Wednesday, August 2, 2017

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

Molecular Pathology/Probes

B-204

SHOX2 and SEP9 genes hypermethylation as biomarkers for plasma-based discrimination between malignant and nonmalignant colorectal lesions

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Background: Colorectal cancer (CRC) is a common malignancy and the fourth leading cause of cancer deaths worldwide. It results from the accumulation of multiple genetic and epigenetic changes leading to the transformation of colon epithelial cells into invasive adenocarcinomas. In CRC, epigenetic changes, in particular promoter CpG island methylation, occur more frequently than genetic mutations. Most cases of CRC are curable if diagnosed early enough. There is a variety of procedures for CRC screening that may be divided into invasive (colonoscopic and sigmoidoscopic examination) and non-invasive methods (fecal occult blood testing (FOBT)). Significant efforts have been invested to develop biomarkers that identify early adenocarcinomas and adenomas with high-grade dysplasia. methylated SEPT9 has been proved to be assay for CRC detection by many studies. However, different analysis methods used currently in data interpretation led to variation in test sensitivity. The aim of the present study was to determine the sensitivity and specificity of SEPT9 and SHOX2 genes hypermethylation as biomarkers for colorectal cancer (CRC). Furthermore, usefulness of these circulating methylated genes will be compared to colonoscopy which is considered as the gold-standard investigation of CRC screening.

Methods: A total of 106 selected individual (25 CRC negative and 81 CRC positive; 50 to 78 years old; 71 male and 35 female) undergoing screening by colonoscopy were included in the study. Circulating DNA was extracted from 3.5 mL plasma samples using Abbott mSample preparation system DNA kit automated on Abbott m2000sp instrument, treated with bisulfite using Abbott Real-time Bisulfite Modification Kit , purified, and assayed by real-time polymerase chain reaction for assessment of DNA methylation of (SHOX2) and (SEP9) genes, these assays were validated, optimized and evaluated before processing of patient samples. A multiplex polymerase chain reaction combining either SHOX2 or SEP9 and the reference gene beta gene (ACTB) was performed in triplicate for all specimens.

Results: SHOX2 and SEP9 genes methylation was significantly higher in patients with malignant colorectal lesions than those with nonmalignant lesions (P<0.001). *In detecting malignant colorectal lesions,* SHOX2 showed higher sensitivity (97.5% vs. 88.8%) and specificity (92.6% vs 73.5%) than SEP9. SHOX2 *revealed a better sensitivity than SEP9 in detecting stage I (92% vs. 72%) and II (100% vs. 93%) CRC, while both markers showed similar sensitivity (100%) in detecting stages III and IV CRC.*

Conclusion:

SHOX2 and *SEPT9* are frequently methylated in CRC patients. Promoter hypermethylation of *SHOX2* and *SEPT9* may therefore serve as minimally invasive biomarkers for detection CRC. SHOX2 methylation was found to be more sensitive than SEP9 in detecting stages I and II of malignant CRC lesions.

B-205

Genomic DNA extraction from whole blood: A comparative study between modified salting out technique and spin - column based method

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Background and Objective:

DNA extraction from different sources is the essential primary step in any genomic research. Methods used for extraction are evaluated based on the duration, feasibility and cost - effectiveness. Generally speaking, all methods used in DNA extraction should involve disruption of cells / tissues, denaturation of nucleoprotein complex, inactivation of DNase in the sample, and removing contaminants from the extracted DNA. The used method is judged based on the quantity and quality of the yielded DNA. The traditional salting - out technique remained the standard DNA extraction method for years. Researchers started to develop several modifications to this method in order to improve the DNA yield, decrease the extraction time and minimize the cost. Over the past years, spin - column DNA extraction kits became widely used in molecular biology labs. This could be attributed to the simplicity of the technique and the possibility of producing a better quality DNA. In the present study we aimed at comparing the extracted genomic DNA using a modified salting out technique versus that produced from the same peripheral blood samples using a commercially available spin - column DNA extraction kit.

Methods:

Peripheral blood was collected from 100 volunteers, in standard EDTA tubes, and DNA extraction from leukocytes was performed using both a modified salting out technique and a commercially available spin - column kit. In this in - house modified salting out technique, proteinase K was not used, 1% sodium dodecyl sulfate was used as a detergent in the white blood cells lysis solution, and protein precipitation was performed using ammonium acetate in high concentration. The concentration of the resulting DNA from both methods was measured using Nanodrop spectrophotometer, and the 260 / 280 ratio was checked for all samples.

Results:

Comparing the DNA extracted from peripheral blood leukocytes using both mentioned techniques showed a significantly higher concentration using the spin column kit ($p = 0.003$) than the resulting DNA from the modified salting out technique. On the other hand, the effective deproteinization of both methods (Using the 260 / 280 ratio) did not show any significant statistical difference ($p = 0.134$). Correlation was tested between the resulted DNA concentration using both methods but it was insignificant ($p = 0.7$).

Conclusion:

Using spin - column based genomic DNA extraction method from peripheral blood results in a yielded DNA with higher concentration than that produced from salting out technique, although the quality (purity) of DNA resulted from both methods is comparable. Further work is needed to assess whether the difference in concentration is cost effective or not taking into consideration that the spin - column technique is more expensive especially when used on a large scale.

B-206

Multi-variant Genetic Panel for genetic risk of opioid addiction

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BACKGROUND

Over 116 million people worldwide have chronic pain and prescription dependence1. In the US, opioids account for the majority of overdose deaths, and in 2014, almost 2 million Americans abused or were dependent on prescription opioids2,3. Genetic factors may play a key role in opioid prescription addiction.

OBJECTIVES

Describe genetic variations between opioid addicted and non-addicted populations and derive a predictive model determining risk of opioid addiction.

METHODS

Design:

Case cohort study comparing the frequency of sixteen single nucleotide polymorphisms involved in the brain reward pathways in patients with and without opioid addiction. Data were modeled with TreeNet 10-fold cross validation (Salford Systems, San Diego, CA), and used to generate a weighted score.

Setting:

Thirty-seven patients with prescription opioid or heroin addiction and thirty age and gender matched controls were used to design the predictive score. Generalizability of the prediction score was tested on an additional 138 samples.

RESULTS Method Derivation: Observed data: ROC statistic=0.92 ,

sensitivity=82%(95% CI: 66-90), specificity=75% (95% CI: 56-87). TreeNet "learn" data: ROC statistic=0.92, sensitivity=92%, specificity=90%, precision=92%, and overall correct=91%. Generalizability: Sensitivity=97% (95% CI: 90 to 100), specificity=87% (95% CI:86 to 93), positive likelihood ratio=7.3 (95% CI: 4.0 to 13.5), and negative likelihood ratio=0.03 (95% CI:0.01 to 0.13).

CONCLUSIONS

The NeurR score can be used for opioid addiction risk assessment. By identifying patients with a lower risk for opioid addiction, our model may inform therapeutic decisions. Further studies are needed to evaluate additional populations and settings.

