Tuesday, July 29, 2014

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

Immunology

A-323

Evaluation of pre eclampsia markers in pregnant women with chronic hypertension and pregnant women without hipertension

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Background: The relationship between two biomarkers, soluble tyrosine kinase (sflt1) and placental growth factor (PIGF) has been described as useful for precocious identification of pregnant women at risk for pre eclampsia

Methods: 20 hypertensive pregnant women were included among the twenty- and thirty-sixth weeks of pregnancy and a control group of non hypertensive pregnant women was the comparison group. All serum samples were frozen at -80C , and processed simultaneously. The markers were processed Cobas 6000 analyzer from Roche Diagnostics.The cut- off ratio for sFlt-1/PLGF exclusion of preeclampsia described in the package insert of the kit is 33 , with 95% sensitivity and 94% specificity . Values greater than 85 are suggestive of disease.

Results: In the group of pregnant women without hypertension, the average ratio was 22 and in the hypertensive group was 284 . There was a significant difference between the groups evaluating the paired t-test ($p < 0.0001$) . Group of hypertensive pregnant women showed no relation to the lower cut- off suggested for exclusion of pre -eclampsia . In the group of healthy patients only 2 patients showed higher values compared to the deleting 33 does not pre- eclampsia are possible. In the group of chronic hypertensive pregnant women 70 % (13/20) had higher values at 85 .

Conclusion: In our sample, the use of markers of preeclampsia was found to be of the most value to clinical practice in the evaluation of patients with potential risk for progression to pre - eclampsia . Clinical trials are necessary to follow up in this group of patients

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Seasonal frequence of the most requested specific IGE

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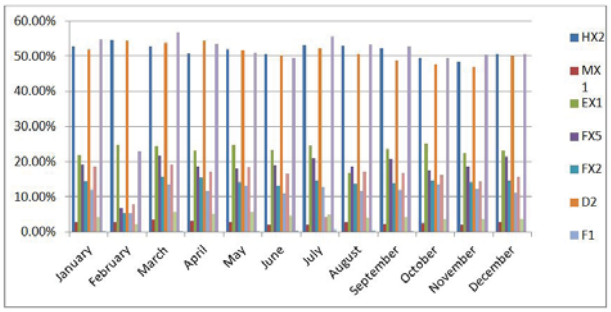
Background: Allergic asthma, rhinitis, and atopic eczema are among the commonest causes of chronic ill health in the world. Asthma is one of the most common chronic conditions affecting both children and adults, yet much remains to be learned of its aetiology. Although genetic predisposition is clearly evident, gene-by-environment interaction probably explains much of the international variation in prevalence rates for allergy and asthma. In our laboratory the ten most requested specific IgE are: IgE specific for dust and mites (HX2), IgE specific to fungi (MX1), IgE specific for epithelial animals (EX1), IgE specific for baby food (FX5), IgE specific for seafood (FX2), IgE specific for D. farinae (D2), IgE specific to egg white (F1), Milk specific IgE (F2), IgE specific to soybean (F14), IgE specific to D. pteronyssinus (D1) and IgE specific for cocoa (F93). Analyse the seasonal frequence of the most requested specific IgE.

Methods: We analyse the frequence of the IgE specific for dust and mites, IgE specific to fungi, IgE specific for epithelial animals, IgE specific for baby food, IgE specific for seafood, IgE specific for D. farina, IgE specific to egg white, Milk specific IgE, IgE specific to soybean, IgE specific to D. pteronyssinus and IgE specific for cocoa throughout the year 2012. The informatics system has given the data and for the statistical analysis we used the dispersion

Results: The results of this data are on the graph.



Conclusion: We conclude that there is no seasonal fluctuation in the incidence of IgE specific studied.

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A Rapid and Effective Tool for Monitoring Monoclonal Antibody Production

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Background: Monoclonal antibodies (Mab) are used in a variety of fields from diagnostics to therapeutics. We examined the effectiveness and utility of tools created with MedMira Miriad RVF Toolkit to assess Mab post-production functionality.

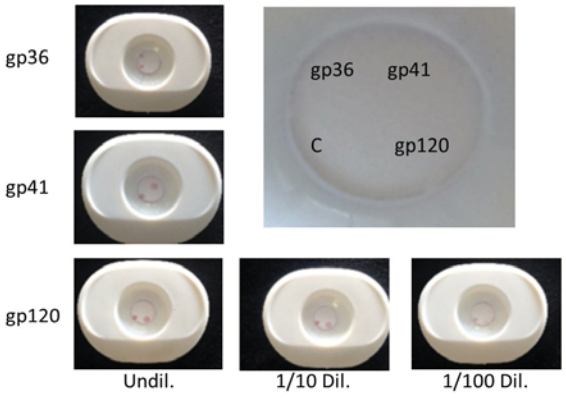
Methods: Six hybridoma supernatants, developed against unique peptide sequences derived from HIV and HCV proteomes, were obtained from a supplier. Miriad RVF Toolkit was used as per manufacturer instructions, with 0.5µL (1mg/ml) of each peptide or mouse IgG (control) spotted on to the cartridges using a micropipettor. Cartridges contained multiple test spots, one spot per peptide and one control spot (see Fig 1 for illustrative example for HIV peptides). Two sets of cartridges, one each for HCV and HIV peptides, were prepared and allowed to dry at room temperature for 30 minutes. Samples of neat or PBS diluted hybridoma supernatants were added during procedural steps.

Results: Reactive results, shown in Fig 1, were scored on a one to three grading scale, three being the highest. Increasing dilutions resulted in decreased reactivity. Two antibodies HCV MDL-1 and 3 became non-reactive at a 1:100 dilution, all others yielded reactive results at the 1:100 dilution. For example, anti-gp 120 antibodies were reactive down to a 1:100 dilution as illustrated in Fig 1. The specificity of the antibodies was evidenced by lack of cross reactivity of each Mab applied to cartridges containing multiple spots.

Conclusion: Testing with the Miriad RVF Toolkit was completed in less than one hour following receipt of antibodies. Miriad RVF Toolkit therefore represents a tool that can be used to efficiently assess production levels, functionality, and specificity of Mab. The time to results also allows Miriad RVF Toolkit to be used as an in-process monitoring tool during production. Results can be documented by recording of visual interpretation or by photographs as illustrated in Fig 1.

Fig 1.

Capture Peptide	Graded Miriad RVF Results/scores at various dilutions		
	neat	1:10	1:100
HCV MDL-1	3+	1+	-
HCV MDL-3	3+	2+	-
HCV MDL-4	3+	3+	2+
HIV gp36	3+	3+	2+
HIV gp41	3+	3+	2+
HIV gp120	3+	3+	1+



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Usefulness of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinima

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Background: Alpha-fetoprotein(AFP) has been used as a diagnostic marker for hepatocellular carcinoma(HCC), and fucosylated fraction of AFP(AFP-L3) has been proposed as a marker for HCC.

M**ethods:** We evaluated performance of the micro-total analyzer system(μ-TAS), on-chip immunoassay analyzer for AFP-L3. The linearity, precision and carry-over rate of μ-TAS were evaluated, and we compared the AFP-L3% levels between patients with early HCC and control group with benign liver diseases.

Results: The linearity was good(R2=0.9995) and coefficient of variation(CVs) of between-day precision in high and low concentration were 0.2% and 0.18%, respectively. AFP-L3% levels were higher in patients with early HCC than in control(13.4%±16.9% versus 4.6%±3.4%). The sensitivity and specificity with AFP-L3% were 57% and 67% at a cut-off value of 5%, and 43% and 83% at a cut-off value of 7%, respectively.

Conclusion: μ-TAS showed good performance of linearity, precision and carry over rate, and AFP-L3% could be a suitable serologic marker for evaluating early HCC.

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What is the Optimal Threshold for an ANA ELISA?

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Context: ELISA ANA screening [Seradyne; Indianapolis, IN] was brought in-house at the VAMC in 2013 using an automated Triturus processor [Grifols; Los Angeles, CA]. Rheumatologists had previously indicated dissatisfaction with excess false positive screens and initial validation studies had suggested improved specificity using a threshold optical index [OI] of 2.5 in place of the manufacturer's suggested value of 1.0. We evaluated this change using clinical data. **Methodology**: Reference lab [RL] confirmation (Lab Corp; Raleigh, NC) was obtained in 34(group 1) and 23 (group 2) consecutive specimens (33 and 21 patients) with OI values 2.5 and between [1.0, 2.5) respectively with adequate specimen volume. RL confirmatory testing entailed either a panel of specific antigens (if ordered) or a lab ordered IFA. Positive results on one of more panel antigens or an IFA titer 1:80 was considered confirmatory. The electronic health record was reviewed for clinical data. **Results**: Median age was 64 [27-91] YO with11 (20%) females [twice the female proportion in our general population]. 26 (76.5%) and 9 (39%) specimens confirmed in groups 1 and 2 respectively [chi sq. = 8.16, p<0.005]. Review of the 7 patients (9 specimens) with false negative screens using the higher threshold revealed 2 with hepatitis C and 2 who were ≥80 YO. The appearance of aberrant autoantibodies in these instances is well known. The fifth patient was status post renal transplant for IgA nephropathy. This patient had two specimens sent 4 months apart with OIs of 2.91 and 1.46 and IFA titers of 1:640 (homogeneous pattern)] and 1:320 (speckled) respectively. A multi-antigen panel done with the second specimen was negative. The sixth patient had been diagnosed with Rheumatoid arthritis with high titer of anti-CCP antibodies. The last patient had been diagnosed with SLE elsewhere in the 1990s based on renal biopsy. RL ELISA screens in 2008 and 2010 were negative. It did not appear that results of ANA screening affected clinical management in any of these patients. **Conclusions**: Our study, although small, suggested that improvement in specificity with a decreased number of workups for false positive ANA screens was possible through adjustment of the OI threshold without negative clinical consequences.

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Evaluation of an Anti-Streptolysin O assay for use on the Binding Site Next Generation Protein Analyser

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Elevated blood serum concentrations of human Anti-Streptolysin O (ASO) can be used to provide serologic evidence of past or present infection by β-Haemolytic Streptococci bacteria. Increasing serum concentration of ASO antibodies are produced in response to Streptolysin-O exotoxins secreted by the bacteria. Measurement of ASO levels in serum can be used as an aid in the diagnosis of diseases such as glomerulonephritis, rheumatic fever, bacterial endocarditis, tonsillitis, and scarlet fever. Here we evaluate an ASO assay designed for the quantitative *in vitro* measurement of human ASO in serum using the Binding Site's next generation protein analyser. The instrument is a continuous throughput, bench top turbidimetric analyser capable of automatic sample dilutions up to 1/10,000 and having a throughput of up to 120 tests per hour. Analyser precision is promoted by single-use cuvettes, whilst the user interface is enhanced through bi-directional communication capability, primary sample ID and fully bar coded reagent management systems. Evaluation of the assay on the next generation protein analyser demonstrated an overall assay time of 12 minutes which was read at end point. The assay auto dilutes a single serum based calibrator to produce a measuring range between 50 - 800.00 IU/mL at the standard (1/10) sample dilution. Samples outside the standard range auto re-dilute to neat (1/1) or a secondary dilution (1/20) as appropriate. Results which are still outside the measuring range following auto dilution are reported as <5.00 IU/mL, or >1600 IU/mL. Total precision studies performed at 5 different levels across the measuring range were assessed in duplicate over 21 working days, using a single kit lot on three analysers. Levels assessed were at 731 IU/mL (SD = 33.696, %CV = 4.6%), 427 IU/mL (SD = 16.403, %CV = 3.7%), 82 IU/mL (SD = 4.575, %CV =5.8%), 236 IU/mL (SD = 9.285, %CV =3.9%), and 151 IU/mL (SD = 6.586, %CV = 4.5%). The assay gave a linear response over the measuring range of 50 - 800.00 IU/mL at the standard 1/10 sample dilution and over a range of 5.00 IU/mL- 80.00 IU/mL at the minimum 1/1 dilution. A linear regression of $y = 0.981x - 16.2$ and $R^2 = 0.997$ was demonstrated at 1/10, whereas a regression of $y= 1.032x - 0.411$ and $R^2 = 0.998$ was demonstrated at 1/1 dilution. No significant interference was seen when the assay was challenged with haemoglobin (500mg/

dL), bilirubin (200 mg/L) and chyle (1500 FTU). Comparison comprising normal and clinical samples (n =121) was carried out against the Binding Site SPA PLUS analyser, covering between 52.000 IU/mL - 822.000 IU/mL. Analysis by Passing-Bablok regression demonstrated a linear fit of y = 0.96x + 0.92. We conclude that the ASO assay for the Binding Site Next generation protein analyser is reliable accurate and precise and shows good agreement with existing assays.

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Cytokine and IFN-γ-Induced Chemokine mRNA real-time PCR Taqman Probe Assay After Mycobacterium tuberculosis Specific Antigen Stimulation in Whole Blood from Infected Individuals

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Background: Recently, the interferon gamma (IFN-γ) release assay (IGRA) was introduced as an alternative immunodiagnostic method to the tuberculin skin test (TST) for detecting latent tuberculosis infection (LTBI). However, IGRAs are known to have limited sensitivity and cannot differentiate between active pulmonary tuberculosis (PTB) disease and LTBI. Numerous cytokines and regulator factors have been implicated in the pathogenesis and control of *Mycobacterium tuberculosis* (MTB) infection. Therefore, additional cytokines including T helper 1 (TH1)-type and T helper 2 (TH2)-type cytokines and chemokines associated with MTB infection may improve the performance of IGRAs. **Methods:** In the present study, a molecular diagnostic method using the real-time RT-PCR TaqMan® assay, which is able to quantitate mRNA expression levels, was developed for eight human targets (IFN-γ, TNF-α, IL-2R, IL-4, IL-10, CXCL9, CXCL10, and CXCL11) and evaluated with three different patient groups (active PTB, LTBI, and healthy non-TB groups). **Results:** Results revealed that positivity of TNF-α, IL-2R, and CXCL10 in the active PTB group was 96.43%, 96.43%, and 100%, respectively. The positivity of IL-2R and CXCL10 in the LTBI group was 86.36% and 81.82%, respectively. Statistical results revealed that TNF-α and CXCL9 (both $p<0.0001$) were the best individual markers for differentiating between the three different MTB infection groups. For optimal sensitivity, the simultaneous detection of multiple targets was attempted. The combination of IFN-γ, TNF-α, and IL-2R and the combination of TNF-α, IL-2R, CXCL9, and CXCL10 showed the best performance for detecting active PTB (both 100% positivity) and LTBI (86.36% and 81.82% positivity). **Conclusion:** These results imply that the combination of suitable single markers is very useful for the efficient diagnosis of MTB infection and the differentiation of MTB infection status.

