, dilutions were made of spike-in samples using the plasma from the "recipient" to dilute the proportion of cfDNA present from the "donor". Plasma was extracted using Qiagen's Circulating Nucleic Acid kit. After extraction, samples were quantified for total cfDNA using a qPCR method while dd-cfDNA was calculated using the AlloSure workflow.

Results: Total recovery of cfDNA as measured by qPCR ranged from 11 to 34ng per 5ml of plasma which is consistent with ranges expected from healthy volunteers. Intra-operator variability for total cfDNA ranged from 1.5% to 11% CV. The inter-operator variability ranged from 9% to 17%. When dd-cfDNA was measured, the intra-operator variability ranged from 5.6% to 7.8% and the Inter-operator CVs ranged from 1.7% to 12.8%, both within the established variability of the assay. Linearity tests showed the assay performed as expected; the measured dd-cfDNA was consistent with expected values based on dilution.

Conclusions: The recovery of cfDNA was reproducible within one operator and between operators. The cfDNA from the donor is consistently recovered at different spike-in amounts indicating that the extraction method is appropriate for use with AlloSure where low levels of donor cfDNA require reproducible and robust recovery.

B-444

Standardized Reagent Formulation and Data-driven QC Criteria Ensure Efficient and Consistent Delivery of Plasma Cell-free DNA Results for Organ Transplant Rejection

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Background: Workflows for complex molecular diagnostic testing often require multiple steps and formulation of specialized reagents. High quality standardized reagents are critical to ensure accurate and consistent test results. AlloSure® is a recently released Laboratory Developed Test that quantifies the amount of donor-derived cell-free DNA (dd-cfDNA) in the plasma of kidney transplant recipients by targeted next generation sequencing of 266 SNPs. We identified key reagents in the AlloSure workflow that would benefit from transfer to our reagent manufacturing group for formulation. Formulation of these reagents under a GMP compliant lab ensures standardized and validated processes, QC prior to release for use in patient testing, and reduces variabilty introduced when reagents are prepared at the time of testing. The transfer of these key reagents to our manufacturing group required establishing independent QC processes and acceptance criteria in addition to training staff and validating the successful transfer of the formulation procedures.

Objectives: The purpose of this study was to establish formulation procedures and defined QC criteria for key reagents used in a cell-free DNA next generation sequencing assay.

Methods: The preparation of 266 AlloSure targeted amplification primers was identified as a key component that warrants transfer to our manufacturing group. Transfer of the primer preparation, both as a pool used in the pre-amp step and the 48 different multiplexes used in the secondary amplification, included creating a training plan, SOPs, batch records, standardized lot labeling, and production of 3 independent lots before the official transfer was complete. QC criteria were developed by testing the NIST NA12878 reference standard in the AlloSure NGS workflow over the course of 1 year. The criteria used for passing lots of primers were set using number of SNPs that pass QC as determined by the same AlloSure QC algorithm. Statistical analysis of the number of AlloSure SNPs successfully sequenced was performed using binomial quantile estimation. The data from this analysis were used to define the QC testing criteria for the formulated primer pools. Additional QC criteria which requires obtaining the expected dd-cfDNA results from spike-in controls is used for CLIA lab acceptance of the materials.

Results: The historical data were used to estimate the binomial probability of an individual SNP passing QC using the mean as calculated from all of the runs. The resulting minimum 1% quantile is 230 SNPs passing QC. Based on this cut-off, 99% of primer sets used succesfully in AlloSure testing to date passed this QC.

Conclusions: Performance and the QC metric for primer lots was defined by the specific requirements needed using data from multiple runs over long periods of time. The metrics can be used to reject lots of primers that do not meet specifications at two levels, primer performance against a reference standard (NIST) and obtaining the expected dd-cfDNA results using well-characterized spike-in controls. This ensures high quality lots of primers are used in AlloSure testing and accurate and consistent results are delivered for managing organ transplant patients.

B-445

Improving the Sensitivity of the Coomassie Brilliant Blue (CBB) Test

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Background: Sensitive and accurate protein quantitation is important for the manufacture of immunoassays. The CBB test is widely used as a sensitive and rapid method.(1) After its introduction, several improvements of the test have been reported, including the use of the ratio of the absorption values at 590 nm and 450 nm(2) instead of measuring at ~590 nm only. Increasing the linearity of a test improves accuracy when linear regression is used for value assignment. Further increasing the sensitivity allows for broader and more flexible application. Since measurement at different wavelengths captures differently protonated forms of the dye, we set out to determine whether altering the pH-value of the reagent could further improve linearity or sensitivity of the assay.

Methods: We determined the impact of different pH-values of Bradford reagent (Bio-Rad) on absorption values and combinations at different wavelengths. Slopes and correlation coefficients (r^2) of bovine serum albumin (Sigma) standard curves ranging from 10 to 100 µg/mL were used as indicators for sensitivity and linearity. 60 µL of sample was placed in a well of PS standard F-bottom microplates (Greiner Bio-One) followed by the addition of 240 µL of reagent that had been pH-adjusted. The absorption measurements were performed using the Spectra Max Plus Microplate Reader (Molecular Devices).

Results: A pH-value of ~0.8 for the original reagent was confirmed to be the optimum for the measurement at 595 nm. However, the highest slope was observed at a pH-value of ~1.0 when using the ratio 595/470. The slope was ~4.7-fold higher compared to the absorption measurement at 595 nm at a pH-value of ~0.8, and the slope was ~1.6-fold higher compared to the absorption ratio 595/470 at the pH-value of ~0.8. r^2 values in the pH-range from 0.5 to 1.0 were comparable (0.9824 and 0.9693 for the 595/470 ratio and 595 nm, respectively).

Conclusion: When using the 595/470 ratio, the slope of the CBB assay can be significantly increased by using a pH-value of ~1 for the reagent. Linearity is not markedly changed, moving the original pH-value of the reagent from about 0.8 to 1. Thus, a reagent pH-value of ~1 represents a simple means to improve the sensitivity of CBB testing, maintaining linearity and consequently accuracy of testing throughout the measuring range.

References:

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B-450

Evaluation of endogenous amino acids as preanalytical controls for blood samples

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Background: Comparison of analyte concentrations between blood sample types including wet and dried plasma/serum and capillary and venous whole blood is difficult. A pre-analytical, endogenous standard would allow normalization between blood sample types to control for sample quality and volume and would allow more precise quantitation and quality analysis. The objective of this study was to determine the feasibility for use of amino acid (AA) levels as a suitable pre-analytical standard (PS) for dried and wet blood samples.

Methods: The free AA concentrations in wet and dried plasma and whole blood for 12 AA was measured for nine healthy donors (ages 29 to 60) by LC-MS/MS. Fasting (early morning) and fed (one hour post lunch) AA levels were determined for three donors on three separate days, using whole blood collected with HemaSpot™-HF devices by finger stick. AA stability over time (1, 7, 30, 60 and 90 days) and temperature (-20, 22, 37 and 45 ° C) was determined for dried whole blood from three separate donors.

Results: Levels of five AA (Val, Thr, Ile, Leu, Phe) showed strong correlation (<11% CV) between nine donors for four sample types: wet and dried plasma, and wet and dried whole blood. Minimal differences in AA levels were observed between fasting and fed state. Levels of Phe, Ile, Pro, Val, Leu, Tyr and Trp were stable (<10% loss) as dried blood up to 90 days at temperatures of -20 to 45 ° C, while Gly, Ser and Thr were not stable over time.

Conclusions: Several AA including Val, Ile, Leu and Phe show promise for use as a pre-analytical standard for dried and wet plasma and whole blood samples.