B-207

Preclinical validation of fluorescence *in situ* hybridization assay for detection of 5p deletions associated to Cri-Du-Chat syndrome

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Background: Cri-du-chat syndrome is a chromosomal disorder caused by a deletion of the short arm of chromosome 5, which may be visible or submicroscopic. The most important clinical features are a high-pitched cat-like cry, facial dysmorphism, microcephaly, severe psychomotor and mental retardation. The patients with 5p deletion show a high variability phenotypic and cytogenetic. A critical chromosomal region involved in the cat-like cry is mapped to proximal 5p15.3, while the region involved in the remaining features of the syndrome mapped to 5p15.2. The CTNND2 gene, mapped in this region, is potentially involved in cerebral development and their deletion may be associated with mental retardation. The first test to perform is karyotype analysis, which will confirm the diagnosis. In doubtful cases, when there is a conflict between the clinical suspicion and an apparently normal karyotype result, Fluorescence *in situ* Hybridization (FISH) analysis should be performed. Although the performance of FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior to implementation of assay. **Objective:** To validate a FISH assay for detection of 5p15.2 and 5p15.31 deletions associated to Cri-Du-Chat syndrome. **Methods:** We used Cri-Du-Chat and Sotos probe combination manufactured by Cytocell®. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its sensitivity and specificity. To establish a normal cutoff was estimate the false positive rate from 10 cultured normal blood samples. Two analysts score 200 cells (100 per analyst). All probe signal patterns were recorded. The cutoffs were calculated using the beta inverse (BETAINV) function. FISH analysis was also performed with a sample whose 5p15 deletion previously detected by the karyotype. **Results:** The Cri-Du-Chat probe presents the FLJ25076 (5p15.31) and CTNND2 (5p15.2) probes labelled respectively with green and red fluorophores. The SOTOS probe (NSD1gene) is labelled in green and is used as a control. In the normal cell, there should be fusion of the red and green signals (2F) and two green signals (2G), whilst a deletion of FLJ25076 probe results in 1F1R2G signal pattern, a deletion of CTNND2 results in 1F3G**.** The probe demonstrated 100% specificity and analytical sensitivity. In the analysis of 10 normal blood samples were did not observe false positive cells. The normal cutoff for the positive signal pattern was 1.5%. The FISH analysis performed in a sample with 46,XY,del(5)(p15.2) karyotype showed the 1F1R2G signal pattern in 100% of the cells. In this case there was only loss of FLJ25076 probe (5p15.31). **Discussion:** FISH analysis confirmed the previously identified 5p15 deletion, allowing more accurate detection of the deleted region. The occurrence of mosaicism is a very rare finding. Although we did not observe false positive cells, resulting in a 1.5% cutoff, a case with low number of positive cells should be carefully evaluated. The probe specificity and sensibility was higher than recommended by the ACMG. This FISH assay showed high quality and reproducibility and was approved for use in our laboratory.

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Validation of dual-color, dual-fusion fluorescence *in situ* hybridization assay for the detection of PML-RARA translocation

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Background: Acute promyelocytic leukemia (APL), comprises 5% to 8% of cases of acute myeloid leukemia (AML). It is typically characterized by neoplastic proliferation of cells in the bone marrow with a promyelocytic phenotype and presence of the fusion gene PML/RARα created by the t(15;17)(q24;q21) translocation. The detection of this translocation by conventional cytogenetic can be hampered by low quantity and quality of metaphases. In this context the fluorescence in situ hybridization (FISH) is applied as an usual and rapid diagnostic tool. FISH can be performed in dividing and nondiving cells, which is important when dealing with leukemia cell with low proliferation. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, each clinical laboratory must individually validate its FISH. **Objective:** To validate a fast FISH assay for detection of translocation PML/RARα following recommendations from the American College of Medical Genetics (ACMG). **Methods:** We use the *FAST* PML/RARα translocation, dual fusion probe manufactured by Cytocell®. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a normal cutoff) were estimate the false positive rate from 10 uncultured normal bone marrow samples and 10 uncultured normal blood samples. Two analysts score 500 interphase cells (250 per analyst). All probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function. The probe hybridization time was only one hour. **Results:** The *FAST* PML/RARα probe presents the PML (15q24) and RARα (17q21) probes labeled respectively with red and green fluorophores. A normal result should show 2 green and 2 red signals (2G2R). Two fusion signals in addition to the one green and one red signals (2F1G1R) indicate the presence of the classical translocation. The probe demonstrated 100% specificity and analytical sensitivity. In the analysis of bone marrow and blood samples were identified six atypical signal patterns, but we did not observe false positive cells with the typical positive signal pattern (2F1G1R). The normal cutoff for the 2F1G1R signal pattern was 1.5%, both bone marrow and blood samples. The cutoffs obtained with BETAINV function were validated for counting 200 cells. The analyses of normal and abnormal samples by FISH were in agreement with the karyotype results. **Discussion:** Immediate treatment of patients carrying the t(15;17) translocation is critical due to the risk of early death. *FAST* PML/RARα FISH probe allows rapid detection of the rearrangement, with only one hour of hybridisation required. The probe specificity and sensibility was higher than recommended by the ACMG. Adopt a protocol without cell culture using the *FAST* PML/RARα probe will allow optimizing the process and reducing the release time of the result with the same quality and reliability obtained with the conventional probe. This FISH assay showed high quality and reproducibility and was approved for use in our laboratory.

B-209

Accelerated Telomere Shortening in Chinese Parkinson's Disease Patients

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Background: Telomeres are the repeated sequences which protect the ends of chromosomes. The shortening of telomere length is regarded as an indicator of cellular aging and enhanced by oxidative stress and inflammation. Parkinson's disease (PD) is a long-term degenerative disorder of the central nervous system characterized by both inflammation and oxidative stress. The telomere length in PD is assumed to be shortened, however, the results of telomere length in Parkinson's disease are inconsistent.

Methods: We performed a hospital-based case-control study of 288 cases (137 women, 151 men) of PD patients and 301 (144 women, 157 men) of healthy normal controls, with sex and age matched, from the Peking Union Medical College Hospital, China.

The mean age of the patients at sample collection was 64.0 ± 12.8 years (range 26-89 years). All patients fulfilled the UK PD Society Brain Bank criteria for clinical

PD. The healthy subjects were recruited from those visiting the hospital for a health examination free from neurological disorders. Circulating leukocytes were collected for DNA extraction. LTL was measured by a quantitative PCR method. Biochemical variables, including total protein (TP), albumin, prealbumin, GLU, total cholesterol (TC), triglycerides (TG), high-sensitivity C-reactive protein (hs-CRP), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and homocysteine (hcy) were measured by use of an automatic analyzer. Linear regression was used to analyze the relationship between clinical characteristics and LTL. Logistic regression was used to determine the risk of PD associated with LTL. To further explore telomere length in PD, we searched relevant articles using the same method to detect LTL in Medline, Embase, Web of Science and Cochrane Library to conduct a meta-analysis. All analyses were conducted using STATA 12.0 (StataCorp, College Station, TX, USA) or SPSS 16.0. A two-sided P<0.05 indicated statistical significance.

Results: LTL was significantly shortened in PD comparing with controls (1.09±0.45 vs. 1.69 ± 0.79, P<0.001) and decreased steadily with age in both controls and PD, respectively (r=-0.474, P<0.001; r=-0.187, P<0.001). Meanwhile, the PD group had relative low levels of TP, albumin, TC, HDL-C, LDL-C but high of prealbumin. Through multi-adjustment, only age (P<0.001), hcy (P=0.016) were stably negatively related with LTL. The age and sex adjusted odds ratio (OR) for PD was 12.96 [95% confidence interval (CI) 7.23-23.23, P<0.001] comparing the lowest to the highest quartile of LTL. After search, 6 studies using qPCR to detect telomere length were found, meta-analysis indicated telomere length was significantly shortened in PD (random SMD=0.63, 95% CI 0.03-1.24, P=0.041).

Conclusion: This is the first study exploring the relationship between telomere length and PD in Chinese. Our study indicated telomere length is shortened in Chinese PD patients, and the result is consistent with the pooled result of meta-analysis. These observations suggest that telomere is accelerating shortened in PD patients in comparison to the normal population and shorter telomeres were associated with increased PD risk.

B-210

Multiplex Ligation-Dependent Probe Amplification (MLPA) as a diagnostic tool for detection of a large deletion on the *MECP*2 gene in Rett Syndrome.