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Toxoplasma gondii IgG avidity among Brazilian IgG+/IgM+ women

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Background: *Toxoplasma gondii* is prevalent in Brazil and and IgG and IgM is routenly requested for pregnant women. The IgG avidity test analyzes the bonding strength of the antigen-antibody complex after treatment with urea. In the acute phase of the disease, the binding of antigen-antibody complex is easily dissociated because the IgG avidity is reduced. This test maybe used as a supplemental assay for samples that are IgM and IgG positive in order to indicate recent infection.

Objective: To describe the results obtained by the IgG avidity test in women that is IgG and IgM positive for *Toxoplasma gondii.*

Methods: We have selected 413 samples from our routine that were IgM positive with Toxo IgM kit (Roche, Mannheim, Germany) and presented IgG level higher than 50 IU/mL by the Toxo IgG kit (Roche). The *Toxoplasma gondii* avidity test was performed using TSI Toxok -G - Plus kit (DiaSorin, Saluggia, Italy) which is an ELISA test. The avidity index was calculated by the ratio of the optical density of the urea buffer treated sample divided by optical density of the sample without urea treatment. Results below 30%, were considered recent infection (less than 12 weeks), results higher than 60 % infection were considered more than 12 weeks of infection. Between 30-60% the result was considered inconclusive.

Result: The results are shown on Table 1. Only 1% of the females were considered recent infected and 2.7% inconclusive

Conclusion: We conclude that the IgG avidity test for toxoplasmosis may be an important tool for the interpretation of IgG/IgM results in pregnant women.

Avidity	Number of samples	%
>60%	398	96,4
30-60%	11	2,7
<30%	4	1,0
Total	413	100

Table 1- IgG avidity detected in the studied samples.

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Neopterin ELISA kit: Analytical evaluation and verification of the reference interval in native population in Argentina.

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Background: Neopterin, 2-amino-4-hydroxy-6-(D-erytrhro-1′,2′,3′-trihydroxypropyl)-pteridine, is a low-molecular compound synthesized from guanosine triphosphate (GTP) by macrophages. The clinical utility is like specific marker for the activation of cellular immunity. Measurement of plasma neopterin concentrations has been proposed as a test for monitoring the activity of infectious, autoimmune and malignant diseases.

Objective: The purpose of this study was to evaluate GenWay Neopterin ELISA kit performance and verification of the reference interval in native population in Argentina.

Materials and methods: Plasma samples were obtained from 38 healthy volunteers (aged 17-60 years). Neopterin was determined by ELISA GenWay Biotech Inc. The verification of the reference interval was calculated using the CLSI guidelines C28-A3. Bias and imprecision, were calculated using the protocol EP-10 from CLSI, with three different standard concentrations (nmol.L^{-1}): 4; 12 and 37. The results were compared with the specifications of folate, due to neopterin desirable specifications are not yet established. *Intra-assay and inter-assay variability were determined in* ten normal plasma samples and ten samples with elevated neopterin concentrations from rheumatoid arthritis patients (RA), QC plasma provided by the manufacturer and compared with literature data. The criteria for acceptable performance were the target value plus or minus 2 standard deviations. To study behavior among different sources of specimens, variance analysis ANOVA was used.

Results: The limits of references values of neopterin were verified (< 10.0 nmol.L^{-1}). The lower and higher limits were 1.36 (percentile 2.5) and 9.93 (percentile 97.5) nmol.L^{-1} respectively. Bias and imprecision, encountered in the standard concentrations were: 13.6 % and 11.2%; 9.8 % and 3.1%; 7.6% and -1.4% respectively. Intra-assay variability in normal and elevated samples was 7.9% and 11.4% respectively; in QC samples (normal and elevated) were 9.8% and 6.1% respectively. Inter-assay variability for the same group of samples was 8.2%; 13.8%; 10.4% and 7.8% respectively. The lot to lot CVs for the two samples used for Quality Control of three lots of the neopterin ELISA kit were 13.1% and 7.4% respectively. Total error allowable (TE_a) for neopterin was calculated for the three standard concentrations: 33.64%; 19.27%; 13.94%, TE_a for folate is 39.0%.Variance analysis had not shown statistical significance among the processed samples.

Conclusions: The verification of neopterin reference interval on healthy volunteers in native population is consistent with literature data. This interval allowed distinguishing between healthy and RA patients. The precision was verified by the good intra and inter-assay coefficients of variation. The lot to lot CV was < 13.5%. Although desirable specifications for neopterin are not yet established, we used folate in terms of comparison; folate is a pterin derivative. We found that the mean neopterin's TEa was 22.3% and the highest value obtained, 33.64 %, was smaller than the folate. We conclude that the results with GenWay ELISA on imprecision and bias were acceptable when were compared with the well-known folate's desirable specifications. Variance analysis had not shown statistical significance between neopterin measurements among different sources. In summary, GenWay ELISA kit characterized here is valuable for clinical applications, is simple and rapid for neopterin determinations in serum or plasma.

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Assessment of a Particle Immunofiltration Assay [PIFA] for Antibodies Associated with Heparin Induced Thrombocytopenia (anti-HIT)

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Background: The Department of Surgery at VAMC queried the clinical laboratory about the feasibility of implementing a rapid assay for anti-HIT. The only assay satisfying their specifications was a PIFA [Akers Biosciences; Thorofare, NJ] The question was how to evaluate this assay given the low incidence of true positive (TP) cases of HIT as indicated by a positive result on a functional assay e.g. the serotonin release assay [SRA]. **Methods**: To circumvent the low incidence of TPs we pooled serum specimens from five patients that had been received for Vitamin D assay. PIFA was negative on the pooled serum that was then used to serially dilute the positive control material supplied by the manufacturer between 1:2 and 1:32. The diluted control material was run using PIFA and the dilutions and the negative pooled serum were then sent to a reference lab (RL) [Lab Corp; Raleigh, NC] where the material was tested using an ELISA assay with a threshold index of 0.4. The entire procedure was repeated using a different lot of positive control material (total of 12 determinations sent to the RL). **Results**: Of the 12 simulated specimens, 5 were positive by both PIFA and ELISA and 4 negative by both methods [overall concordance=75%] Three specimens were positive by PIFA and negative by ELISA. All discordant specimens had ELISA indices >0.3 but less than 0.4. **Conclusions**: The study was small and SRA results were not available on the simulated samples. However it would appear that FPs already known to be a problem when employing ELISA would be increased when using PIFA as the latter apparently begins generating positive results when the ELISA index is >0.3. A negative PIFA result is however concordant with ELISA. PIFA may therefore be usable as the first step in an algorithm insofar as a negative result would suggest that anti-HIT is unlikely and a positive result could be referred for additional testing.

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New immunological assays for diagnosis of Schistosoma mansoni for clinical acute and/or chronic forms

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Background: Control constraints of schistosomiasis include the lack of diagnostic methods with high sensitivity. We initiated a prospective study in southeast Brazil in order to develop sensitive diagnostic methods for Schistosoma mansoni infection, with 4 endemic areas together with 80 travelers infected in a freshwater pool.

Methods: Sera, whole blood, urine and saliva samples from the patients were used for the standardization of innovative diagnostic methods. Comparisons were performed with eggs in feces, IgG titers, encephalomyelitis by NMR and clinical symptoms. The new methods used were immunochromatography (dipstick), Immunomagnetic Separation and ELISA with highly purified monoclonal antibodies.

Results: We could diagnose acute patients 10 days post-infection, also more than 95% of positive cases from chronic and low endemicity patients. New methods for IgG detection using purified glycoprotein or recombinant protein or peptides (10 aminoacids) were superior to conventional ELISA.

Conclusion: Best results were seen for recombinant protein with 100% of sensitivity. Data showed 100% of sensitivity of chronic patients and 98% of acute patients.

Financial support: Fapemig, CNPq, Capes, Fiocruz, Fiotec, PDTIS (Brazil). Fulbright, NIH, University of Georgia (USA).

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Audit of Autoimmune Neurological Antibodies requesting in a Singapore institution

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Background: There is increased testing of autoimmune neurological antibodies for evaluation of neurological diseases, but there is limited information on its prevalence in our local population. We aimed to determine the neurological antibody requesting patterns and seropositivity rates in patients presenting with neurological disorders in our institution.

Materials and Methods:

Autoimmune neurological antibodies Anti-NMDA, Anti-VGKC, Anti-AQP4, Anti-Hu, Anti-Yo and Anti-Ri requested from January 2010 to December 2013 in our institution were reviewed; only the first positive antibody result was considered for patients with multiple requests. For Anti-AQP4, cerebrospinal fluid oligoclonal bands (OCB) and pleocytosis information were reviewed as well.

Statistical analysis was performed using SPSS Version 17.0.

Results:

There were 443 requests of neurological antibodies for 203 patients, of which three most common presenting complaints were seizures, altered mental state and encephalitis.

The median age was 48.4 years old, with male to female ratio of 1.09, and 118 Chinese, 19 Malays, 17 Indians and 49 belonging to other ethnic groups. The median age and ethnic breakdown for each antibody is shown in the table.

7.6% of these patients were positive for any of the neurological antibodies. Anti-AQP4 had the highest seropositivity at 23.4%, followed by Anti-VGKC(9.9%), Anti-NMDA(9.2%), Anti-Yo(3.9%), Anti-Hu(1.3%) and Anti-Ri(0%). No patients were positive for more than 1 antibody.

Amongst patients with positive Anti-AQP4, they are older (55.6 versus 36.9 years old in negative patients) and less likely to have a positive OCB or pleocytosis. Young females were more likely to be positive for Anti-NMDA compared to older females or males, as shown in the table.

Indian ethnicity was positively associated with Anti-VGKC positivity.

Conclusion:

Our data showed that autoimmune neurological antibody positivity were not uncommon, however this could be due to requesting bias. Further studies will be helpful to identify their prevalence in patients with different neurological complaints.

Type of neurological autoantibody and characteristics	All patients	Patients with positive result	Patients with negative result
Anti-Aquaporin 4 Antibody (AQP4)			
Number of patients (%)	47	11 (23.4)	36 (76.5)
Median age (years)*	44.6	55.6	36.9
Ratio of chinese:malay:indian: other ethnic groups	22:5:6:14	8:0:0:3	14:5:6:11
Anti-N-methyl-D-aspartate Antibody (NMDA)			
Number of patients (%)	120	11 (9.2)	109 (90.8)
Median age (years)*	42.5	26.5	44.9
Ratio of chinese:malay:indian: other ethnic groups	71:13:6:20	5:1:0:5	66:12:6:25
Anti-Voltage gated K channel (VGKC)			
Number of patients (%)	81	8 (9.9)	73 (90.1)
Median age (years)	53.8	50.1	54.1
Ratio of chinese:malay:indian: other ethnic groups	53:3:8:17	3:1:3*:1	50:2:5:16
Anti-Yo			
Number of patients (%)	76	3(3.9)	73 (96.1)
Median age (years)	57.3	56.7	57.5
Ratio of chinese:malay:indian: other ethnic groups	53:5:6:12	2:1:0:0	51:4:6:12
Anti-Hu			
Number of patients (%)	77	1 (1.3)	75 (97.4)
Median age (years)	57.3	73.4	57.2
Ratio of chinese:malay:indian: other ethnic groups	53:7:4:12	0:1:0:0	53:6:4:12
* Difference between patients with positive antibody compared to those with negative antibody is significant with p value <0.05.			

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Deficiency of CD16 in polymorphonuclear neutrophils. Myelodisplastic disorder, paroxysmal nocturnal hemoglobinuria or neutrophilic FcGRIIIB gene deficiency?. A case report.

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Background CD16 antigen, the human Fcγ receptor III (FcgRIII), is a protein constitutively expressed on polymorphonuclear neutrophils (PMNs), monocytes and NK-cells. CD16 participates in phagocytosis and in antibody-dependent cellular cytotoxicity. It exists in 2 different forms encoded by 2 nearly identical linked genes, FcgRIIIA and FcgRIIIB that generate alternative membrane anchored molecules: FcgRIIIA (50-65 kd) is a transmembrane form expressed on NK cells

and macrophages; FcγRIIIB (48 kd) is anchored through a phosphatidylinositol (PI) linkage and expressed only on neutrophils.

Abnormal CD16 expression in cell surface might indicate myelodysplastic disorder or indicate an acquired clonal hematologic disorder such as paroxysmal nocturnal hemoglobinuria (PNH).

In the present study we report a case of total CD16 deficiency on PMNs.

Methods We studied the peripheral blood from a 72-year-old male that only presented neutropenia. A 6-color flow cytometry tube was used including the markers CD16, CD24, CD14, FLAER, CD33 and CD45. The cells were run on a 3-laser FACSCanto II (BD) with FACSDiva software (BD) and analyzed using Infinicyt software (Cytognos).

Results Immunophenotypic analysis of peripheral blood sample from patient showed a lack of CD16 expression on neutrophils, but NK cells were CD16+. The GPI-anchored proteins such as FLAER, CD24, and CD14 and all other myeloid antigens were expressed normally in neutrophils and monocytes.

Conclusion These results suggest a neutrophilic FcGRIIIB gene deficiency and reject a myelodysplastic disorder and paroxysmal nocturnal hemoglobinuria. Previous studies indicate deficiency of CD16 does not compromise the host defence. Apparently, the other receptors for IgG, CD32 and CD64, can compensate for the lack of CD16 Eur J Clin Invest. 2004 Feb;34(2):149-55.

A-336

Diagnostic efficacy evaluation of IL-1RI, IL-1 β and CDK2 in peripheral blood and synovial fluid with rheumatoid arthritis

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Background: To explore the diagnostic efficacy of interleukin 1 receptor typeI(IL-1RI), interleukin 1β(IL-1 β) and cyclin dependent kinase 2(CDK2) in peripheral blood and synovial fluid with rheumatoid arthritis (RA).

Methods: There were selected 94 cases with RA patients in rheumatology outpatient and inpatient department, 40 cases with systemic lupus erythematosus (SLE) patients in outpatient department, 20 cases with acute upper respiratory tract infection(AURTI) patients in emergency department, 20 healthy persons. All subjects were eligible for inclusion criteria. All subjects were drew vein blood 3 ml besides 1 ml knee joint fluid from 24 patients with active RA patients. The level of IL-1RI, IL-1β and CDK2 were detected in serum and synovial by quantitation ELISA, then the three items were evaluated diagnostic efficacy.

Results: There were all significant differences among experimental groups on either IL-1RI, IL-1β or CDK2 by square variance analysis(P<0.001), respectively. On IL-1RI, there were significant differences between RA patients group and RA patients joint synovial fluid group or SLE patients group or acute upper respiratory tract infection group and control group (P<0.001), respectively. On CDK2, there were significant differences between RA active phage and RA relieve phage, between RA patients group and RA patients joint synovial fluid group or SLE patients group or acute upper respiratory tract infection group or control group (P<0.001) , respectively. On IL-1β, there were significant differences between RA active phage and RA relieve phage, between RA patients group and RA patients joint Synovial fluid group or healthy people group (P<0.001), respectively.

Compared RA patients active phage with RA relieve phage, area under curve of ROC in CDK2 was largest, followed IL-1β andIL-1RI. Compared RA patients group with SLE patients group, area under curve of ROC in CDK2 was largest, followed IL-1RI. Compared RA patients group with acute upper respiratory tract infection patients group, area under curve of ROC in IL-1RI was largest, followed CDK2. Compared RA patients group with control group, area under curve of ROC in CDK2 was largest, followed IL-1RI.

Conclusions:IL-1RI had low diagnostic efficiency next to CDK2, but it could efficiently differentiate RA and acute upper respiratory infection (AURI). CDK2 had higher diagnostic efficiency, which could efficiently differentiate active phase and relieve phase of RA, and differentiate RA and SLE, but had low diagnostic efficacy next to IL-1RI, in differentiating RA and AURI. CDK2 plus IL-1RI plus IL-1β paralleling joint diagnosis may increase diagnostic value of RA. CDK2 plus IL-1RI plus IL-1β tandem joint diagnosis may increase early diagnostic value of RA.

A-337

Evaluation of an IgG3 assay for use on The Binding Site Next Generation Protein Analyser

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Assays for measurement of IgG subclasses in serum are routinely used in many immunology laboratories in the diagnosis of IgG deficiencies. Abnormal levels of one or more subclass may be associated with conditions including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia. Deficiency of IgG3 has been reported to be associated with viral infections of the urinary tract. Here we describe the evaluation of an IgG3 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 55 - 2200 mg/L at the standard 1/20 sample dilution. Samples lower than the bottom of the standard measuring range are automatically retested at the lower 1/2 sample dilution, providing a measuring range of 5.5-220 mg/L. Precision studies (CLSI EP05-A2) were performed at five levels, over 21 days with 2 runs per day. Antigen levels of 8.9mg/L, 117.8 mg/L, 159.85mg/L, 258.3 mg/L and 1774.5mg/L were assessed for total and within run precision over 3 reagent lots on 3 analysers. The coefficients of variation were 12.1% and 8.4% for the 8.9mg/L sample, 8.3% and 5.1% for the 117.8mg/L sample, 9.9% and 8.2% for the 159.8mg/L sample, 6.6% and 3.8% for the 258.3mg/L sample and 5.8% and 2.0% for the 1774.5mg/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (55-2200mg/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y= 1.0136x - 11.368, R^2 =0.9971). No significant interference (within 10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 FTU) when spiked into a sample with known IgG3 concentration. Correlation to the Binding Site IgG3 assay for the SPA PLUS was performed using normal and clinical samples (n=102, range 102.10-1836.90 mg/L). Good agreement was observed between assays when anlysed by Passing-Bablok regression; y=1.07x -20.80. We conclude that the IgG3 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-338

Validation of a multiplex electrochemiluminescence assay for quantitation of synovial fluid cytokines and establishing reference interval in non-infected arthroplasty patients

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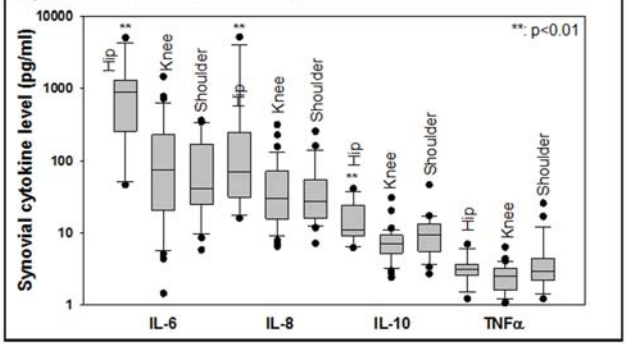
Background: Periprosthetic joint infection (PJI) is a severe complication following arthroplasty. Emerging studies suggest that proinflammatory cytokines in synovial fluid are promising markers for PJI diagnosis. However no assay has been validated for measurement of cytokines in synovial fluid and baseline levels have not been established in non-infected artroplasty patients. We developed and validated an electrochemiluminescence (ECL) multiplex assay for quantification of 9 cytokines in human synovial fluid and established reference intervals in artroplasty patients. Method: The human ultrasensitive cytokine assay (Meso Scale Discovery. Rockville, MD) was modified to measure IL-1β, IL-2, IL6, IL-8, IL-10, IL-12p70, GM-CSF,IFN-γ, TNF-α in synovial fluid. The assay was validated following CLSI guidelines. Patients who underwent primary arthroplasty or arthroscopic rotator cuff repair in hip, knee or shoulder, were enrolled prospectively. These patients were known not have infections and thus were ideal controls for PJI. Synovial fluid was collected intraopratively. Results: The intra- and inter-assay imprecision (n=20) for all the cytokines was less than 15%. Assay accuracy was evaluated by spiking recombinant cytokines into synovial fluid. Percent recovery was within 100±20%. The assay sensitivity and linear range was summarized in the table. No difference was observed between different genders or the two surgical groups. However, patients >=70 years old had significantly higher synovial IL-6 and IL-8 levels than other age groups. Knee and shoulder joints showed similar cytokines levels while hip synovial fluid contained significant increases in IL-6 and IL-8(Fig1). Reference intervals were established for synovial cytokines of knee/shoulder joints in non-infected arthroplasty

patient (<70 years old, see table). Conclusion: The ECL assay provides a reliable method for quantitation of multiple cytokines in less than 25µL synovial fluid. IL-6 and IL-8 are the major cytokines in synovial fluid. Age and joint specific reference interval was necessary for diagnosis of PJI using synovial cytokines.

Analytes	Limit of detection (pg/ml)	Limit of quantitation (pg/ml)	Linear range	Reference interval n=52(pg/ml)
IL-1β	0.9	5.5	0.9 - 50000	<5.5
IL-2	0.7	2.1	0.7 - 10000	<3.1
IL-6	0.6	0.9	0.6 - 50000	<793.1
IL-8	0.3	0.3	0.4 - 50000	<192.7
IL-10	1.0	4.8	0.4 - 50000	<21.9
IL-12p70	1.1	11.5	1.1 - 50000	<11.5
IFNγ	1.3	4.8	0.4 - 50000	<9.4
TNFα	0.9	2.1	0.9 - 50000	<9.6
GM-SF	0.8	3.0	1.6 - 50000	<3.0

Cytokine levels in synovial fluid in non-infected arthroplasty patients
(hip: n=11; knee: n=36; shoulder: n=26)



A-339

Evaluation of the immunoassay reagent kit for high sensitive troponin-I (ARCHITECT® *STAT* high sensitive Troponin-I) with fully-automated chemiluminescent immunoassay analyzer, and the clinical trials in Japan using medical checkup examinees

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Background: Troponin-I is widely used as an aid in the diagnosis of acute myocardial infarction because of its cardiac specificity. In Japan, the measurement of troponin-I belongs to class-I in Guidelines for Management of Acute Coronary Syndrome without Persistent ST Segment Elevation(Japanese Circulation Society (JCS) 2007)and Class-II in Guidelines for Treatment of Chronic Heart Failure (JCS 2010). Use of a high sensitivity troponin assay is recommended due to improved analytical performance. An assay is considered to be high sensitive if the %CV value is 10% or less at the 99th percentile and if at least 50% of normal subjects are detectable. The goal of this study was to evaluate the analytical performance characteristics of ARCHITECT *STAT* high sensitive Troponin-I assay on the fully automated chemiluminescent ARCHITECT analyzer and to determine the 99th percentile upper reference limit and the factors which affect troponin-I in Japanese populations undergoing routine health checks.

Methods: The ARCHITECT *STAT* high sensitive Troponin-I assay is a double monoclonal antibody sandwich assay. This assay has an assay time of approximately 18 minutes and an analytical range of 0.0 to 50,000.0 pg/mL. Precision was evaluated with 3 different sera in duplicate, twice a day for 5 days (n=20). The limit of blank (LoB) and limit of quantitation (LoQ) were evaluated with 20 consecutive measurements of calibrator A (zero concentration) and 5 different patient sera in duplicate for 5 days. Dilution linearity was determined by diluting 3 serum samples with the dedicated diluted solution. Correlation was performed with 50 samples spanning the range of 32-35,389 pg/mL on ARCHITECT *STAT* Troponin-I assay (the conventional troponin-I assay). The 99th percentile of Japanese healthy population and the factors which affect troponin-I were evaluated with 283 patients undergoing routine health checks.

Results: Precision showed %CVs of 2.6-5.9 over the range of 34.18 to 3799.79 pg/mL. LoB and LoQ were 0.35 pg/mL and 3.11 pg/mL, respectively. Method comparison showed a correlation coefficient of r = 0.98 and a slope = 0.98. The 99th percentile value of 166 Japanese apparent healthy subjects was 22.4 pg/mL. This value was equivalent to the 99th percentile value reported in the package insert from Abbott Laboratories. The multiple regression analysis revealed that male sex and age were independent factors for increased cardiac troponin-I levels. Cardiac troponin-I levels were significantly higher in males and positively associated with the age.

Conclusion: The ARCHITECT *STAT* high sensitive Troponin-I assay demonstrated good analytical performance and improved imprecision at low concentration in comparison to the conventional ARCHITECT *STAT* Troponin-I assay. ARCHITECT *STAT* high sensitive Troponin-I meets the definition of high-sensitivity troponin reagent proposed by the IFCC Task Force. The 99th percentile value which was established by the manufacturer may be used in Japan, but it would be necessary to consider the effects of age and gender.

A-340

A Lyophilized Quality Control Material for Human Allergen Specific IgE Testing.

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In vitro measurements of circulating IgE antibodies specific for allergens (sIgE), such as pollens, foods, drugs, venoms, insects, mites, molds and epidermals, are useful for the diagnostic assessment of a patient's allergies. Lyphochek® Allergen sIgE Control Negative and Panel A is a lyophilized, human serum based third party quality control material to monitor the precision of laboratory testing procedures for specific IgE antibodies.

Procedure: Control material was tested for fifteen sIgE on various testing platforms for recoveries, precision, opened vial stability, and accelerated temperature studies for shelf-life prediction.

Results: The control material was assayed to determine sIgE recovery values using several test platforms. The Negative control results were all below the detection limit of the test method used. The table below lists results for the sIgE present in the Panel A control.

Allergen		Specific IgE		
		Siemens Immulite 2000 (KU/L)	Thermo Fisher Phadia® ImmunoCAP (KU/L)	Hycor HYTEC 288 (IU/mL)
D1	D. pteronyssinus	25.01	16.70	8.73
D2	D. farinae	29.38	22.15	16.78
E1	Cat dander	33.93	17.80	8.17
E3	Horse dander	17.99	7.15	3.69
E5	Dog dander	32.95	55.44	14.81
F1	Egg white	3.74	1.20	1.19
F2	Cow's milk	10.29	5.92	2.29
F13	Peanut	3.20	4.15	1.37
G2	Bermuda grass	19.64	15.99	7.57
G3	Orchard grass	29.33	17.63	13.25
G6	Timothy grass	24.81	13.97	12.86
M3	Aspergillus fumigatus	2.76	3.82	0.35
M6	Alternaria tenuis	10.96	8.45	3.69
T3	Birch	20.55	10.10	3.13
W6	Mugwort	2.33	6.12	0.54

Open vial stability studies showed that the sIgE in the control is stable for a minimum of 28 days at 2-8°C. Accelerated temperature studies predicted a shelf-life of over 3 years when stored in lyophilized form at 2-8°C.

Conclusion: Lyphochek Allergen sIgE Control, Negative and Panel A levels are suitable to monitor the precision of laboratory testing procedures for sIgE in human serum or plasma.

A-341

Effect of CD95 on inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes

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Background: Many CD95-expressing cells don't always undergo apoptosis after stimulation with CD95 ligation. To investigate the role of expression of CD95 (Fas/

Apo1) on inflammatory response in fibroblast-like synoviocytes(FLS) obtained from rheumatoid arthritis(RA) and to evaluate the role of phosphatidylinositol 3-kinase(PI3K)/protein kinase B(PKB or Akt) pathways within this process.

Methods: The expression levels of CD95 were measured by immunohistochemistry and reverse transcription polymerase chain reaction(RT-PCR). Apoptotic cells were detected by in situ apoptosis detection (TUNEL) assay. The RA-FLS were treated with agonistic anti-CD95 antibody or CD95 siRNA, then the proliferation was detected by CCK-8,and mRNA level of inflammatory cytokines were detected by RT-PCR. After the RA-FLS were treated with agonistic anti-CD95 antibody, the total Akt and pAkt protein expression was analyzed by western blot, and the changes mentioned above were observed while incubated with the PI3K inhibitor LY294002. **Results:** A significant increase of CD95 antigen was found in RA compared with osteoarthritis (OA) samples, while apoptosis in RA synovial tissue were not obvious. Low concentrations of agonistic anti-CD95 antibody could promote RA-FLS growth and IL-6 mRNA expression, while high concentrations could induce apoptosis and both of these phenomenons were inhibited by CD95 siRNA. Agonistic anti-CD95 antibody could stimulate the expression of pAkt, and PI3K specific inhibitor LY294002 could induce opposite change.

Conclusion: Stimulation of CD95 could promote RA-FLS proliferation and inflammation, and activation of the PI3K/Akt signaling pathway might be the potential mechanism.

A-342

Quantitative detection of Plasmodium falciparum Histidine Rich Protein 2 in saliva

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Background: Malaria is a global health priority with a heavy burden of fatality and morbidity. Improvements in field diagnostics are needed to support the agenda for malaria elimination. Saliva has shown significant potential for use in non-invasive diagnostics, but the development of off-the-shelf saliva diagnostic kits requires best practices for sample preparation and quantitative insight on the availability of biomarkers and the dynamics of immunoassay in saliva. This study measured the levels of the PfHRP2 in patient saliva.

Methods: Matched samples of blood and saliva were collected between March and August, 2011 from forty patients at the ER and OPD of the pediatric unit of Korle Bu Teaching Hospital. Parasite density was determined from thick-film blood smears. Concentrations of PfHRP2 in saliva of malaria-positive patients were measured using a custom chemiluminescent ELISA in microtitre plates. Forty negative-control patients were enrolled. Saliva samples were stabilized with protease inhibitor

Results: Of the forty patients with microscopically confirmed P. falciparum malaria, thirty seven tested positive for PfHRP2 in the blood using rapid diagnostic test kits, and forty for PfHRP2 in saliva. All negative-control samples tested negative for salivary Pf HRP2. The ELISA greed with microscopy with 100 % sensitivity and 100 % specificity. Salivary levels of PfHRP2 ranged from 15 to 1,162 pg/mL in the malaria-positive group.