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Background: Rett syndrome (RS; MIM #312750) is a neurodevelopmental progressive disorder, X-linked that occurs almost exclusively in females. It is caused by mutations in the *MECP2* (MIM #300005), *gene* located on chromosome Xq28, which comprises four exons. The prevalence of RS has been estimated to be between 0.25 and 1 per 10 000 female live births. The disorder is characterized by arrested development between 6 and 18 months of age, followed by developmental regression with loss of acquired skills. The patients first lose purposeful hand movements and an interest in the surrounding world, along with speech. They develop apraxia with characteristic stereotypical hand-wringing movements that resemble hand washing, autistic behaviour and learning disabilities. With increasing age, they can also develop additional complex neurologic findings. The most of the pathogenic mutations described in *MECP*2 gene are located in exons 3 and 4. About 5-10% of the pathogenic mutations described are large deletions spanning whole exons of the *MECP*2 gene. Multiplex Ligation-Dependent Probe Amplification (MLPA) has been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance, such as gene large duplications and deletions. **Aims:** Validate the MLPA kit P015 in Brazilian patients with RS. **Methods:** All of the patients include in this study had a consistent clinical diagnosis for this disorder. Thirteen patients with RS were tested using the commercial MLPA kit P405 version A1 (MRC-Holland), following manufacturer's instructions. Three patients were also tested with Sanger sequencing and one patient were tested by array-comparative genomic hybridization (CGH). The analysis was performed using the Coffalyser v.140721.1958 software. **Results:** The MLPA results were concordant in all patients tested with same kit. Three patients presented MECP2 mutations. One of them presented deletion in exons 1 and 2. Another patient presented deletion in exon 3 and one of them presented duplication in exons 1, 2, 3 and 4. Two patients tested by Sanger sequencing presented pathogenic *MECP*2 point mutations. The same patients not presented alterations in MLPA because the test detect large deletions or duplications. Array-CGH identified duplication in *MECP*2 gene in one patient. This finding was also confirmed by MLPA. **Conclusions:** Until recently, no suitable screening method for detecting whole-exon deletions was available. MLPA has become available for the detection of a large deletion on the MECP2 gene allowing genetic confirmation of previously unconfirmed cases of clinical Rett syndrome.

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Serological performance of AESKU.Seven-up compared to DiaSorin and Euroimmun ELISA Borrelia kits

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Lyme borreliosis, caused by spirochaetes of the Borrelia burgdorferi genospecies complex, is the most commonly reported tick-borne infection in Europe and North America.

Lyme disease is expanding and its latency, multi-phenotypes and complications deserve a quick, reliable serological diagnosis. Since neither gold standard, nor diagnostic standardized serology exists, 3 commercial ELISA kits were compared for diagnostic accuracy.

Combined IgM+IgG anti-Borrelia antibodies were checked using AESKU.SEVEN-UP, DiaSorin, Liaison® and Euroimmun ELISA kits, on 236 adults suspected to be infected. History and clinical presentation and specific serology distinguished between 61 positive and 177 negative patients. The 61 infected individuals presented early or late, cutaneous, neurological, musculoskeletal, cardiac or ocular manifestations. The table below summarizes the main results:

Parameters	Liaison IgM & IgG	AESKU.SEVEN-UP IgM & IgG	Euroimmun IgM & IgG
Sensitivity%	70.5	57.4	63.9
Specificity%	78.5	**91.5**	85.9
PPV	0.53	**0.70**	0.61
NPV	0.88	0.86	0.87
Negative likelihood ratio	0.38	0.47	0.42
Positive likelihood ratio	3.38	**6.77**	4.53
Diagnostic odds ratio	8.74	**14.54**	10.78

It is concluded that all kits can detect anti-Borrelia antibodies, however, the AESKU.SEVEN-UP outperformed DiaSorin and Euroimmun diagnostic performances in specificity, positive predicted value, positive likelihood and diagnostic odds ratio. Hence, positive predictive values in combination with specificity values indicated that the exclusion of these infections was more relevant than its confirmation, thus, avoiding

unnecessary false alarms and therapy. Taking together, AESKU.SEVEN-UP outperforms DiaSorin and Euroimmun performances in ruling out the Borrelia infection.

B-212

Cytogenetics findings in a brazilian male population with infertility

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Background:

The causes of infertility are diverse, and can occur in men, women, or both. We can mention among the genetic causes of male infertility the aneuploidies, such as Klinefelter Syndrome, translocations between the pseudo-autosomal regions of sex chromosomes, additions or deletions of chromosomal material, mosaicism and inversions.

Objectives:

To report the incidence of cytogenetic findings in men referred for karyotype to investigate infertility or gestational loss in a brazilian clinical laboratory.

Methods:

Men between the ages of 18 and 50 who performed karyotype examination between 2014 and 2016, with clinical diagnosis of: infertility, miscarriage and complications related to the male reproductive system; hormonal dysfunction; or Klinefelter syndrome.

Results:

In this period, 4,843 cases were referred to the laboratory. We identified 108 altered karyotypes (2.2%), 46 cases (0.9%) of Klinefelter's Syndrome (47, XXY), followed by translocations in several chromosomes in 0.6% (27 cases) and inversions in 0.26% (4 cases with inv(Y) and 7 cases of varied inversions). Mosaicism was present in only

4.6% (5 cases) and all presented alterations in the sexual chromosomes, being 2.8% mosaicism for the Klinefelter Syndrome (3 cases).

Conclusion:

The study for chromosomal alterations is indicated in cases of complications in fertility, when anatomical and physiological causes have been investigated. In most cases, the patient is unaware of being a carrier for a chromosomal alteration until reaching the reproductive age and experiencing difficulty to conceive. The percentage of altered karyotypes it is still low when compared to the number of exams requested, we can explain this discrepancy by the incorrect indication of the exam, by the technical limitation itself (by not visualizing micro deletions) and finally by the very low incidence of structural rearrangements and aneuploidies. However, the importance of the karyotype examination is maintained for the confirmation of chromosomal alterations as a possible cause of infertility/abortion, as well being able to direct the patient to a possible genetic counseling in case of discovering other cytogenetic alterations.

B-213

An evaluation of Roche cobas MRSA/SA test on the Roche cobas 4800 system

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Introduction

Routine screening of patients for methicillin-resistant *Staphylococcus aureus* (MRSA), an important nosocomial or hospital-acquired pathogen, followed with cohorting of patients tested positive for MRSA, is one of measures to prevent and control spread of MRSA in healthcare institutions.

We evaluated cobas® MRSA/SA test, a qualitative polymerase chain reaction (PCR) assay for detection of MRSA and *S. aureus* (SA) on cobas 4800 system (Roche Diagnostics, Switzerland), against MRSA*Select*™ II chromogenic agar plates (Bio-Rad Laboratories, USA).

The cobas 4800 system consists of software-driven fully automated sample processing and pre-PCR preparation module and PCR thermo-cycler.

Materials and Methods

Total of 53 anonymised nasal, axillary and groin swab samples that have been suspended into Mswab™ liquid media (Copan Diagnostics, USA) were tested for MRSA concurrently using PCR and chromogenic culture methods. Data was analysed using binary matrix.

Limit of Detection (LOD) study was conducted using serially diluted liquid media that was spiked with ATCC MRSA strain. LOD was defined as lowest concentration detectable by method.

Within-run precision studies were conducted using assay quality control material and variance of MRSA, SA and Internal Control (IC) cycle threshold values (Ct) were analysed.

For interference studies, liquid media with MRSA concentration 3 times the LOD was spiked with *P. aeruginosa* and *E.coli* at up to 1 McFarland equivalent concentrations, methicillin-susceptible SA (MSSA) at greater than 4 McFarland equivalent concentration and 0.1 g/L haemoglobin. Difference in Ct as well as the obtained response of test and control samples were evaluated.

Cefoxitin-resistant *S. epidermidis* and *S. lugdunensis* at up to 1 McFarland equivalent concentrations were used to assess analytical specificity.

Results

cobas MRSA/SA test yielded diagnostic sensitivity and specificity of 100.00% and 96.43% respectively compared with chromogenic culture. Concordance between both methods was 98.11%. Discrepant results, when investigated, agreed with the PCR findings. Assay LOD was assessed at 1950 CFU/mL.

Precision studies gave coefficients of variation of 0.821%, 0.690% and 0.707% respectively for positive control MRSA, SA and IC Ct and 1.208% for negative control IC Ct.

Compared with control sample, difference of 0.5, 0.3 and 0.3 Ct for MRSA were observed with test samples with haemoglobin, *E. coli* and *P. aeruginosa* respectively.

In presence of high MSSA concentration, assay was able to detect MRSA in test samples. Difference of 0.1-0.3 Ct between test and control samples was obtained. The assay did not yield any false negative results. All tubes of cefoxitin-resistant *S. epidermidis* and *S. lugdunensis* yielded 'not detected' for both SA and MRSA.

Conclusion

Our data suggests that cobas MRSA/SA test correlates well with chromogenic culture method with good sensitivities and specificities. MRSA detection in samples was also not affected by common interferences such as haemoglobin, high concentration of MSSA and commonly isolated organisms such as *P. aeruginosa and E.coli*. No cross reactivity was observed with cefoxitin-resistant coagulase negative staphylococcus. As the cobas MRSA/SA test is largely automated and has high throughput, laboratory productivity is increased which translates to cost savings. cobas MRSA/SA test therefore can play an important role in the epidemiological control of MRSA in hospitals.

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Application of Matrix Assisted-Laser Desorption Ionization Time-of-Flight Mass Spectrometry for *CYP2D6* Genotype and Copy Number Analysis

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Background: Cytochrome P450 (CYP) 2D6 enzyme activity is known to affect individual responses to pharmacological treatments, particularly variation in drug levels and risk of dose-related adverse reactions. The prediction of *CYP2D6* phenotype from genotype is complicated by more than 100 single nucleotide variants (SNV), copy number variations (CNV), presence of pseudogenes and hybrid rearrangements. Recently, matrix assisted-laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) has been used to analyze *CYP* genes including *3A4, 3A5, 2C9*, *2C19 and 2D6*. The objective of this study was to use MALDI-TOF/MS to identify *CYP2D6* SNVs and CNVs in samples collected from venlafaxine treated patients. Of note, venlafaxine is a serotonin-norepinephrine reuptake inhibitor used for the treatment of anxiety and major depressive disorders. SNVs and CNVs of *CYP2D6* have been reported to associate with venlafaxine pharmacokinetics and optimal dose.

Analytical Methods: seventeen whole blood samples from venlafaxine treated patients were collected pre-dose at steady state from consenting patients. Concentrations of venlafaxine and metabolites were determined and therapeutic doses were recorded in a previous study. Genomic DNA was extracted (PureGene) and samples were de-identified according to institutional protocols. The MALDI-TOF/MS based MassARRAY® System combined with iPLEX® *CYP2D6* panel assay (Agena) was used for *CYP2D6* SNV and CNV analysis. The iPLEX® *CYP2D6* assay includes a panel of 35 pre-designed SNV assays and 5 CNV assays. Each DNA sample was subjected to a multiplexed PCR amplification followed by shrimp phosphatase treatment to neutralize unincorporated dNTPs. Subsequently, samples were subjected to the iPLEX reactions. In an automated Chip prep module the extension products were desalted, and a nanoliter of each reaction was dispensed onto a SpectroCHIP® Array (Agena). Mass of each allele was detected via MALDI-TOF/MS. Data were analyzed using the Typer™ software (Agena). Report tables were generated with presumed haplotypes to predict allele calls and copy number. *CYP2D6* genotypes identified by MALDI-TOF/MS were compared to those obtained previously using the Luminex Bioscience Tag-It Assay.

Results: We identified one poor metabolizer (PM) with two copies of nonfunctional alleles (*3/*4) and 4 intermediate metabolizers (IM) with one copy of nonfunctional allele and one copy of decreased functional allele (*5/* 41, *4/*41, and *5/*9). Furthermore, 12 out of 17 samples were identified as extensive metabolizer (EM) with two copies of functional alleles (neg/neg,*35/neg) or one copy of functional allele and one copy of decreased allele (*41/neg, *35/*41, *2A/*41, and *2A/*9) or one copy of functional allele and one copy of nonfunctional allele (*5/neg, *4/neg, and *2A/*4). Among seventeen samples five samples were detected with only one copy of CYP2D6 and one sample was detected with more than 2 copies. MALDI-TOF/MS results were found to be 100% concordant with the findings obtained by Luminex technology.

Conclusion: The MassARRAY® System combined with iPLEX® *CYP2D6* panel assay reported here is a suitable and reliable platform for *CYP2D6* SNV and CNV analysis.

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Comparison of the *Realtime*HPV HR-S Detection with the Cobas4800 HPV test for the detection of high-risk types of human papillomavirus

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Background: The *Realtime*HPV HR-S Detection (SEJONGMEDICAL, Paju, Korea) is one of the recently developed assays, which is a real-time PCR based test designed for detecting 14 types of high-risk (HR) HPVs (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). This study was to compare the performance of *Realtime*HPV HR-S Detection to Roche Cobas4800 HPV test (Roche Molecular Systems, Branchburg, NJ, USA) for the detection of high risk (HR) HPV. In addition, we analyzed the distribution of HPV genotypes in the patients with abnormal pathologic test result.

Methods: A total of 305 cervical swab specimens were retrospectively collected from patients whose mean age was 43.3 years (22-90 years) between June and September 2016. We tested all the specimens by *Realtime*HPV HR-S Detection and Cobas4800 HPV test. HPV DNA sequencing was subsequently analyzed to confirm the discordant results. HPV distribution by age, results of cytology for 286 patients and biopsy for 43 patients was also analyzed.

Results: Cobas 4800 detected one of 13 HR HPV types in 58.7% of specimens, while *Realtime*HPV HR-S detected in 59.0% of specimens. The overall agreement rate between the assays was 96.1% with 0.947 kappa coefficient. One of the discordant sample was revealed that the result from Cobas was equal to sequencing and the rest 11 samples were revealed that the results from *Realtime*HPV were equal to sequencing. Sensitivity and specificity of 16, 18 and other high HPV detections were high enough (Cobas: 95.9%-100% and 97.8%-100%, and *Realtime*HPV: 98.0-100% and 100.0-100.0%). At the distribution by age, 31-40 age group showed 76.7% (69/90) positive rate while 51-60 age group showed 40.8% (20/49). Upon cytological examination, HPV positive rate of the patients in high-grade squamous intraepithelial lesions (HSIL), LSIL, and atypical squamous cells of undetermined significance (ASCUS) was 72.7% (8/11), 82.4% (14/17), and 82.8% (24/29), while in normal cytology 51.6% (112/217). The patients who were reported CINI, II, III, carcinoma in situ, and invasive cancer by cervical biopsy showed 78.6% (22/28) of HPV positive rate but normal and benign patients showed 33.3% (5/15) of positive rate.

Conclusion: Considering the high agreement rate with Cobas 4800 HPV test, more than 95.9% of sensitivity and specificity, and ability to differentiate HPV 16/18 from other HPV types, *Realtime*HPV HR-S Detection could be a reliable laboratory testing method for the screening of HPV infections. Moreover, as HPV detection rate is markedly higher in patients with precancerous lesion than normal or benign patients, early detection of HPV is very important to screen for early detection of cervical cancer.

B-216

Comparison study of manual and automated extraction systems for cell-free circulating DNA

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Background:

Cell-free circulating DNA (cfDNA) is of vastly growing diagnostic interest in cancer treatment and prognosis therefore there is a need for automation of the respective extraction methods in order to standardize procedures. Since cfDNA can be present at low concentrations in plasma, the method needs to maximize the possible output whilst maintaining a high enough purity and quality to work optimally in downstream analyses such as qPCR, digital PCR or Next Generation Sequencing (NGS).

Methods:

For this study, an automated extraction method (InviMag Circulating DNA Kit / Stratec) was compared with a manual method (QIAamp Circulating Nucleic Acid Kit/ Qiagen). Both methods isolated the DNA from a starting volume of 4 ml of plasma into an elution volume of 80 µl. 8 ml of 68 plasma samples from: No Cancer (4), Breast Cancer (3), NSCLC=Non-small cell lung cancer (16), SCLC = Small cell lung cancer (3), and Metastatic Melanoma Patients (42) were aliquoted in two portions of 4 ml and processed by each method. To determine the potential influence of storage duration, three groups of samples were used: short term stored (less than 12 months; 16), mid-term stored (up to 5 years; 45) and long term stored (>7 years; 7). After extraction, all DNA samples were measured by fragment analysis on a TapeStation 4200 (Agilent) and by qPCR concentration measurement (InviQuant GeneCount - Stratec). Digital droplet PCR (BioRad) was performed for BRAF V600K mutation (8 samples – Melanoma), BRAF V600E mutation, (28 samples – Melanoma) and EGFR T790M mutation (5 samples, NSCLC).