Conclusion: Saliva is a promising diagnostic fluid for malaria when protein degradation and matrix effects are mitigated. Systematic quantitation of other malaria biomarkers in saliva would identify those with the best clinical relevance and suitability for off-the-shelf diagnostic kits.

A-343

Evaluation of an Alpha 2-Macroglobulin assay for use on the Binding Site Next Generation Protein Analyser

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Alpha 2-Macroglobulin is a 725 kDa protein which, due its high molecular weight, is distributed almost exclusively in the intravascular pool. Increased levels of Alpha 2-Macroglobulin are associated with nephrotic syndrome, liver cirrhosis and diabetes mellitus. Measurement of Alpha 2-Macroglobulin can also aid in the diagnosis of blood-clotting or clot lysis disorders. Here we describe the evaluation of a serum Alpha 2-Macroglobulin assay for use with the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 0.1928 - 6.17 g/L at the standard 1/10 sample dilution. Precision studies (CLSI EP5-A2) were performed at five levels in duplicate over 21 working days. Five antigen levels were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 3.2%, 1.0%, 1.2% and 2.8% for the 5.3g/L sample, 2.8%, 0.7%, 1.0% and 2.5% for the 4.1g/L sample, 3.1%, 1.1%, 1.1% and 2.7% for the 3.5g/L sample, 2.4%, 0.9%, 0.9% and 2.1% for the 2.3g/L sample and 3.5%, 2.1% 1.3% and 2.5% for the 0.34g/L sample. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the extended measuring range (0.184 - 6.363 g/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y=1.002x - 0.01903, R^2 = 0.9998). No significant interference was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into samples with known Alpha 2-Macroglobulin concentrations at the standard sample dilution. Correlation to the Binding Site Alpha 2-Macroglobulin assay for the SPA PLUS analyser was performed using normal and clinical samples (n=146, range 0.233-5.554g/L). Good agreement was demonstrated by Passing-Bablok regression; y=1.03x - 0.01g/L. We conclude that the Alpha 2-Macroglobulin assay for the Binding Site Next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-345

Evaluation of the performance characteristics of a new assay for Ferritin on Toshiba 2000FR

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Background:

Ferritin has been a well known marker over the past 20 years as its level in blood is abnormally increased with certain disorders. Furthermore, more recently high sensitivity is increasingly required to diagnose the iron deficiency anemia, for which a lower threshold is set at 12 µg/L or below. On the other hand, serum Ferritin levels may become very high in some disorders such as hemochromatosis, hemosiderosis, etc. Thus, assays for Ferritin levels in serum and plasma are required to have a wide assay range with good prozone (high dose hook effect) tolerance and with a high sensitivity for accurate and reliable measurements. We evaluated the FERNX, a new latex particle-enhanced turbidimetric immunoassay for serum and plasma Ferritin levels with ultra-sensitivity and good prozone tolerance, available from Denka Seiken Co., Ltd. We evaluated the FERNX comparing to the current reagent and also monitored the on-use stability at lower end.

Methods:

We carried out the study on a TBA-2000FR automated clinical chemistry analyzer (Toshiba). The performance data was compared to the current reagent available from Denka Seiken by testing lower detection limit, precision, linearity, prozone, interferences and correlation. The on-use stability was assessed by testing lower detection limit every week with the reagent which was set on the analyzer during the whole study.

Results: The FERNX showed the correlation coefficient 0.99 against the current reagent with 170 clinical samples. No interferences were observed with hemolysis (hemoglobin: 487 mg/dL), icterus (Bilirubin: 20 mg/dL) or lipemia (1410 FTU) with both reagents. CVs (SDs) of the new assay from within-run imprecision with 3 different samples (10 µg/L, 60 µg/L and 310 µg/L) were smaller than the current reagent. The new assay showed prozone tolerance better than the current reagent. Recoveries by the new assay were higher than the upper limit of the measuring range (1,000 µg/L) with samples containing actual Ferritin concentrations up to 50,000 µg/L, on the other hand recoveries by the current reagent were lower than the upper limit with the samples with actual Ferritin concentrations at 6,000 µg/L or higher. Lower detection limit was 3-fold smaller than the current reagent (2 µg/L and 6 µg/L, respectively). The lower detection limit remained at the sample level (2-3 µg/L) during the on-use stability study up to 5 weeks.

Conclusion: The FERNX showed better performance compared to the current reagent. The FERNX showed excellent prozone tolerance and also excellent sensitivity even in the on-use stability study. The FERNX, a new latex particle-enhanced turbidimetric immunoassay reagent for serum and plasma Ferritin, is useful for diagnosis of wide variety disorders including the iron deficiency anemia.

A-346

Development of a latex-enhanced immunoturbidimetric assay for the measurement of MMP-3 levels on automated clinical chemistry analyzers

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Matrix metalloproteinase-3 (MMP-3) is a proteolytic enzyme produced by synovial cells and which participates in joint destruction. The concentration of serum MMP-3 in normal subjects is reported to range from approximately 17.3 to 121 ng/mL, while patients with rheumatoid arthritis (RA) display significantly increased serum MMP-3 levels as the condition worsens. It has been demonstrated that measuring serum MMP-3 levels is a useful marker for evaluating inflammatory activity, prognostic outcome and therapeutic effect in RA.

We have developed a new method for measurement of serum and plasma MMP-3 levels for use in clinical chemistry analyzers. This method is based on latex-enhanced immunoturbidimetry, using anti-human MMP-3 mouse monoclonal antibodies. The concentration is determined by measuring the change in absorbance that results from agglutination of latex particles.

The reagents are supplied ready-to-use, and the assay can be completed within 10 min. Using a Roche/Hitachi 917 auto analyzer, 2.4 µL of human serum or plasma was mixed with 120 µL of the first buffer solution and incubated for 5 min at 37ºC. Subsequently, 40 µL of the second reagent, which contains the monoclonal antibody-coated latex particles, was added and the absorbance was monitored at 570 nm/800 nm (main/sub wavelengths) for 5 min.

The lower detection limit for MMP-3 was 10 ng/mL, and the upper quantitation limit was 1,600 ng/mL. No prozone effect was observed in MMP-3 samples of concentrations from 1,600 through 2,500 ng/mL. The within run C.V. (n=10) at 100 ng/mL, 200 ng/mL, and 400 ng/mL was 1.2 %, 0.6 %, and 0.9 %, respectively. The between run C.V. (n=10) at 100 ng/mL, 200 ng/mL, and 400 ng/mL was 1.7 %, 2.1 %, and 2.6 %, respectively. Interference studies showed no effect from bilirubin, hemoglobin, rheumatoid factor (RF), or chyle at concentrations of 20 mg/dL, 500 mg/dL, 500 IU/dL, and 2,000 formazin turbidity units, respectively.

Comparison of our assay kit with the approved IVD reagent, the principle of which is enzyme immunoassay, yielded a correlation coefficient of 0.980 and an equation of Y (present method) = 0.95X (the ELISA kit) + 10.53 (n = 115 serum samples). We concluded that this assay reagent provides an accurate, precise, and simple method for routine measurement of MMP-3 levels in serum and plasma samples.

A-347

Development of a Duplex Assay for the Simultaneous Detection of Anti-Thyroglobulin and Anti-Thyroid Peroxidase Autoantibodies Employing Biochip Array Technology.

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Background: Autoantibodies (aAb) are established clinical markers of autoimmune disease; in particular, they aid in the diagnosis of autoimmune thyroid disease (AITD) and distinguish it from other forms of thyroiditis. AITD causes cellular damage and alters thyroid gland function by humoral and cell mediated mechanisms. A characteristic feature of AITD is the production of aAb to thyroglobulin (Tg) and thyroid peroxidase (TPO), key regulatory proteins in the synthesis of the hormone thyroxine. Elevated serum levels of Tg aAb and TPO aAb have been shown to be associated with chronic thyroiditis such as Hashimotos' thyroiditis (which results in hypothyroidism) and Grave's disease (which results in hyperthyroidism). TPO aAb are considered a more sensitive marker of thyroid autoimmunity; however, depending on the patient, TPO aAb may be low while Tg aAb are elevated, thus dual measurement of TPO aAb and Tg aAb will facilitate a more accurate diagnosis of thyroid autoimmunity. The aim of this study was to develop a duplex detection system that will allow for the simultaneous detection of Tg aAb and TPO aAb from a single serum sample using biochip array technology. This represents a useful analytical tool for applications in clinical settings. **Methods:** Human TPO and Tg proteins were immobilized to discrete testing regions (DTR) on a biochip surface using an indirect sandwich assay format. Chemiluminescent signal from each DTR was detected by digital imaging technology on the Evidence Investigator analyser. The multi-analyte calibrators for the standard curve were developed from serum samples containing high levels of TPO aAb with low levels of Tg aAb and vice versa. The calibrators were then standardised using NIBSC reference material 65/93 and 66/387 for Tg aAb and TPO aAb respectively. A correlation study was conducted with a cohort of 236 clinical serum samples, which were assessed for both Tg aAb and TPO aAb, using the biochip array technology and commercially available immunoassays. **Results:** Cross-reactivity and interference testing demonstrated that each individual assay was specific for its target analyte. Both assays demonstrated high sensitivity with detection levels of 0.08 IU and 0.002 IU for Tg aAb and TPO aAb respectively. Mean %recovery for the reference material was 107% with a %CV of 5.22 for Tg aAb and 103% with a %CV of 18.63 for TPO aAb. Correlations to the assigned values resulted in a correlation coefficient of 0.968 and a slope of 0.9372 for Tg aAb and a correlation coefficient of 0.918 and a slope of 0.8446 for TPO aAb. **Conclusion:**This study reports on the development of a clinical diagnostic product for the simultaneous measurement of TPO aAb and Tg aAb in the detection of AITD. Using biochip array technology, this duplex assay simultaneously measures levels of both Tg and TPO aAbs from a single sample, offering advantages over current diagnostic tools which use individual tests for the measurement of these aAbs. This newly developed assay uses low sample volume and will provide a highly sensitive and specific test for the detection of each analyte in a clinical setting.

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Comparison of the AESKU HELIOS IFA system with another ANA Screening Method

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Background: This study compares the AESKUSLIDES® HEp-2 cell line processed on the fully automated HELIOS IFA analyzer (AESKU.SYSTEMS) to the NOVA Lite® HEp-2 cell line (INOVA) processed on the PhD system (Bio-Rad Laboratories).

Methods: 82 de-identified serum samples were tested on the HELIOS automated analyzer at AESKU, Oakland and processed on the PhD system at University of Pittsburgh Medical Center (UPMC). Base dilutions were performed at 1:80 and titrated to an end-point dilution of 1:1280. Several discrepant positive samples were subsequently run in the BioPlex® 2200 system using the BioPlex 2200 ANA screen (Bio-Rad Laboratories). Two independent clinical laboratory scientists evaluated the results at both locations.

Results: Qualitatively, 80/82 (97.6%) HELIOS results were concordant with the PhD system. Titration results also correlated well: 19 samples were double negative, 10 were borderline (1:80 or negative), 41 within 1 titer, 7 within 2 titers, 4 within 3 titers, and 1 within 4 titers Borderline outcomes were treated as comparatively equal when paired results were 1:80 and negative.

5 out of 7 samples within two titers of the PhD/INOVA results had higher HELIOS end-point titrations (range 1:320-1:1280). 3 out of 4 samples with a distance of 3 titers had higher HELIOS end-point titrations. Clinical data and BioPlex results of these 3 samples indicated autoimmune hepatitis or Sjgrens syndrome and an SSA or Centromere result >8 (BioPlex cutoff <1.0). Two additional samples with no clinical history of connective tissue diseases (1:320/negative and 1:80/1:1280, PhD:HELIOS) had a BioPlex result of negative and SSA/SSB >8 respectively. The clinical and diagnostic accuracy of the AESKUSLIDE/HELIOS reagent system is favorable based on higher end-point titrations and confirmatory data.

Conclusion: Many laboratories allow a comparative titer discrepancy of 1 dilution as a diagnostic convention when determining precision. Therefore, the combination of AESKUSLIDES® and HELIOS analyzer has a higher sensitivity and specificity than the INOVA/PhD system. AESKU ANA IFA reagent systems are designed to be more clinically relevant to disease state individuals and are therefore more diagnostically significant.

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Evaluation of an IgG1 assay for use on the Binding Site Next Generation Protein Analyser

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Assays for measurement of IgG subclasses in serum are routinely used in many immunology laboratories in the diagnosis of IgG deficiencies. Abnormal levels of one or more subclass may be associated with conditions including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia. Reduced IgG1 levels are often indicative of general immunodeficiency. Here we describe the evaluation of an IgG1 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser

capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 1500-36,000mg/L at the standard 1/10 sample dilution, with sensitivity of 150mg/L. High samples were remeasured at a dilution of 1/40 with a measuring range of 6000-144,000mg/L. Precision studies (CLSI EP5-A2) were performed at nine levels in duplicate over 21 working days and were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 3.2%, 1.7%, 2.4% and 1.1% for the 522 mg/L sample, 5.5%, 2.6%, 2.5% and 4.2% for the 2871mg/L sample, 3.0%, 1.6%, 1.4% and 2.1% for the 3083mg/L sample, 3.3%, 1.8%, 1.8% and 2.1% for the 4869mg/L sample, 5.5%, 1.2%, 5.4% and 0% for the 7179mg/L, 4.1%, 1.5%, 3.6% and 1.4% for the 12,131mg/L sample, 3.4%, 1.2%, 3.0% and 1.1% for the 14,542mg/L sample, 5.4%, 2.0%, 3.6% and 3.5% for the 13,847mg/L sample and 4.7%, 3.1%, 2.5% and 2.5% for the 28,132mg/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (1500-36,000mg/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y= 0.97x + 101.70, R^2 = 1.00). No significant interference (within ±10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into a sample with known IgG1 concentrations when run using the minimum sample dilution. Correlation to the Binding Site IgG1 assay for the SPA PLUS was performed using 142 samples (range 1064 - 16,822mg/L). Good agreement was observed between assays (mean 6273mg/L; range 1064 - 16,822mg/L v mean 6199mg/L; range 1055-15,177g/L), Passing-Bablok regression; y=1.05x - 228.61. We conclude that the IgG1 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

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Evaluation of an IgG2 assay for use on the Binding Site Next Generation Protein Analyser