Results:

Fragment analysis by TapeStation showed the characteristic cfDNA fragments of 170 bases in most of the extractions from both methods. Nevertheless some QIAamp extractions showed additional signals of fragments above 500 bases. Quantification by TapeStation showed a lower mean concentration of cfDNA with the automated method than with the manual method, whereas with qPCR mean concentration values, InviMag extracted samples were slightly higher than QIAamp samples. In general, cfDNA concentrations measured in InviMag samples by TapeStation showed a stronger correlation with concentrations measured by qPCR, than those isolated with the manual QIAamp method. Nevertheless, both methods delivered comparable cfDNA yield, especially measured by qPCR.

No differences between the short term stored, mid-term stored and long term stored samples could be observed. We conclude, that storage at -70°C can stabilize cfDNA in plasma samples for years.

Digital PCR results were comparable in both methods in terms of accepted droplet counts and fractional abundance of detected mutations. Excluding invalid samples, both methods displayed 100% correlation for the detected mutations in this study.

Conclusion:

We conclude that the automated method is appropriate for cfDNA extraction.

B-217

Non-invasive fetal sex determination using cell-free fetal DNA isolated from maternal capillary blood obtained by fingertip puncture: the elimination of exogenous male DNA from the collection site is crucial.

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Background:

Noninvasive fetal sex determination is a test capable to indicate the baby's sex at the beginning of pregnancy. This assay searches the Y-chromosome in a maternal blood sample drawn by venipuncture. Its presence indicates the gestation of a boy and its absence indicates a girl. Despite its clinical indications, the greatest demand is from mothers eager to know the sex of their unborn child. With the intention of bringing comfort to the mothers and evaluate the presence of fetal DNA in maternal microcirculation, we idealized to perform it in the capillary blood. Thus, the aim of the present study was to investigate whether fetal sex determination performed on plasma isolated from capillary blood is comparable to venous blood. The latter is a well-established method in our laboratory.

Methods:

This study enrolled 101 pregnant volunteers. The gestation weeks ranged from 8 to 20 weeks, and the median was 11 weeks. After asepsis with isopropyl alcohol, venous and capillary bloods were collected at the same occasion in appropriated EDTA tubes by cubital fossa venipuncture and fingertip puncture, respectively. EDTA-plasma was isolated within two hours (150 uL for capillary and 1mL for venous bloods) and submitted to an automated DNA extraction. The multicopy sequence DYS-14 was assessed in quadruplicate by qPCR. RNAse P gene was co-ampified in all instances. After testing the first 27 volunteers, it was noted that exogenous Y-chromosome is present on women's fingertips, what results in a strong false-male (positive) signal in almost half of the samples from mothers bearing female fetuses by the reference method. Thus, a fingertip's asepsis with diluted sodium hypochlorite solution was implemented and the volunteers were divided in 3 groups: the isopropyl alcohol (n=27; 15 males, 12 females), the 0.5% buffered sodium hypochlorite twice (n=39; 20 males, 19 females) and 1% buffered sodium hypochlorite once (n=35; 17 males, 18 females). The total, male (positive) and female (negative) agreements between the results for each specimen were computed in all groups. The degree of agreement was also quantified by kappa statistics.

Results:

For the isopropyl alcohol group, the total, male and female agreements between capillary and venous bloods were 81%, 100% and 58%, respectively (Kappa = 0.6, good). For the 0.5% buffered sodium hypochlorite twice group they were 100%, 100%, and 100%, respectively (Kappa = 1, perfect). For the 1% buffered sodium hypochlorite once group, the total agreement was 100%, the male agreements was 100% and the female agreement was 100% (Kappa = 1, perfect).

Conclusion:

Fetal DNA is present in the maternal microcirculation allowing the execution fetal sex determination on the capillary blood. The capillary blood collection is much less invasive than venipuncture bringing comfort to the mother. Moreover, it offers the possibility of home self-sampling. However, exogenous male DNA could be present at the women's fingertips and for a reliable fetal sex determination by using the above-described method, the elimination of exogenous male DNA from the collection site is critical. Furthermore, the knowledge gained in this study can also impact the forensic sciences, specifically, the touch DNA field.

B-218

Simplified workflow for BCR-ABL1 e14a2/e13a2 fusion quantification in whole blood by semi-automated nucleic acids extraction, multiplex one-step RT-qPCR and delta-delta Cq method.

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Background:

Molecular testing for the BCR-ABL1 fusion gene by RT-qPCR is the most sensitive approach for monitoring the response to therapy of patients with chronic myeloid leukemia. The most frequent fusions subtypes are e13a2 and e14a2 (found in 97-98% of the patients). Moreover, there are guidelines describing consensus best practices for monitoring these transcripts. These documents report multi-steps workflows that are not routine-friendly. Thus, the objective of the present study was to validate simplified e14a2/e13a2 quantification in whole blood by RT-qPCR.

Methods:

Positive samples were constructed by the spiking of known amounts of k562 cells into the negative peripheral blood (5 mL) or in-vitro transcribed e14a2 or e13a2 RNA into the samples during the nucleic acid extraction. Red blood cells were removed by using PharmLyse (BD Biosciences). Nucleic acid was extracted by using EasyMag (Biomerieux). e14a2/e13a2 and ABL1 mRNA were co-amplified with the Europe Against Cancer primers/probes in a duplex one-step RT-qPCR reaction performed with QuantiNova RT-qPCR master mix (Qiagen) on light cycler 480 II (Roche). For quantification, standard curves were constructed with ssDNA oligos. Amplification efficiencies were retrieved from these curves and the applicability of the ΔΔCq method was evaluated. The absolute and relative quantification were compared using samples spiked with K562 (n=28, $1.39x10^6$ to 15 cells) by linear regression and Bland-altman statistics. The limits of detection for e14a2, e13a2 and K562 RNAs were determined applying probit regression analysis to a serial dilution of each target. Precision was evaluated by testing samples spiked with 10^5, 10^3, and 10^2 k562 cells in triplicate during 6 days. The accuracy was investigated comparing the proposed method with Xpert BCR-ABL Monitor IS G2 (Cepheid) (n=53, with K562 cells from $1.39x10^6$ to 0) using linear regression and Bland-altman. Experiments with K562 were calibrated to international scale (IS) using calibrator panel e14a2 (Asuragene).

Results:

The median (Max-Min) of ABL1 achieved by the proposed workflow was $1.1x10^6$ ($1.75x10^7$-$4.3x10^5$) copies/sample. e14/a2, e13/a2 and ABL1 RT-qPCR efficiencies did not differ in all tested occasions, meaning that the ΔΔCq method is applied (absolute and relative quantification comparison reveled: R^2 of 0.99 and bias of -0.0073 log). The limits of detection were 0.138 (95%CI 0.041-3.7), 0.017 (95%CI 0.004-1.23) and 0.093 IS (95%CI 0.075-0.131) percent of BCR-ABL1/ABL1 for e14/a2, e13/a2 and K562 RNAs, respectively. In the precision assay, the medians (Max-Min) were 32.8% (33.9-31.6), 0.52 (0.66-0.42) and 0.059 (0.022-0.075) for the samples with 10^5, 10^3, and 10^2 k562 cells, respectively. Qualitatively, the total agreement between the proposed method and expected results was 98.1% (95%CI 90-99%) and between Xpert and expected result was 86.8% (95%CI 75-93%). Quantitatively, the R^2 and bias for the proposed method *versus* Xpert were 0.89 and 0.57 log, respectively.

Conclusion:

We described a reliable and routine-friendly workflow for BCR-ABL e14a2/e13a2 fusion quantification in whole blood that reduces the number of steps proposed by the current guidelines. The proposed workflow showed acceptable sensitivity, precision and accuracy. The assay reached the molecular response 5 (MR^5) based on the ABL1 molecules extracted per sample and MR^3, MR^4 and MR^3 for 14/a2, e13/a2 and K562 RNAs, respectively.

B-219

Incidence of Turner syndrome in a brazilian female population with infertility

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Background: Infertility affects approximately 10% of women between the ages of 15 and 44 and the female factor accounts for 30% of the causes of infertility. Some of the causes of female infertility are: anatomical abnormalities, genetic alterations and age. Among the genetic alterations, we can mention Turner syndrome, translocations between the pseudo-autosomal regions of the sex chromosomes, mosaicism and inversions. For every 2,500 girl born, 1 is a carrier of Turner syndrome, often discovered when there are recurrent miscarriages or amenorrhea.

Objectives: To report the incidence of cytogenetic findings in women referred for karyotype to investigate infertility or gestational loss in a brazilian clinical laboratory.

Methods: Analysis of the cytogenetic findings of female karyotype between the ages of 18 and 40, referred for the investigation of infertility, abortion and amenorrhea during the years of 2015 and 2016.

Results: 3,330 cases were referred to the laboratory. We identified 60 altered karyotypes (1.8%), with 24 cases (0.7%) related to the X chromosome, 4 cases (0.1%) of women with karyotype 46,XY and 32 cases (0.9%) with other changes such as inversion, translocation, robertsonian translocation or the presence of a marker chromosome. Among the related to X, 9 cases were 45,X0 (0.27%), 8 cases of mosaicism (0.24%), 2 cases with a deletion on Xq (0.06%), 1 derX (0.03%), 1 isoX (0.03%), 1 dupX (0.03%), 1 translocation (X;5) (0.03%) and 1 mosaic of Turner with a derX (0.03%).

Conclusion: The karyotype is essential for the discovery of chromosomal alterations related to infertility, such as Turner syndrome. Genetic counseling becomes a possibility for those patients with the presence of mosaicism for Turner syndrome. Only 2-10% of syndromic women achieve spontaneous pregnancy, but anomalies on the X chromosome can be transmitted to offspring, increasing the risk of miscarriages, congenital malformations and chromosomal abnormalities.

B-220

PRKAR1A Sanger Sequencing and Deletion/Duplication Analysis for the Clinical Diagnosis of Carney Complex

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Background:

Carney Complex (CNC) is an autosomal dominant condition, characterized by spotty skin pigmentation, endocrine hyperactivity, and myxomas (both cardiac and non-cardiac). Cardiac myxomas may result in an obstruction of blood flow, embolism, and/or heart failure. The phenotype overlaps with Cushing Disease, Primary Pigmented Nodular Adrenocortical Disease (PPNAD) and other adrenal hyperplasias. The majority of CNC is due to pathogenic variants in *PRKAR1A*, coding for the type 1-alpha regulatory subunit of protein kinase A. More than 125 pathogenic variants in *PRKAR1A* have been identified. The estimated prevalence is 1:1,000,000 with 100% penetrance and approximately 160 index cases reported hitherto. Approximately 70% of individuals diagnosed with CNC have an affected parent, and approximately 30% have a de novo pathogenic variant. Sequence analysis of *PRKAR1A* will detect a pathogenic variant in 60% of probands, while an additional 10% of probands may harbor a large deletion or duplication variant. While immunohistochemical (IHC) staining analysis may be used to screen for CNC, identification of a *PRKAR1A* pathogenic variant can confirm diagnosis.

Materials and Methods: We have developed and validated a clinical assay for *PRKAR1A* variants using Sanger sequencing and qPCR for Deletion/Duplication. In this procedure, genomic DNA is first extracted from whole blood, followed by polymerase chain reaction (PCR) amplification of all exonic regions and intron/exon boundaries of the gene. Following enzymatic digestion to purify the PCR product and remove it from unincorporated primers and nucleotides, bi-directional Sanger sequencing is performed using universal primers and fluorescent-dye terminator chemistry. Sequencing products are separated on an automated sequencer and trace files are analyzed for variations in the exons and intron/exon boundaries of all exons using Mutation Surveyor™ software and visual inspection. Deletion/Duplication Analysis consists of a very short (80-150 bp) PCR amplification in a reference gene and in all *PRKAR1A* exons. During PCR, SYBR Green intercalates into the double stranded DNA structure and the fluorescence increases greatly. The fluorescent signals are used to calculate a relative copy number.

Results: To validate the accuracy of the assay, we compared our Sanger sequencing results with results reported in the 1000 Genomes Project for 4 Corriell samples, and we compared our Deletion/Duplication results with array-CGH results for 3 samples that were previously identified with large deletions or duplications. Assay imprecision was also assessed.

Conclusions: In summary, we have developed and validated a clinical assay to detect *PRKAR1A* variants including large deletions and duplications. This test will aid in the clinical diagnosis of CNC in individuals, specifically in confirmation of those cases that exhibit loss of immunohistochemical PRKAR1A expression.

B-221

Cytogenetic findings in Brazilian patients investigating for autism spectrum disorders

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Background: The array-CGH technics utilize both molecular and the cytogenetics approach to perform the whole genome analysis with a higher resolution than previous technics such as the karyotype. Now it is possible to detect microdeletions or microduplications (loses or gain of genetic material) previously not visualized by karyotype. These changes are often found in patients with behavioral disorders such as autism. Therefore, the array analysis was established as a first line test to investigate autism spectrum disorders However, there are still many requests for karyotype analysis for these patients.

Objective: To identify the cytogenetic findings in patients referred to investigate autism spectrum disorders.

Methods: Statistical analysis between the years 2014 and 2016 from all cases referred to perform a peripheral blood karyotype with a autism diagnostic hypothesis.

Results: 488 patients were analyzed in this period, 478 without any abnormalities, 5 cases with chromosome 9 inversion; 1 case with chromosome 9 duplication; 2 patients with a marker chromosome; a Robertsonian translocation involving the chromosomes 14 and 21; and finally a chromosome 22 ring.

Conclusion: In the evaluation of the 488 patients, only one had a relevant alteration for autism (the chromosome 22 ring). This is justified because most of the alterations are undetectable using the karyotype. Studies have shown that array-based tests make it possible to diagnose 15 to 20% of patients with autism spectrum disorders and other behavioral disorders, while the G-banding karyotype detects changes in about 3% of cases not associated with clinically recognizable chromosomes syndromes. We can explain the still high demand for karyotypes by the particular scenario of health insurance companies that require a normal karyotype to perform the array, medical education to request and interpret a new test, and the higher cost than conventional karyotype. We expect that addressing this issues we will help change this scenario.

B-222

Cytogenetics findings in Jacobsen Syndrome

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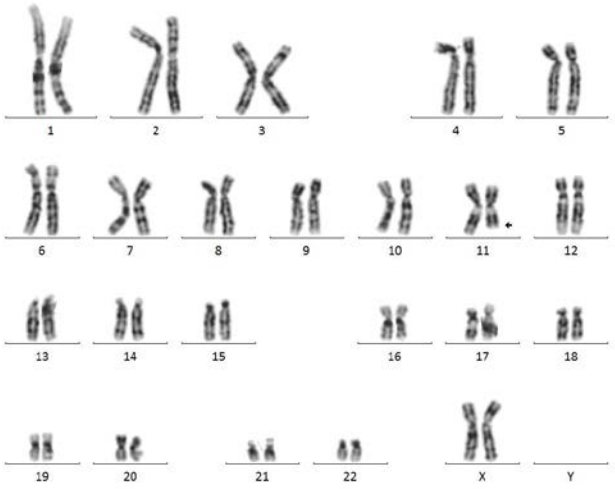
Background: Jacobsen syndrome is rare and caused by the terminal deletion of the long arm of chromosome 11, more specifically in the 11q23 band. This region is home to a hereditary folate-sensitive site. The first time this disorder was observed was in 1973 by Jacobsen. This syndrome results in a complex phenotype characterized by delayed neuropsychomotor development, craniofacial anomalies, varied cardiac defects, and blood dyscrasias. The diagnosis is initially based on karyotype analysis when the deletion involving the q23 band is identified. It is also possible to obtain confirmation through molecular studies (FISH), where the absence of the FLI-1 and JAM-3 genes is visualized. Studies confirm that only 15% of these alterations may have been inherited and 85% occur *de novo*.

Objective: To report the efficiency of cytogenetics in the diagnosis of Jacobsen Syndrome in a Brazilian population.