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Assays for measurement of IgG subclasses in serum are routinely used in many immunology laboratories in the diagnosis of IgG deficiencies. Abnormal levels of one or more subclass may be associated with conditions including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia. In particular, reduced production of IgG2 in children is associated with recurrent infection. Here we describe the evaluation of an IgG2 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 194-7000mg/L at the standard 1/10 sample dilution, with sensitivity of 2.96mg/L. High samples were remeasured at a dilution of 1/40 with a measuring range of 776-28,000mg/L. Precision studies (CLSI EP5-A2) were performed at five levels in duplicate over 21 working days and were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 4.8%, 1.5%, 2.4% and 3.9% for the 80mg/L sample, 5.7%, 3.3%, 1.6% and 4.4% for the 390mg/L sample, 3.5%, 1.0%, 1.3% and 3.0% for the 1828mg/L sample, 2.6%, 1.0%, 1.4% and 2.0% for the 2982mg/L sample and 2.9%, 1.2%, 1.4% & 2.3% for the 5855mg/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (194-7000mg/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y= 0.9612x + 0.0298, R^2 = 0.9878). No significant interference (within ±10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into a sample with known IgG2 concentrations when run at the minimum sample dilution. Correlation to the Binding Site IgG2 assay for the SPA PLUS was performed using 143 samples (range 386 - 8031mg/L). Good agreement was observed between assays when analysed by Passing-Bablok regression; y=0.99x - 0.02. We conclude that the IgG2 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

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Examining the Frequency of Autoantibodies in the Brazilian Population in 2013 Using the Multiplex Technique

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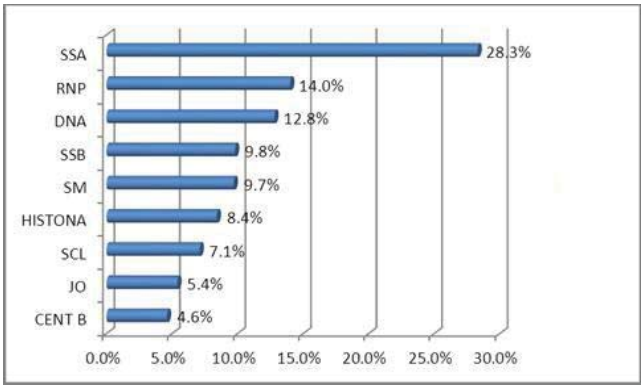
Background: Autoantibodies are antibodies that react against the organism's own components. The appearance of a large number of autoantibodies is a pathological condition that can occur in a number of diseases, such as diabetes mellitus type 1, systemic lupus erythematosus, Sjögren's syndrome, Hashimoto's thyroiditis, Graves' disease and rheumatoid arthritis, among others.Objective: Describe the frequency of autoantibodies in Brazilian samples using the Luminex technology.

Methods: In the period from January to December 2013, 42,249 serum samples of patients from all over Brazil were analyzed by DASA's Manual Immunology laboratory. The samples were tested for the following autoantibodies: dsDNA, SSA, SSB, Sm, RNP, Scl-70, Jo-1, Centromere B and Histone, using the Athena Multi-Lyte ANA II Plus Test System (Zeus Scientific, Raritan, NJ) and the Luminex 200 IS equipment, version 2.3 (Luminex, Austin, TX). The results were interpreted using the Luminex 200 software, version 2.3, according to the reference value indicated by the kit's manufacturer.

Results: Of the samples tested for autoantibodies using the Luminex method, 27,884 (66%) presented a negative result and 12,674 (30%) presented a positive result, while 1,689 (4%) presented an inconclusive result. Among the samples that presented a positive result, the most prevalent autoantibody observed was SSA, present in 28.3 % of the samples analyzed. SSA is related to diseases such as Sjögren's syndrome, Systemic Lupus Erythematosus, Rheumatoid Arthritis, SS/SLE Overlap Syndrome, Subacute Cutaneous LE (SCLE), Neonatal Lupus and Primary Biliary Cirrhosis, among others. Graph 1 represents the autoantibodies detected and their respective frequencies in the population studied.

Conclusion: The Luminex xMAP technology enables the detection of multiple analytes in a single reaction well, reducing operating costs and physical space, and may be widely applicable to various processe Graph 1 - Frequency of the autoantibodies detected.



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Evaluation of a Cystatin C assay for use on the Binding Site Next Generation Protein Analyser

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Cystatin C has been shown to be superior to creatinine as a marker of glomerular filtration rate and is increasingly used in the diagnosis of renal dysfunction. Here we describe the evaluation of a Cystatin C assay for the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay is programmed to automatically dilute a single calibrator to create a 6-point calibration curve with a measuring range of 0.33-7.09mg/L at the standard 1/10 sample dilution, with sensitivity of 0.33mg/L. High samples are automatically re-measured at the 1/20 sample dilution with a measuring range of 0.67-14.18mg/L with a total assay time of 11 minutes. Within-run precision was assessed by running 5

samples at concentrations across the measuring range twenty times with a single kit lot, on one analyser. The coefficients of variation for these 5 levels were as follows; 2.10% for the sample at 0.57mg/L, 1.21% at 1.22mg/L, 1.13% at 2.52mg/L, 1.08% at 3.69mg/L and 2.36% at 6.14mg/L. Linearity was assessed using a high Cystatin C sample which was serially diluted across the width of the curve at the 1/10 sample dilution (range 0.24-8.4mg/L) and tested in triplicate at each dilution. Results were compared to expected values and demonstrated good linearity when results were analysed by linear regression; y=1.003x-0.0327, R^2=0.9998. No significant interference (within ±6%) was observed when serum pools of known Cystatin C concentrations were spiked with bilirubin (20mg/dL), haemoglobin (5g/L), Intralipid (2000mg/dL) or triglyceride (1000mg/dL) and tested at the standard sample dilution. Comparison was made to the Cystatin C assay for use on the Binding Site SPA PLUS analyser by comparing 115 normal and clinical serum samples (range 0.53-8.54mg/L). Good agreement was observed when the data was analysed by linear regression analysis; y=1.0175x - 0.2179, R^2=0.9917. We conclude that the Cystatin C assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

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Evaluation of a liposome-based CH50 assay for use on the Binding Site Next Generation Protein Analyser.

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The complement cascade is made up of around 20 serum proteins that form part of the innate immune system. A major function of complement is to lyse bacteria through formation of the membrane attack complex (MAC). The complex interactions of the complement cascade mean that functionality of the MAC cannot necessarily be inferred by apparently normal levels of any single component. Measurement of complement activity is therefore desirable, however existing methods involving antibody-sensitised erythrocytes are limited by the need to create appropriate serum dilutions and by the instability of the erythrocytes. These issues have been overcome through the use of liposomes encapsulating glucose-6-phosphate dehydrogenase (G6PDH) in place of erythrocytes. On addition of sample, antibodies in the reagent combine with dinitrophenyl on the liposomes. The resultant complex activates complement in the sample, which lyses the liposome, releasing G6PDH to react with glucose-6-phosphate and NAD in the reagent. The change in absorbance can be measured turbidimetrically and is proportional to the complement activity in the sample. Complement activity has been correlated with the active stage of systemic lupus erythematosus, rheumatoid arthritis, cryoblogulinemia-vasculitis, some forms of nephritis, and inherited deficiencies of the complement system. Here we describe evaluation of a CH50 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of. The analyser is programmed to construct a six point calibration curve from a single, lyophilized serum-based calibrator. The standard curves are validated by assay of control fluids. The assay range was 12.5 - 100 U/mL using a 1/2 sample dilution. The assay showed a high degree of linearity when serially diluted serum samples were assessed using a weighted linear regression analysis of measured values against expected values (y = 1.01x + 0.26 r^2= 0.998, range 9.987 - 102.230 U/mL). Precision was assessed at five levels across the measuring range. Coefficients of variation for within run and total precision respectively were 20 U/mL - 4.8% and 8.3%, 31 U/mL - 3.7% and 5.8%, 42 U/mL - 1.5% and 4.4%, 52 U/mL - 1.4% and 3.5%, and 80 U/mL - 2.1% and 4.4%. No significant interference (within ±10%) was observed upon addition of haemoglobin (500 mg/dL), bilirubin (20 mg/dL), ascorbic acid (50 mg/dL) or Intralipid (250 mg/dL) to samples with known CH50 values. Good agreement was observed with the Binding Site SPA PLUS CH50 assay when normal and deficient samples (n=28, range 23.091 - 66.143 U/mL) were compared and analysed by Passing-Bablock regression: y=0.96x - 0.24. We conclude that the CH50 assay for the Binding Site next generation protein analyser measures complement activity rapidly, precisely and accurately and shows good agreement with existing assays.

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Evaluation of the urine utility of a multipurpose albumin assay for use on the Binding Site Next Generation Protein Analyser

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Albumin measurement in urine is routinely performed for the diagnosis of kidney disease in conjunction with other laboratory and clinical findings. Here we describe the evaluation of a multipurpose albumin assay for use on the Binding Site's next generation protein analyser with respect to the measurement of urine. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay is programmed to automatically dilute a single calibrator to create a 6-point calibration curve with a measuring range of 11-332mg/L at the standard 1/1 sample dilution, with sensitivity of 11mg/L. High samples are automatically re-measured at the 1/50 sample dilution with a measuring range of 550-16600mg/L and a total assay time of 10.5 minutes. Precision studies (CLSI EP5-A2) were performed testing five urine concentrations in duplicate over 21 working days. Each antigen concentration was assessed for total, inter-lot and inter-instrument precision using three reagent lots on three analysers. The coefficients of variation were 3.9%, 0.97% and 1.24% for the 23.0mg/L sample, 3.2%, 0.26% and 0.50% for the 39.0mg/L sample, 2.1%, 0.87% and 0.31% for the 143.4mg/L sample, 3.2%, 1.21% and 1.23% for the 275.1mg/L sample and 2.6%, 0.94% and 0.84% for the 1490.2mg/L sample. Linearity was assessed using a pool of urine samples spiked with purified human albumin. The fluid was serially diluted and results were compared to expected values. The assay showed good linearity when observed results were compared to expected results and analysed by linear regression; y=0.9933x-0.7688, R^2=0.9995. No significant interference was observed (<±3%) when urine samples of known albumin concentration were spiked with bilirubin (20mg/dL), haemoglobin (25mg/dL), ascorbic acid (20 mg/dL) or total protein (100mg/dL). Comparison was made to the albumin urine assay for use on the Siemens BN™II analyser by comparing clinical urine samples (n=71, range 12.8-1334.8mg/L). Good agreement was observed when the data was analysed by linear regression; y=1.048x + 7.3231, R^2=0.9959. We conclude that the urine albumin assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

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Patient sensitization profile by ImmunoCAP Solid Phase Allergen Chip (ISAC) in a large Brazilian laboratory.

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Background: Most atopic patients have positive test results to numerous allergens and the true cause of symptoms can be difficult to identify due to an inconclusive medical history regarding the role of different allergens and reactions. ImmunoCAP ISAC is a microarray technology developed by allergy specialists to allergy specialists for investigation of multisensitized patients. It can also reveal unexpected sensitizations, cross reactive components and risk assessment, providing physicians with relevant information to handle allergen avoidance and individualized patient management.
Objective: The aim of this study was to evaluate the allergy sensitization to some of the important molecular allergens and evaluate the incidence of Latex and Insect Venom. Samples were collected at SP and RJ DASA laboratories from 05 Jun 2012 to 30 Jan 2014. All 66 patients were tested with a panel of 112 different allergens components summing up 7,392 results with ImmunoCAP ISAC (Phadia AB, Thermo Fisher).
Methods: ImmunoCAP ISAC is a semi-quantitative test and results are reported in ISAC Standardized Units (ISU) giving indications of specific IgE antibody levels. The allergen components are spotted in triplets and covalently immobilized to a polymer coated slide. IgE antibodies from the patient sample bind to the immobilized allergen components. The complex is detected by a fluorescence labeled anti-IgE antibody which is measured with a laser scanner. **Results:**
Results for components were evaluated using Phadia Microarray Image Analysis (MIA) software. 27,3% of the reports were found negative (66,7% women/ 33,3% men) and 72,7% of the reports were found positive (56,3% women / 43,7% men). Shrimp Tropomyosin (nPen m1) and Grass Pollen (nCyn d 1) had almost similar positive frequency (16%) and Ovomucoid (nGal d 1) was 50% less compared to

the first two ones (8,33%). Taking patient as calculation basis, the higher incidence was found for insect venom components (15.58%), followed by Latex (8,33%) and Peanuts (2,03%)

Molecular Allergens: % positive

Shrimp Tropomyosin nPen m1 16

Ovomucoid nGal d 1 8,33

Grass Pollen nCyn d 1 16,67

Patients: % positive

Latex 8,33

Insect Venom 15,58

Peanuts 2,03

Conclusion: A high positivity of Tropomyosin allergens, which might indicate cross reactivity between mites, cockroach and shrimp is important to handle shrimp allergy, since these patients might also react to mites. Patients with sensitization to peanuts components rAra h 1 rAra h2 are among the group with high risk to severe allergic reaction to peanut. Ovomucoid is an allergen which indicates that egg symptoms might persists after childhood as well as indicates severity. Positivity to pollen allergens indicates that pollen allergy might be underestimated in our population. The results to Latex and Insect venom show the importance of this toll, since other sensitizations may be found in parallel providing more patient information.