Methods: A survey of karyotypes performed at DASA Cytogenetic Laboratory of São Paulo from January 2014 to January 2017, with a deletion in the 11q23 region visible by microscopy.

Results: During this period 6,286 constitutional karyotypes of patients up to 20 years old were performed, with the diagnostic hypothesis compatible with some of the features found in Jacobsen Syndrome. Of these karyotypes, only 3 cases presented the 11q23 deletion.

Conclusion: The results demonstrate the small incidence of Jacobsen Syndrome. We can conclude, due to the size of the deletion, that it is possible to be visualized through classical cytogenetics in the analysis of the karyotype with resolution of at least 400Mb, confirming the Jacobsen Syndrome, and guaranteeing the efficacy of the analysis method. However, deletions less than 7.5 Mb which involve the genes for the development of the syndrome may not be identified in the cytogenetic study, being necessary the complementation with the analysis by FISH.



B-223

IL28B genotipe and liver fibrosis in patients with chronic hepatitis C

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Background: Interleukin (IL)28B polymorphisms are strongly associated with spontaneous clearance of hepatitis C virus (HCV) infection and response to therapy, but whether IL28B genotype affects liver fibrosis severity is unclear. Our aim was to study the relationship between IL28B genotype and markers of liver fibrosis.

Methods: We studied a population of 120 chronic hepatitis C patients. Liver fibrosis was assessed by shear wave velocity (Vc) determined by Arfi technique. In all patients was determined IL28B polymorphism, biomarkers of fibrosis hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP-1), tissue inhibitor of metalloproteinase 1 (TIMP-1), platelets, AST and ALT. Moreover algorithms for estimating the degree of fibrosis ELF, Apri, Forns, Fibrotest, Fibrometer, Fib-4, Fibro-Q and Hepascore were calculated. An abdominal ultrasound was also performed to evaluate liver disease.

Results: Patients had a mean age of 49.5 years and 64% were men. Prevalent viral genotype was 1 (79.5%) and distribution of IL28B genotypes was as follows: 22% CC, 64.7% CT, and 13.3% TT. Patients with CT genotype had significantly lower concentrations of PIIINP ($p=0.006$), TIMP-1 ($p=0.039$) and higher platelet count ($p=0.001$) compared to those with CC and TT. Apri, Forns, Fibrotest, Fibrometer, Fib-4 and Fibro-Q also were significantly lower in CT patients ($p<0.05$). AST, ALT, ELF and Hepascore were also lower in CT patients but differences were not significant. Furthermore CT patients have lower degree of liver fibrosis according to a lower Vc ($p=0.039$) and lower presence of ultrasound findings of liver disease ($p=0.008$).

Several authors have suggested that CC genotipe is associated with a state of enhanced immunity that can promote viral clearance but alternately can increase the liver damage. Other authors have noted that T allele is more prevalent in patients who develop cirrhosis. According to this and based on our results, the combination of the two alleles (CT) could be the most favorable option in relation to the severity of fibrosis.

Conclusions: The genotype CT of IL28B is associated with a lower degree of liver fibrosis determined by biomarkers, imaging techniques (ultrasound) and elastography techniques (Arfi).

B-224

Minimal Residual Disease Monitoring in AML by RT-qPCR of NPM1 mutations

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Background: While many markers exist for cytogenetically abnormal AML, standard-risk AML offers a limited number of markers with which to base treatment decisions. In order to offer physicians at our hospital a better tool for prognostication and treatment, we recently investigated the use of a newly published method to track NPM1 mutation status in peripheral blood as a marker of residual disease. **Methods:** An RT-qPCR method was devised based on work by Ivey et al published last year. We modified the protocol by analyzing various PCR conditions on positive and negative cell line DNA to determine the best conditions for the RT-qPCR reaction. This was then followed by patient samples with various mutational statuses confirmed by the Illumina TruSight Myeloid Sequencing Panel. **Results:** We determined that the RT-qPCR reaction run with primers designed by Ivey et al were able to determine the presence of mutated NPM1 transcripts in peripheral blood of positive patients, though it was not able to distinguish Type A (TCTG insertion) from Type B (CATG insertion). Efficiency of the PCR reaction was determined to be 102% and 97% for the NPM1 and Abl reactions, respectively, with linear correlations of greater than .99 across over 1000-fold dilution of RNA. Limit of detection was determined to be .01% mutated RNA in wild-type RNA background, with potential for lower limits. Concordance with the TruSight Myeloid panel was 100% between wild-type and Type A mutation patients, with an N of 5 and 2, respectively. In addition, we determined that the RT-qPCR reaction could be run at a single annealing temperature for both the NPM1 and control ABL reactions as well as with a simpler intercalating dye reaction instead of the costlier quenched probe assay without sacrificing specificity or sensitivity. A larger cohort study is now underway in our institution. **Conclusion:** We now have a successful pre-validation this assay for use in determining minimal residual disease in NPM1-mutated AML patients. While it is unfortunate that the assay cannot distinguish Type A and Type B mutations, the prognostic information is currently identical for both types, allowing this assay to be used for additional patients. We have also successfully simplified the assay through several means from the original paper, reducing necessary tech time and potential mistakes.

B-225

Association between angiotensin-converting enzyme gene insertion/deletion polymorphism and risk of recurrent miscarriage in Middle Eastern population

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Background Angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin-aldosterone system, converts angiotensin I to angiotensin II. Recurrent pregnancy loss (RPL) had said to be related to the angiotensin converting enzyme insertion/deletion gene polymorphisms (ACE I/D). But the conclusions were controversial. This study was conducted to investigate the real association in ACE I/D polymorphisms and RPL.

Method A total of 786 women (496 with history of RPL and 290 with no history of RPL; 21 to 36 years old) from 4 different Middle Eastern countries (Egypt (213), Saudi Arabia (297), Qatar (173) and United Arab Emirates (103)) whose know to be wild type for both factor V Leiden and factor II (prothrombin) genes mutation included in the study. Genomic DNA extraction was performed using Qiagen DNA blood extraction kit, ACE I/D genotype was assayed using validated reverse hybridization polymerase chain reaction (PCR) kit from ViennaLab Diagnostics GmbH Vienna, Austria.

Results We found that the genotype frequencies of ACE I/D (24.1% vs. 18.3%) and D/D (59.7& vs. 4.5%) were more seen in women with RPL compared with the women with no RPL history, the difference between the two groups is statistically significant (p<0.001).

Conclusion: Our data shows a significant association between ACE I/D polymorphism and recurrent miscarriage risk. ACE polymorphic D allele contributes to increased risk of recurrent miscarriage.

B-226

Confirmation of CGG Repeats in Brazilian Women Tested for Fragile X Syndrome

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Background: Fragile X syndrome (FXS) is a common genetic condition that causes intellectual disabilities, including deficits in development. It affects both males and females of all ages, but the frequency of the disease is higher in males. The repetition of the trinucleotide CGG in the 5´UTR region and the methylation status of *FMR1* (Fragile X Mental Retardation 1) gene are the major causes for this disorder. Clinical interpretation is based on the number of CGG repeats and alleles with more than 200 CGG characterizes a full mutation with *FMR1* gene inactivation, 55-200 CGG is considered a premutation, 45-54 CGG is considered a grey-zone and in healthy people, this segment is repeated around 5-45 times. Diagnostic testing for FXS usually relies on *FMR1* gene PCR and conventional sequencing. The *AmplideX™ FMR1 PCR kit* (Asuragen, Austin, USA) enables the conversion of the size of PCR products to number of CGG repeats. After sequencing, the electropherograms are analyzed and it is possible to identify gene-specific product peaks, however in some female samples it is difficult to observe both alleles (two peaks) due to the very similar number of CGG repeats.**Objective:** Here we report the incidence of the identification of just one allele in blood samples from female patients submitted to FXS diagnostic test in a large Brazilian private laboratory.

Methods: 199 samples collected during the year of 2016 from women from zero to 66 years old were evaluated using the *AmplideX™ FMR1 PCR kit*. Briefly, a PCR was performed using specific primers that spun the CGG repeat region. PCR products were sequenced at the 3730xl DNA analyzer and the electropherograms analyzed using the GeneMapper v4.0 software. The archives originated in the 3130xl were analyzed using GeneMapper and the sizes of the alleles were plotted in an excel file that converted sizes to number of CGG repeats using a specific formula.