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Autoantibodies directed against moesin N_{1-297} /$C_{471-577}$ are specific serum biomarkers for immune thrombocytopenic purpura (ITP)

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Moesin is a member of the ezrin/radixin/moesin (ERM) protein family, and is localized in the cytoplasmic side/end of membrane in filopodia and other micro-extensions on the cell surface. Moesin consists of three domains: N-terminal (N) membrane-binding domain, α-helical (α), and positive charge C-terminal domain. In comparison to other cell types, human platelets demonstrate high expression of moesin but not ezrin or radixin. Phosphorylation of threonine558 in carboxy-terminal actin-binding domain of moesin is associated with the activation of platelets. We have cloned three polypeptides of human moesin: N_{1-297} terminal, $\alpha_{298-470}$ helix domain and $C_{471-577}$ terminal as well as investigated autoantibodies against moesin in patients with ITP. Following informed consent, serum samples from patients with ITP (n=77), patients with non-immune thrombocytopenia or other hematologic diseases (n=47), and gender-matched healthy control subjects (n=50) were evaluated. The titers of moesin autoantibodies were significantly elevated in the sera from patients with ITP compared with healthy subjects (Mean of autoantibodies titers = N_{1-297} 0.515 vs 0.155; P =0.0001). The levels of moesin autoantibody against $C_{471-577}$ in ITP patients were also markedly higher than healthy subjects (Mean of autoantibody titer = $C_{471-577}$ 0.430 vs 0.103, $P < 0.0001$). In contrast, the titer of autoantibody against moesin $\alpha_{298-470}$ helix domains was similar in ITP and healthy subjects. When an autoantibody cut-off value of ±2D in normal control subjects (n=50) was assigned, the serum levels of moesin autoantibodies in ITP patients were found to be elevated in 91% (70/77) for N_{1-297}, 72% (56/77) for $C_{471-577}$ but only 1.3% (1/77) for $\alpha_{298-470}$. Patients with other hematologic diseases, including non-immune thrombocytopenia, anaphylactic purpura, multiple myeloma, pure red cell aplasia, and myelodysplastic syndrome were all negative for moesin autoantibodies. Regardless of the cause of ITP, the autoantibodies against moesin N_{1-297} or $C_{471-577}$ were significantly high than among patients with non-immune-related thrombocytopenia and healthy subjects**.** We used Western blot analysis to confirm the presence of moesin autoantibodies in ITP patients. The results showed that only ITP patients' serum specifically recognized the moesin N_{1-297} and $C_{471-577}$ terminal domains as well as commercial moesin antibodies. In contrast, the antoantibodies from ITP patients' serum did not recognize $\alpha_{298-470}$ helix domains. We also confirmed using Western blot analysis that serum from patients with thrombocytopenia with other hematologic diseases and healthy subjects did not show the presence of autoantibodies against moesin N_{1-297} or $C_{471-577}$ terminal. To further confirm that the autoantibodies against moesin N_{1-297} or $C_{471-577}$ terminal domains were present in ITP patients, antigen competitive inhibition assay was accessed. This N_{1-297} terminal polypeptide (0.5ug/ml or 2.5ug/ml) blocked the detection of autoantibodies against moesin N_{1-297} in the standards whereas an isotype-matched ITP serum did not have any impact on the detection of this autoantibody. Similarly, $C_{471-577}$ terminal polypeptide in the presence of both low (0.5ug/ml) and high (2.5ug/ml) concentrations of the blocking autoantibody against moesin $C_{471-577}$ terminal portion. We propose that autoantibodies against moesin N_{1-297} and $C_{471-577}$ may be specific serum biomarkers for clinical diagnosis and differentiation of ITP from non-immune thrombocytopenia and other hematologic diseases.

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Analytical evaluation of third-generation allergen-specific IgE assay "3gAllergy™" Measurement by Automated Immunoassay System " IMMULITE 2000 XPi "

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Introduction: With the changes in the living environment including diet and air pollution, allergic diseases have been expanded and diversified. Accordingly, laboratory testing for any kinds of allergy has become important and important. Recently, a new generation of allergy blood testing, 3gAllergy, coupling with IMMULITE has been launched. The full automated random-access multiparameter luminescence immunoassay system, IMMULITE 2000 XPi is based on a solid phase two-site chemiluminescent enzyme immunoassay (CLEIA) and provides quantitative determination of allergen-specific IgE. Here we evaluated analytical performance of the IMMULITE 2000 XPi measurement system of 3gAllergy.

Specimens: We used serum and plasma samples collected from our inpatients/ outpatients and the employees who volunteered, as well as control samples commercially available. This study has been approved by the ethical committee in Hamamatsu University School of Medicine.

Methods: In this study, we evaluated the basic performance and compared IMMULITE 2000 XPi Immunoassay System and its dedicated reagents (Siemens Healthcare Diagnostics, USA) with the ImmunoCAP (Phadia AB, Sweden).

Results: 1. Dermatophagoides pteronyssinus (D1), House dust mites (H1) and Japanese cedar (T17) are the major allergen in Japan. The 3gAllergy reagents showed the following performance.

1) Within-run precision The CVs(%) for control samples measured 20 times with 7 cconcentrations in sequence were 1.7 to 5.98% (D1), 2.7 to 8.7% (H1) and 4.3 to 6.1% (T17).

2) Linearity Linearity observed in high concentration range and in low concentration range indicated favorable results, respectively. However, a downward trend were observed in the range of more than 250 IUA/mL.

3) Minimal detection limit By use of sequential dilution of the specimen with low concentration, the detection limit were shown to be 0.02 IUA/mL.

2. Fourteen tests for Allergen (mite, cedar, alder, cypress, orchard grass, ragweed, mugwort, dog, cat, egg white, ovomucoid, egg yolk, cow's milk and peanuts)

1) Correlation We evaluated correlation with immuno-CAP for the 14 allergens. The class concordance rates and measurement correlation of 14 tests were comfortable. However, slightly higher tendencies in 3gAllergy are observed in the egg white, ovomucoid and egg yolk.

Conclusion: The basic performance of IMMULITE 2000 XPi of "3gAllergy™" and correlation with immunoCAP was satisfactory. The slight higher tendencies in 3gAllergy for egg white, ovomucoid and egg yolk were considered due to the higher sensitivity, and then patients with low titer of allergen-specific IgE could be discovered and followed. 3gAllergy is a third-generation allergen-specific IgE assay, delivering fast and accurate results to help enhance the quality of care and service provided to the patient. In addition, the device has some excellent properties, for example, offering excellent precision, accuracy and lot-to-lot reproducibility, a wide range of test menu, easy handling and maintenance, reducing workloads of manual sample sorting with the innovative sample rack system and time for reporting. The properties could lead to improved patient care.

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Droplet Digital PCR (ddPCR) for Quantitative Analysis of Treg-specific Demethylation Region (TSDR) in peripheral blood compared to Flow Cytometry (FACS)

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Background: Regulatory T cells (Tregs) are crucial for immune homeostasis and regulate several immune responses, especially tolerance induction. In transplanted patients they may be useful for monitoring immune status, whereas the value for tolerance prediction of peripheral Treg determination is still debated. Several methods for Treg detection are in use, either based on analysis of FoxP3 protein expression by FACS analysis or the determination of a specific de-methylated region in the FOXP3 gene (TSDR).

Methods: The TSDR of the FoxP3 gene de-methylation was determined with the QX100™ Droplet Digital™ PCR System (Biorad) using methylation specific assays. DNA was extracted after CD4+ T-cell isolation with Dynabeads® CD4, followed by bisulfite conversion. We analyzed Treg numbers by FACS compared to the TSDR ddPCR in 60 blood samples (31 from male, 29 from female) from 38 patients during the first 13 months after liver transplantation (LTx). Peripheral blood mononuclear cells (PBMC) were isolated with Lymphoprep™ (Axis-Shields) and frozen at -80°C. After thawing the cells were stabilized in RPMI 1640/FCS/Penicillin overnight at 37°C, and subsequently stained. Treg were identified as CD4+CD25+CD127lowFoxP3+/CD4+ cells in a BD FACSCANTO™ II (BD Biosciences). For the CD25 staining an antibody (clone M-A251) recognizing a different epitope than the therapeutically used Basiliximab was chosen to eliminate interference.

Results: Compared to an earlier conventional qPCR method (LightCycler480), the precision was substantially improved with the TSDR ddPCR (6.3% CV) between runs at a level of 1.3% de-methylated TSDR compared to 30% with qPCR using the same control sample. FACS analyses were based on a minimum count of 30,000 CD4+ cells and showed an imprecision of 6.1% (CV) at 2.6% Tregs. In LTx patients TSDR ddPCR showed higher values (5.2%±2.6) compared to the FACS assay (1.4%±1.0). Noteworthy, if FoxP3+/CD4+ cells were considered, resulting values were still lower (1.9%±1.5) compared to TSDR. No significant correlation was found between Treg percentages determined by both methods (r=0.218, p=0.095).

Discussion: The de-methylated FoxP3-TSDR is highly specific for natural Treg cells. It has already been proven that human non-regulatory T cells conserve their methylation status after being activated (imprinting). Hence TSDR ddPCR provides a more robust measure than the analysis of protein expression by the use of antibodies in FACS. The precision of ddPCR is comparable to FACS analyses. Our new ddPCR assay is able to measure all TSDR T-cells, even those, which are undetectable by FACS assay such as "latent" Tregs, which have lost their phenotypic FoxP3 expression. Another reason for the deviation and the lack of agreement might be due to the complexity of the flow cytometric analyses in particular if intracellular staining is needed. In addition in critically ill patients high background signals are common, but hard to compensate for. This new ddPCR assay allows for specific Treg quantification with adequate precision, good reproducibility, fast turn around, low costs and only requires 20% of blood compared to FACS. Further studies will show if the exact ratio of Treg/CD4 cells measured by ddPCR is useful as a biomarker for tolerance assessment in transplanted patients.

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The Association of Serum Free Light Chain Levels with Markers of Renal Function

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BACKGROUND: The kidney is often affected in plasma cell dyscrasias, usually due to the effects of nephrotoxic monoclonal free light chains. Renal failure due to a monoclonal gammopathy may be detected by the highly sensitive serum free light chain (sFLC) ratio yet missed by electrophoretic assays. The aim of this study was to assess sFLC levels in relation to markers of renal function.

METHODS: 513 patients were included in this study. sFLC levels were measured by Freelite® (The Binding Site Group Ltd, Birmingham, UK) assay using the BNII nephelometer (Siemens Diagnostics, Germany). κ/λ sFLC ratio was calculated. Serum creatinine levels were analysed by modified Jaffe method in Cobas 8000 analyser. GFR was estimated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. Patients were assigned to 2 groups depending on their eGFR values: ≤ 60 mL/min/1.73m2 (group 1, n=103) and >60 mL/min/1.73m2 (group 2, n=410). Data were expressed as median and min.-max. All statistical analyses were done with SPSS version 20.0 and a significance level of 0.05 was considered.

RESULTS: Serum kappa FLC median value was 36.4 (5.62-16000) mg/L, serum lambda FLC was 21.7 (4.91-8770) mg/L, κ/λ sFLC ratio was 1.33 (0.01-3258), serum creatinin was 1.56 (0.63-7.21) mg/dL in group 1. Both of Lambda sFLC and κ/λ sFLC ratio were correlated with eGFR (r=-0.318, r=0.198, p<0.05, respectively). We did not find any significant correlation between κ/λ sFLC ratio and eGFR in group 2.

CONCLUSIONS: We examined the association between polyclonal sFLC concentrations and renal function. Our preliminary findings suggest that serum Lambda FLC might be considered useful marker of predicting renal function. Prospective studies are needed to clarify the usefulness of these parameters for identifying renal failure due to a monoclonal gammopathy.

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Evaluation of a Caeruloplasmin assay for use on the Binding Site Next Generation Protein Analyser

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Caeruloplasmin is synthesised in the liver and has a major role in copper metabolism, carrying approximately 95% of the total copper in serum. Decreased levels of Caeruloplasmin can be caused by hereditary disorders of copper metabolism, for example; inability to transport oxidised copper (Cu2+) from the gastrointestinal epithelium into the circulation (as in Menkes disease), or the inability to insert Cu2+ into the developing Caeruloplasmin molecule (as in Wilson's disease). Dietary copper insufficiency, including malabsorption, also reduces serum Caeruloplasmin concentrations. Here we describe the evaluation of a serum Caeruloplasmin assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is enhanced by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems.The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 0.03-0.82g/L at the standard 1/10 sample dilution, with sensitivity of 0.03g/L. High samples are automatically re-measured at a dilution of 1/20, with an upper measuring range of 0.06-1.64g/L. Precision studies (CLSI EP5-A2) were performed at five levels in duplicate over 21 working days. Five antigen levels were assessed in duplicate, twice daily for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 9.4%, 1.5%, 5.5%, 7.4% and 8.8% for the 0.061g/L level, 7.6%, 1.6%, 3.4%, 6.6% and 6.2% for the 0.156g/L level, 5.6%, 1.7%, 2.3%, 4.8% and 2.9% for the 0.247g/L level, 5.7%, 1.7%, 2.1%, 5.0 and 3.7% for the 0.442g/L level and 6.4%, 2.0%, 1.8%, 5.9% and 3.7% for the 0.867g/L level respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (0.03 - 0.82g/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values ($y=0.96x + 0.00$, $R^2 = 0.999$). No significant interference (within 10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1250 formazine turbidity units) when spiked into a sample with known Caeruloplasmin concentrations and measured using the minimum sample dilution. The assay was compared to the Caeruloplasmin assay for the Binding Site SPA PLUS analyser by running clinical samples (n=36). Good agreement was demonstrated by Passing-Bablok regression; $y = 1.00x + 0.01$. We conclude that the Caeruloplasmin assay for the Binding Site next next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

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Identification of an IgD biclonal gammopathy

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Background: Monoclonal abnormalities are confirmed by immunofixation electrophoresis (IFE) which identifies the immunoglobulin heavy chain and/

or light chain type. Routine IFE includes antisera to gamma, alpha and mu heavy chain and kappa and lambda light chain. When a free light chain is detected without a corresponding heavy chain, IFE is performed with antisera to delta and epsilon heavy chains to rule out a monoclonal IgD or IgE. We do not routinely reflex to anti-delta and epsilon IFE when we detect a free light chain in the presence of an intact immunoglobulin with the same light chain type. We recently initially reported a "monoclonal IgG lambda plus monoclonal free lambda" that eventually was confirmed as a biclonal IgG lambda and IgD lambda. Because the presence of a monoclonal IgD protein is often associated with either multiple myeloma or primary amyloid, this IgD/IgG biclonal gammopathy triggered an evaluation of our anti-delta and epsilon reflex process for free light chains.

Methods: Protein electrophoresis is performed on Helena agarose gels and IFE is performed with Sebia reagent kits. IFE reflex testing uses BioWhittaker antiserum for IgD and Binding Site antiserum for IgE.

Results: During a 1 month period we detected 1245 patients with a serum monoclonal protein. Twenty-eight of the samples (2.2%) were also tested with IgD and IgE antisera. Of these 28 patients, 19 had a monoclonal free light chain and the remaining 9 were eventually reported as having a biclonal gammopathy of 2 intact immunoglobulins with differing light chain type. In addition, there were 26 patients with an intact monoclonal immunoglobulin plus a monoclonal free light chain of the same type as the intact immunoglobulin. As per the laboratory protocol, these samples were not reflexed to the expanded IFE.

Conclusion: Our protocol to reflex monoclonal free light chains to anti-delta and epsilon IFE does not include free light chains that are associated with an intact monoclonal immunoglobulin with the same light chain. This protocol resulted in 2.2% of positive IFE tests being reflexed. If all new monoclonal free light chains are reflexed (regardless of the presence of an intact immunoglobulin with the same light chain), our reflexed IFE testing would approximately double.

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Clinical Evaluation of the New BioPlex Celiac IgA and IgG Kits

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Background: Serologic testing, specifically for tissue transglutaminase (TTG) and deamidated gliadin peptide (DGP) antibodies, is increasingly being relied upon to establish a diagnosis in patients with suspected celiac disease (CD). Therefore, diagnostic accuracy of CD-specific autoantibodies is critically important. Although monitoring patients with CD who have instituted a gluten-free diet (GFD) is routine practice, how responses compare between various serologic markers is not always clear. This study compares performance of bead-based multiplex immunoassays (MIAs) for TTG and DGP antibodies to that of currently available individual enzyme immunoassays (EIAs) for diagnosis and monitoring.

Methods: Retrospective samples were obtained from healthy controls (n=210), inflammatory disease controls (n=101), and patients with CD and related diseases (total n=105), including adult patients with biopsy-proven CD (n=96; 7 with IgA deficiency), pediatric patients with CD (n=6), and patients with dermatitis herpetiformis (DH) (n=3). An additional retrospective CD cohort was included (n=10), consisting of paired samples pre- and post-GFD (time on GFD 1.6 to 52.6 months). MIA testing was performed on BioPlex® 2200 Celiac IgA and IgG kits (Bio-Rad); EIA testing was performed on QuantaLite R h-tTG and Gliadin IgG II IgA and IgG kits (INOVA Diagnostics). All testing was performed according to manufacturers' instructions.

Results: The specificity of TTG-IgA, TTG-IgG, DGP-IgA, and DGP-IgG by MIA in healthy donors ranged between 96.2% and 99.5%. These were not significantly different from the EIA specificities (97.6% to 99.5%). For the disease control cohort, TTG-IgA and DGP-IgA showed similar specificities between MIA (97% and 100%) and EIAs (100% for both). However, the EIAs had lower specificities for TTG-IgG and DGP-IgG at 92% and 96%, compared to 100% and 99% for the MIA. In the CD/DH cohort, excluding IgA deficient patients, MIAs and EIAs demonstrated identical sensitivities at 92% for TTG-IgA and 86% for DGP-IgA. For TTG-IgG and DGP-IgG, the total CD/DH cohort was analyzed, including IgA deficient patients. The sensitivity of DGP-IgG was 83% and 79% for MIA and EIA; for TTG-IgG, overall sensitivities were 41% and 48% for MIA and EIA. If only IgA deficient patients were analyzed, a sensitivity of 57% was obtained for DGP-IgG by both methods. However, TTG-IgG by MIA had a higher sensitivity than EIA (71% vs 29%). Among patients on a GFD, for individuals positive for DGP-IgA at baseline, 25% remained positive by both MIA and EIA. For those patients positive for TTG-IgA, 11% and 14% remained positive on MIA and EIA, respectively. In contrast, 62.5% and 71.4% of patients positive for DGP-IgG remained positive by MIA and EIA.

Conclusion: The diagnostic accuracy of autoantibody serology for diagnosis of CD appears comparable between MIA and EIA methods. The only significant differences identified between the methods were for TTG-IgG and DGP-IgG. These results also confirm that TTG-IgA displays the best combination of sensitivity and specificity for CD, as demonstrated by previous studies. Further, the IgA isotypes appear to have the most utility for monitoring, based on conversion to a negative serology following implementation of a GFD.

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Diagnostic utility of autoantibodies and HLA-DRB1 Shared Epitope in patients with recent onset Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic polyarthritis. Anti-cyclic citrullinated peptide (anti-CCP) antibodies have diagnostic value in RA but the role of shared epitope (SE) is unclear. We assessed the diagnostic value of anti-CCP antibodies and SE in patients with symptoms of arthritis in their first visit to the rheumatologist.

Methods: We measured anti-CCP antibodies with QUANTA LiteTM enzyme-linked inmmunosorbent assay (ELISA) kit for the detection of IgG anti-CCP3 (Cyclic Citrullinated Peptide 3) antibodies in patient sera (cut-off value, 40 UI/ml). SE was determined with GenID® Reverse Hybridization kit for detection of SE in HLA-DRB1 alleles kit in patient plasma. They were tested for 211 patients with suspected rheumatoid arthritis. The American College of Rheumatology (ACR) criteria for RA were fulfilled for 106 patients. These patients were diagnosed of RA. The other 105 patients were diagnosed with other rheumatic disease. We also determined rheumatoid factor (RF) with BAYER® FR IgM immunoturbidimetric assay for ADVIA 2400 (cut-off value, 20 UI/ml). We determined the diagnostic value (sensibility, specificity and likelihood ratios) for anti-CCP antibodies, SE and RF. We determined the area under the curve (AUC) for anti-CCP antibodies and RF. Statistical analyses were performed using IBM SPSS Statistics version 19 for Windows (New York, USA).

Results: Sensitivity of anti-CCP antibodies, SE and RF for RA were 66,0%, 72,6% and 81,1%, and specificity were 96,2%, 38,1% and 76,2%, respectively. The AUC for anti-CCP antibodies was 0.875 with 95% CI of 0.828 to 0.922 and the AUC for RF was 0.864 with 95% CI of 0.815 to 0.913. The positive likelihood ratio for anti-CCP antibodies, SE and RF were 17.37, 1.06 and 3.41 respectively. The negative likelihood ratio for anti-CCP antibodies, SE and RF were 0.35, 0.72 and 0.25. Anti-CCP antibodies were positive in 30.0% of RF-negative RA patients. Anti-CCP antibodies and RF were positive in 60.4% of total RA patients and were negative in 13.2% of total RA patients.

Conclusion: the anti-CCP antibodies have a very good diagnostic value in their first visit to the rheumatologist. However, the SE doesn´t have diagnostic value in our population.

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4-Phenyl butyric acid attenuate apoptosis via inhibition of endoplasmic reticulum stress against diabetic cardiomyopathy

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Background: Diabetic cardiomyopathy (DCM) is a major cause of death of patients with diabetes. It is known that apoptosis has been considered to play a critical role in DCM. Our recent studies have demonstrated the important role of endoplasmic reticulum stress (ER stress) in diabetes-induced cardiac cell death. The aim of this study was to investigate cardiac protection by 4-Phenyl butyric acid (PBA), a low molecular weight compound that acts as a chemical chaperone to enhance protein folding and ameliorate ER stress, in the development of DCM.

Methods: At 2 weeks, and 2 and 5 months after diabetes onset with Type 1 diabetic mouse model induced with multiple low-dose streptozotocin, cardiac remodeling and dysfunction were determined using echocardiography and hemodynamic evaluation and cardiac fibrosis was detected by Picric acid-Sirius red staining; ER stress signal pathway and apoptosis were detected by western blotting assay and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Mechanism of cardiac protection by PBA was used by cell culture with embryonic rat heart derived cells (H9c2).

Results: Apoptotic cells and CHOP, the activated form of caspase-3 and caspase-12 delineated that diabetes mainly induced cardiac cell death at the early stage of diabetes (2 weeks), but not in the late stages (2 and 5 months). However, there was no apoptotic cell death in the hearts of diabetic mice treated with PBA. In parallel with apoptotic effect, significant up-regulation of the ER chaperones, including phosphorylated eIF2α (p-eIF2α), GRP78, GRP94 and cleaved ATF6 proteins were significantly increased in the heart of diabetic mice at 2 weeks after diabetes onset. However, none of these increased ER chaperones in the hearts of diabetic mice were observed in the heart of diabetic mice treated with PBA. Pre-exposure of H9c2 cells to PBA significantly prevented high glucose or tunicamycin-induced ER stress and apoptosis, while same pretreatments did not have any effect on normal H9c2 cells.

Conclusion: These results suggest that ER stress exists in the diabetic heart, which may cause the cardiac cell death. PBA can attenuate diabetes-induced cardiac cell death via suppression of cardiac ER stress and associated apoptotic effects. This study could provide important theoretical support for prevention and treatment of DCM. The study also might open up a new way for the drugs in research and development for diabetic cardiovascular complications.

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Vitamin D Receptor Polymorphisms and HLA-Class II Genotypes Among Lebanese with Multiple Sclerosis - A Pilot Study

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Background: Multiple sclerosis (MS) is an autoimmune disease with multifactorial etiology. Previous studies showed that HLA-DRB1*15 allele is a major genetic risk factor for MS in other populations possibly through regulation of vitamin D receptor (VDR) complex. In this study, we investigated the HLA class II genotypes and VDR gene polymorphism among a group of Lebanese MS patients and controls.

Methods: Fifty MS patients (remitting/relapsing, aged: 19-74 years, male:female=1:2.1) were selected for this study, based on the Expanded Disability Status Scale. The controls included: 49 healthy subjects (aged: 15-59 years, male:female=1:2) and 51 neurologic patients other than MS (Non-MS, aged 13-70 years, male:female=1:1.12). After a thorough history, blood in EDTA tube was collected. Extracted genomic DNA was used for molecular analysis of VDR genotypes (*ApaI*, *TaqI* and *BsmI*) and HLA class II typing (low resolution HLA-DRB1/3/4/5) (Luminex, San Diego, CA). Differences between groups were evaluated using Mann Whitney-U test. Chi-square test was used for association between various categorical variables. (P<0.05 statistically significant*)

Results: All determined variables were not statistically different between healthy and non-MS patients (p>0.05); therefore both were combined into one control group for analysis. Results are summarized below.

Groups	**Age**(years) (Mean±SD)	**HLA-DRB1*15**(%)	**VDR Gene**								
			ApaI			*TaqI*			*BmsI*		
			AA(%)	Aa(%)	aa(%)	TT(%)	Tt(%)	tt(%)	BB(%)	Bb(%)	bb(%)
MS (n=50)	42.8±13.5	11(22)	15(30)	26(52)	9(18)	20(40)	24(48)	6(12)	7(14)	22(44)	21(42)
Control (n=99)	33.7±12.7	8(8)	37(37)	48(49)	14(14)	31(31)	47(48)	21(21)	20(20)	51(52)	28(28)
P value	<0.001*	0.017*	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Frequency of HLA-DRB1*15 was significantly higher in MS patients compared to controls. None of the VDR genes differed between the two groups. Odds ratio (OR) for MS in the presence of DRB1*15 allele is 3.21 (p= 0.016; 95%CI= 1.20-8.59). Cosegregation of HLA-DRB1*15 and VDR genotypes indicated a slightly increased risk for MS in the presence of A-allele (OR=3.40; p= 0.022; 95%CI= 1.14-10.19). Similarly, combination of DRB1*15 with b-allele resulted in even higher OR of 4.22 although not statistically significant (p= 0.08; 95%CI= 0.75-23.89).

Conclusion: Our results confirm that HLA-DRB1*15 is a strong predisposing factor for MS in Lebanese patients. Furthermore, the interaction between specific VDR alleles and HLA polymorphism may synergistically influence the susceptibility to MS.

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Frequency of antinuclear antibodies (ANA) by indirect immunofluorescence in Brazilian samples

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Background: Antinuclear antibodies (ANAs) are considered a hallmark of autoimmune rheumatic diseases (ARDs), and the indirect immunofluorescence assay (IFA) on HEp-2 cells is the standard method for ANA detection. During the past decade, as the demand for ANA testing increased, new automated methods have arisen for screening/detection of ANAs.Objective: To describe the frequency of positive ANAs and the most common patterns found in Brazilian samples, following the IV Brazilian Consensus on FanHep2 in 2013.

Methods: During the 2013 year, 192.593 serum samples were screened to ANA detection by the Immunology department of DASA, using IFA technique. In the first semester, a semi-automated technique was used, with slides preparation performed by Quanta-Lyser 240 equipment (Inova Diagnostics, San Diego, CA) and manual reading. In the second semester, the "Integrated Laboratory" (Inova Diagnostics) system was implemented. This system is composed by Nova View equipment and Quanta-link software. It allows the performance of full automated process, from slides preparation to reading. During both phases, the initial dilution of samples was 1/160.

Results: We found that 73% of the analyzed samples showed negative result and 27% showed positive result for ANA. The most frequent pattern found among positive samples was Speckled Nuclear Fine Dense - PSNFD, followed by Speckled Nuclear Fine, as described at Table 1. Some patterns were found in less than 1% of the positive samples as NUMA 1 (Pattern Nuclear Fine Speckled with Mitotic Apparatus), Fine Dense Speckeld Cytoplasmitic, Cytoplasmatic with Isolated Dots, NUMA2 (Mitotic Apparatus Type Mitotic Fuse), Nuclear Type Nuclearmembranous, Polar Speckled Cytoplasmatic, Mitotic Apparatus type Intercellular Bridge, Cytoplasmic Fibrilar, Fine Speckled Cytoplasmatic, Mitotic Apparatus type Centriole and Pleomorphic Nuclear Speckled (PCNA).

Conclusion: Our findings demonstrated that 35.43% of the positive samples presented PSNFD pattern, which according to previous reports are rarely found in ARDs.

Table 1. ANA's patterns detected. Legend: *Other patterns: frequency less than 1%.

Pattern	Number of samples	%
Nuclear Fine Dense Speckled (PSNDF)	18609	35.43
Nuclear Fine Speckled	13832	26.33
Nuclear Quasi-Homogeneous Speckled	5179	9.86
Nuclear Homogeneous	2752	5.24
Nucleolar	2423	4.61
Nuclear Coarse Speckled	2419	4.61
Nuclear Centromere	2121	4.04
Nuclear Coarse Speckled Reticuladet	1401	2.67
Reticular Specled Cytoplasmatic	1364	2.60
Nuclear dots	588	1.12
*Other patterns	1838	3.51
Overall	52526	100

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THE SERUM LEVELS OF DIKKOPF-1 (DKK-1) IN AXIAL SPNDYLOARTHRITIS (AXSPA) ARE RELATED TO DISEASE DURATION

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Background: Tumor necrosis factor (TNF) alpha is responsible for induction of dkk-1 which down-regulates bone formation. Therefore, it was expected that TNF-blocker therapy would inhibit radiographic progression in patients with axSpA but this effect has not been observed yet. Nevertheless, most of the studies have included patients with long disease duration and it is unknown whether or not this effect would be the same in patients with an early stage of the disease.

Objectives: To investigate if disease duration influences on the serum levels of dkk-1 in patients with axSpA. Methods: Observational study including consecutive patients with axSpA according to ASAS criteria visiting a tertiary hospital between January 2011 and June 2013. All patients were receiving NSAIDs and none of them was under biologic therapy. The following characteristics were recorded at one visit: Demographic (age, gender), symptoms duration, HLA-B27, disease activity indices (BASDAI, CRP, ESR) and function (BASFI). Blood samples to determine dkk-1 serum levels by enzyme immunoassay were collected at the same visit too. Patients were classified as early axSpA (symptoms duration ≤ 5 years) and established axSpA

(>5 years) and the characteristics enumerated above were compared between both groups. Univariate and multivariate linear regression models were employed to identify the characteristics related to dkk-1 serum levels.

Results: Thirty one patients with early axSpA and 21 patients with established disease were included. Patients with early axSpA were younger (32.6 ± 9.3 vs 41.0 ± 10.2 years; p<0.01), had lower degree of disease activity (BASDAI: 4.6 ± 2.7 vs 6.6 ± 1.9; p<0.01 and ESR: 7.7 ± 9.2 vs 18.1 ± 15 mmHg; p<0.05) and worst function (3.2 ± 2.9 vs 5.8 ± 2.5; p<0.01) compared with patients with established disease. Serum levels of dkk-1 were significantly higher in patients with early disease (25.9 ± 11.5 vs 13.9 ± 13.5; p<0.001 ng/dl). No statistically significant differences were found between both groups for the rest of characteristics. In the univariable analysis, symptoms duration and BASDAI were inversely related to dkk-1 levels (std β: -0.435; p<0.01 and Std β: -0.283; p<0.05, respectively). However, only the relationship with symptoms duration remained statistically significant in the multivariable analysis (std β: -0.415; p<0.01).

Conclusions: Serum Dkk-1 levels in patients with axSpA depend on disease duration, being higher in patients with recent onset of the disease. The effect of TNF-blocker therapy on radiographic progression may be different in patients with an early stage of the disease compared with patients with established disease.

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Impact of genetic (HLA-DRB1 Shared Epitope) and environmental (Smoking) factors in the presence of anti-CCP antibodies in Rheumatoid Arthritis

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Background: Reumatoid Arthritis (RA) is a multifactorial autoimmune disease where environmental and genetic factors interact in the etiology of the disease and development of anti-cyclic citrullinated peptide antibody. The aim of this study is to investigate whether HLA-DRB1 shared epitope (SE), tobacco exposure (TE) and smoking dose (SD) are associated with the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies in Spanish patients with RA.

Methods: A cohort of 106 patients with early diagnosed RA was studied. Anti-CCP antibodies and rheumatoid factor (RF) were measured at diagnosis and HLA-DRB1 genotyping was performed for SE. TE was categorized as never or ever. Smoking dose (SD) was categorized in pack-years with a cut-off of 20 pack-years. Contingency tables and models of logistic regression were used to calculate the association between SE and smoking with the presence of anti-CCP.

Results: In univariate analysis, SE (OR=2.68; 95% CI 1.11 to 6.46), TE (OR=2.79; 95% CI 1.12 to 6.97), SD (OR=6.04; 95% IC 1.68 to 21.74) and the presence of RF (OR=8.73; 95% IC 2.84 to 26.80) were associated with the presence of anti-CCP antibodies. In logistic regression analysis, only SE-TE interaction (OR=7.083; 95% IC 1.01 to 49.50) and presence of RF (OR=3.07; 95% IC 1.26 to 7.49) were independently associated with the presence of anti-CCP antibodies.

Conclusion: SE-TE interaction and the presence of RF were significantly and strongly associated with the presence of anti-CCP antibodies.

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Evaluation of the CSF and serum utilities of a multipurpose albumin assay for use on the Binding Site Next Generation Protein Analyser

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The integrity of the blood-brain barrier may be compromised in certain neurological conditions. Measurement of albumin in CSF and serum, and calculation of a ratio, is widely used as a diagnostic tool in these circumstances. Here we describe the evaluation of the serum and CSF utilities of the low level albumin assay for the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay is programmed to create a 6-point calibration curve from a single serum based calibrator. Total, inter-lot and inter-instrument precision was assessed for both sample types by running serum and CSF pools at five concentrations across the respective curves. Each sample was run in duplicate, twice daily for 21 days across three analysers. Linearity was assessed using pools of normal serum and CSF samples spiked with purified human albumin. Both fluids were serially diluted across the width of the standard curve and results were compared to expected values. No significant interference (±3.03%) was observed when CSF and serum samples of known albumin concentration were spiked with bilirubin (20mg/dL), haemoglobin (500mg/dL) or Chyle (1500 FTU's). Comparisons were made to both the serum albumin and CSF albumin assays for use on the Siemens BN™II analyser by comparing clinical samples. The main assay characteristics are summarised in the table below:

Assay		Serum	CSF
Initial sample dilution		1/200	1/1
Initial range		2200-66400mg/L	11-332mg/L
Maximum sample dilution		1/200	1/10
Maximum range		2200-66400mg/L	110-3320mg/L
Sensitivity		11mg/L	11mg/L
Assay time (mins)		10.5	10.5
Total precision (concentration)(C.V)		(3.7g/L) **(2.9%)**	(145.5mg/L) **(5.92%)**
		(13.0g/L) **(3.0%)**	(281.5mg/L) **(5.37%)**
		(28.5g/L) **(2.88%)**	(439.9mg/L) **(3.62%)**
		(37.0g/L) **(2.6%)**	(593.1mg/L) **(3.52%)**
		(54.4g/L) **(3.3%)**	(975.2mg/L) **(8.08%)**
Inter-kit precision (concentration)(C.V)		(3.7g/L) (**0.6%)**	(145.5mg/L) **(1.15%)**
		(13.0g/L) **(0.4%)**	(281.5mg/L) **(2.89%)**
		(28.5g/L) **(1.0%)**	(439.9mg/L) **(1.36%)**
		(37.0g/L) **(0.6%)**	(593.1mg/L) **(1.88%)**
		(54.4g/L) **(0.6%)**	(975.2mg/L) **(1.87%)**
Inter-instrument precision (concentration)(C.V)		(3.7g/L) **(1.1%)**	(145.7mg/L) **(0.77%)**
		(13.0g/L) **(0.5%)**	(282.3mg/L) **(1.59%)**
		(28.5g/L) **(0.5%)**	(441.8mg/L) **(2.30%)**
		(37.0g/L) **(0.5%)**	(594.6mg/L) **(1.30%)**
		(54.4g/L) **(1.4%)**	(977.8mg/L) **(1.41%)**
Comparison	**Sample no.**	106	62
	Range	19,868-55,847 mg/L	33.9-961.9 mg/L
	Passing and Bablock analysis	Y=1.01x + 1010.77	Y= 1.08x +14.88
	R^2	0.9541	0.9782
Linearity	**Linear regression**	y=0.9978x +589.88	y=1.0x-4.72
	R^2	0.9996	0.9995

We conclude that the low level albumin assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing albumin assays.

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Multiplex Assay of Circulating Inflammatory Biomarkers in Patients with Stroke

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Background: Neuroinflammation is involved in the pathophysiological mechanisms of stroke. However, the role of inflammatory blood biomarkers in relation to the clinically relevant information remains unclear. This study determines the association of various circulating inflammatory biomarkers with stroke severity, classification into different stroke subtypes and patient 3-month outcome.

Methods: 215 stroke patients (Large artery (LAA), n = 93; Cardioembolic (CE), n = 47; Lacunar (LAC), n= 33; Cryptogenic (CR), n = 7 and Hemorrhagic stroke (HS), n = 35) were prospectively evaluated in the Faculty Hospital Plzen between 2012 and 2013. Stroke severity (National Institutes of Health Stroke Scale; NIHSS) was measured at hospital admission. Functional outcome (modified Rankin Scale; mRS) was assessed after 3 months. A multiplex panel of 14 biomarkers (IL1, IL6, IL10, IL12, MCP-1, OPG, OPN, VEGF, MMP1, MMP2, MMP7, MMP9, 25-OH-Vitamin D, 1,25-OH-Vitamin D) was assessed in plasma samples (collected within 4 hours from symptom onset) by Luminex xMAP™ technology. The associations of circulating inflammatory biomarkers with the stroke severity, classification into different stroke subtypes and patient outcome were evaluated by Spearman's rank correlations and Wilcoxon test.

Results: Positive correlations with stroke severity (NIHSS at baseline) were found for IL6 (r = 0.15, P = 0.02), IL10 (r = 0.16, P = 0.014) and MMP9 (r = 0.14, P = 0.03). The worse 3-month outcome (mRS) was correlated with IL6 (r = 0.14, P = 0.036) and blood leukocytes (r = 0.14, P = 0.038). Higher plasma levels of IL6 (P = 0.02), IL10 (P = 0.006) and MMP9 (P = 0.029) and startlingly lower cholesterol (P = 0.029) were found in patients with more severe stroke at baseline (NIHSS > 10). Patients with

worse 3-month outcome (mRS ≥ 3) had higher plasma level of MMP9 (P = 0.04), glucose (P = 0.025), higher NIHSS at baseline (P < 0.0001), lower cholesterol (P = 0.0082) and trend to higher IL6 (P = 0.084). The biomarkers that varied by the stroke classification were OPG (osteoprotegerin, P = 0.018), IL10 (P = 0.015), MMP2 (P = 0.0004), cholesterol (P = 0.0066), NIHSS at baseline (P < 0.0001) and 3-month mRS (P < 0.0001). Patients with ischemic (LAA + CE + LAC) in comparison to hemorrhagic stroke had lower NIHSS at baseline (P = 0.0192), mRS at 3 months (P = 0.003), OPN (osteopontin, P = 0.016), OPG (P = 0.0087) and MMP2 (P = 0.0004).

Conclusion: In our study, various inflammatory circulating biomarkers correlated with stroke severity, such as IL6, IL10 and MMP9. IL6 and blood leukocytes correlated with 3-month outcome. Lower level of OPN, OPG and MMP2 were found in ischemic in comparison with hemorrhagic stroke patients and should be further studied as diagnostic biomarkers of stroke classification. Patients with hemorrhagic stroke had more severe neurological deficit at baseline and worse 3-month outcome. Circulating inflammatory biomarkers should be further examined in patients with stroke.

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Evaluation of an IgM assay for use on the Binding Site Next Generation Protein Analyser

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IgM is the first immunoglobulin produced in a primary immune response. It constitutes around 10% of the total serum immunoglobulin, and is largely in a pentameric form. Pentameric IgM is able to flex into a "crab" or "staple" formation when binding to antigen and activates the classical pathway of complement. Here we describe the evaluation of a serum IgM assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 0.2 - 7.5g/L at the standard 1/20 sample dilution, with sensitivity of 0.1g/L. High samples are remeasured at a dilution of 1/400 with an upper measuring range of 4.0 - 150.0g/L. The assay time is 10.5minutes and is read at end point. Precision studies (CLSI EP5-A2) were performed at eight levels in duplicate over 21 working days. Antigen levels of 0.19g/L, 0.28g/L, 0.38g/L, 1.44g/L, 1.65g/L, 2.68g/L, 5.11g/L and 10.21g/L were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 6.2%,1.4%, 2.3% and 5.5% for the 0.19g/L sample, and 5.6%, 1.5%, 3.8% and 3.8% for the 0.28g/L sample, 5.6%, 1.2%, 3.7%, 4.1% for the 0.38g/L sample, 4.0%, 2.0%, 3.4% and 0.0% the 1.44g/L sample, 3.4%, 1.6%, 2.6% and 1.4% for the 1.65g/L sample, 3.6%, 1.2%, 3.0%, 1.6% for the 2.68g/L sample, 4.0%, 2.2%, 3.0% and 1.3% for the 5.11g/L sample and 5.6%, 2.5%, 3.6% and 3.4% for the 10.21g/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (0.198 - 7.662 g/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values; y=0.9953x + 0.0235, R^2 = 0.9993. No significant interference (within 10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into a sample with known IgM concentrations and measured at the minimum sample dilution. Correlation of this assay with the equivalent assay for the Binding Site SPA PLUS was performed using both normal and clinical samples (n=115, range 0.135 - 60.424 g/L). Good agreement was demonstrated by Passing-Bablock regression; y=0.98x - 0.02 g/L. We conclude that the IgM assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

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Antibodies-to-Infliximab: Assay Development and Correlation with Infliximab Concentrations in Serum Samples of Treated Patients

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Background: Infliximab (IFX) is a chimeric therapeutic monoclonal IgG1 kappa antibody targeting tumor necrosis factor-α and is FDA-approved for treatment of several inflammatory disorders. Patients undergoing therapy may form antibodies-to-Infliximab (ATIs), which can reduce circulating drug concentrations. Measurement of IFX concentration and ATIs is useful for guiding therapy in patients who have lost clinical response. Here we present the development of an electrochemiluminescent immunoassay (ECLIA) for detection of ATIs. Methods: A "bridging" ECLIA was developed in which the ATI forms a link between biotin- and sulfo-tag-labeled IFX (MesoScale Discovery LLC). An 8-point standard curve was established using a high-affinity human IgG1 ATI (AbD Serotec), and an acid-dissociation step was performed to disrupt immune-complexes. Residual sera with physician-ordered IFX and ATI were evaluated (n=37) and compared to results from a reference laboratory (Esoterix). Serial sera from patients on IFX were collected at trough levels (n=36), 48-72 hours post-infusion (n=33) and 28-32 days post-infusion (n=33). IFX was measured using clonotypic heavy chain peptides by LC-MS/MS. Results: The analytical measurable range for the ECLIA ATI assay was established as 19.5-2,500ng/mL (R^2=0.9911). Limit of quantitation was defined as 19.5ng/mL; results >19.5ng/mL were classified as positive. Using residual serum samples, the ECLIA showed an overall qualitative concordance of 86.4% to a commercial method. In the cohort of serial samples, 22% (8/36) of trough samples, with IFX concentrations of 8.5±8.8µg/mL, were positive for ATI; in 7/8 samples the ATIs persisted 28-32 days after IFX infusion (IFX concentration 15±11µg/mL). Samples obtained 48-72h after IFX infusions were negative, suggesting that high concentrations of IFX (77±40µg/mL) interfere with ATI measurement. Presence of ATIs was associated with lower concentrations of IFX at trough (8.8±8.9µg/mL in ATI negatives *vs.* 3.3±4.8µg/mL in positives, p=0.0038), 48-72h post-infusion (78±41µg/mL *vs.* 47±12µg/mL, p=0.0357) and at 28-32 days after (15±7µg/mL *vs.* 7±10µg/mL, p=0.0048). Out of the 8 positives measured at trough, 7 had IFX<5µg/mL. In the residual sera cohort used for method comparison samples with IFX<5µg/mL (n=18) showed 100% concordance for ATI by the two methods. In contrast, samples with IFX≥5µg/mL (n=19), had a concordance of 73.7%. In the presence of detectable trough levels of IFX, significance of ATIs is unclear. Based on these data, we propose an algorithm for assessment of patients showing a loss of response to IFX. Initial testing would be performed for quantitation of trough IFX by LC-MS/MS with a reflex to ATIs in cases when IFX concentrations are <5µg/mL. Conclusions: Using a newly-developed ECLIA bridging method, we have demonstrated that the presence of ATIs correlated with lower IFX concentrations and that the majority of ATIs were found in patients with trough IFX<5µg/mL. In addition, interference by endogenous IFX may be an issue, specifically when assessed at peak levels. An algorithmic approach starting with the quantitation of IFX measured at trough with a reflex to ATI could provide guidance to clinicians in identifying patients who might respond to increased doses of IFX compared to patients for whom another biologic agent might be more appropriate.