Results: From all 199 samples, 40% (79) presented only one peak, which represented only one allele; 54% (107) were considered normal; 4% (8) were classified as premutaded; 2% (3) grey-zone and 1% (2) fully mutated.

Conclusion: Since it was not possible to define whether the other allele was fully mutated or the difference between the amounts of CGG in both alleles were so small making it impossible to distinguish the difference between then, we established a routine to solve this issue. The injection time during sequencing was decreased from 5 to 2.5 seconds, in order to avoid signal saturation, and after decreasing injection time, it was possible to confirm that the samples that showed only one peak in fact presented signal saturation and looked like CGG repeats of only one allele. After the decrease of injection time, it was possible to observe a small but clear difference of CGG repeats between the two alleles with the separation of the peaks and ensuring the correct result.

B-227

Development and Validation of a Quantitative Digital Droplet PCR Assay for Detection of *KRAS* Mutations in Codons 12, 13, 61 and 146 in Plasma cfDNA

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Introduction: Approximately 30-50% of colorectal cancers (CRC) have mutations in *KRAS*. Most occur in hotspot regions in codons 12, 13, 61 and 146. These mutations lead to constitutive activation of the RAS/MAPK pathway downstream of EGFR, limiting the effectiveness of anti-EGFR therapies, such as cetuximab and panitumumab, which inhibit ligand-mediated activation of EGFR. Therefore, identification and quantitation of these mutations is critical in selecting the appropriate therapy. Liquid biopsies might provide a less invasive and cost effective alternative to tissue biopsies to establish KRAS status. **Objective:** To assess the analytical performance of three commercially available KRAS assays: A screening KRAS12, 13 assay for detection of mutations in codon 12 and 13 (G12A, G12C, G12D, G12R, G12S, G12V, G13D), a screening KRAS 61 assay for detection of mutations in codon 61 (Q61L, Q61H, Q61R, Q61K) and a targeted assay for detection of KRAS A146T mutation (BioRad, Hercules, CA) in patient plasma. **Materials and methods**: We used *KRAS* reference standards (Horizon Discovery), nucleosomal *KRAS* cell line DNA (ATCC H747, SW948, SW48), formalin-fixed paraffin embedded (FFPE) tissue DNA, and cell free DNA (cfDNA) from patients and healthy volunteers. Blood

samples were collected into Streck Cell-Free DNA BCT® tubes. DNA was extracted using Qiagen's QIAmp Circulating Nucleic Acid kit. Simultaneous quantitation of the wild type (WT) and KRAS mutants (MT) was performed using the AutoDG and QX200 ddPCR multi-well BioRad system (Hercules, CA).We assessed sample stability, limit of detection (LOD), limit of quantitation (LOQ), analytical precision, dilution linearity, analytical specificity, and cross reactivity. For accuracy assessment, we performed spike recovery and re-testing of genotyped FFPE DNA samples (n=60) and Horizon Discovery reference standards (n=10). **Results:** Plasma stability was acceptable up to 21 days at -80°C. For all three KRAS assays the LOD of plasma DNA was established at 5 positive droplets (15 copies/ml plasma), based on CLSI guidelines. Intra- (n=20) and inter -assay (n=20) imprecision were <20% CV at 42 copies/ml plasma. Based on this observation, the LOQ was established at 42 copies/mL plasma. Serial dilutions of KRAS-positive cell lines and KRAS-positive plasma-derived DNA were linear down to 34 copies/mL for KRAS 12, 13 assay, 31 copies/mL for KRAS 61 assay, and 27 copies/mL for KRAS A146T assay. Analytical specificity was assessed by adding increasing amounts of WT DNA (up 10 times the normal level) in a mutant sample (0-5772 copies/ml plasma). There was no significant change in MT copies measured. Cross-reactivity between KRAS 12/13, KRAS 61 and KRAS A146T assays was undetectable using KRAS reference standards. Recoveries for spike in experiments were 100% for KRAS 12/13 (range 85-118%), 109% for KRAS 61 (range 97-117%) and 105% for KRAS A146T (range 92-121%). Plasma DNA from 60 normal donors was negative for each KRAS mutation tested, while all FFPE and reference standards were KRAS mutation positive. **Conclusions:** The tested KRAS assays are fast, reliable and accurate in detecting and quantitating KRAS mutations in liquid biopsies for therapy selection and might also prove useful in assessing patients' responses to treatment.

B-228

FISH Microdeletion in the Diagnosis of Complete Androgen Insensitivity Syndrome

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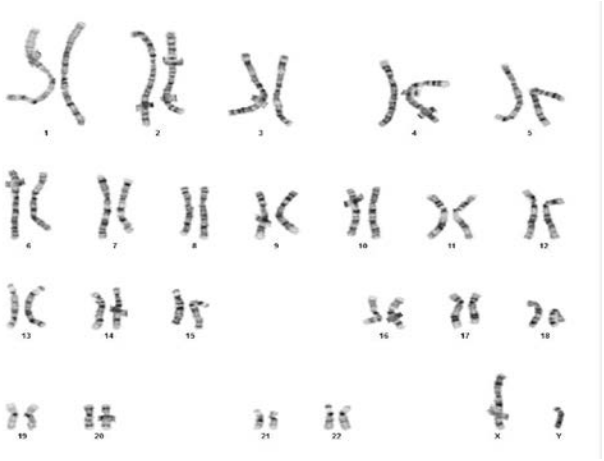
Complete androgen insensitivity syndrome occurs when the body cannot use androgens at all. Individuals with this condition have the external sex characteristics of females, but do not have a uterus and therefore do not menstruate and are unable to conceive a child (infertile). Affected individuals have male internal sex organs (testes) that are undescended, which means they are abnormally located in the pelvis or abdomen. Undescended testes have a small chance of becoming cancerous later in life if they are not surgically removed. Laboratory diagnosis is rendered by conventional karyotyping (46, XY) and confirmed if androgen receptor gene sequencing reveals a mutation. Our patient is a four-year old phenotypical female who was brought to the outpatient clinic by her mother for evaluation of a painless right inguinal swelling. On physical examination, she had a normal vaginal introitus and urethral meatus with absence of clitoromegaly. The right inguinal swelling was reducible. Pelvic ultrasound showed bilateral inguinal hernias, absence of the uterus, and presence of bilateral gonads in the inguinal canals. Total testosterone was 6.5 ng/dl (normal range 1-3 ng/dl). Karyotype was 46, XY. FISH Microdeletion showed an SRY hybridization signal Yp11.3(SRY+). AR gene sequencing detected no mutation. A conventional karyotyping or fluorescent in situ hybridization (FISH) probes for either the SRY region of the Y chromosome or a subtelomeric Y chromosome probe are essential to differentiate an undermasculinized male from a masculinized female. FISH microdeletion can detect upwards of 98% of the SRY signal hybridization for complete androgen insensitivity syndrome and partial androgen insensitivity syndrome. The analysis is performed on DNA obtained from buccal swabs. Mutation analysis of the androgen receptor gene is now available. Hence, chemistry and genetics analysis play a pivotal role in the diagnosis of androgen insensitivity syndrome.



B-229

Determination of apoE isoforms in young with Alzheimer's disease in the family and its association with cognitive tests and dyslipidemias

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Background: The association with Alzheimer disease (AD) reinforces the need for faster and cheaper methods for Apolipoprotein E (ApoE) genotype and their isoforms E2, E3 and E4 as well as early cognitive methods. Many studies in this area are being developed but are still in inconclusive phase regarding the risk factors that lead to the establishment of AD, so the authors of the study aims to study the lipid profile and the determination of ApoE and its isoforms in active young A community, comparing with cognitive tests in two groups: control individuals without AD in the family and a group of individuals with AD in the family.

Methods: Initially we selected 56 volunteers from the community, with a mean age of 39.7 ± 11.5 years, composed of active youngsters, the majority of whom were women (n = 44). N = 19 individuals had a history of AD in the family and n = 37 stated they did not. All will sign the TCLE. The project was sent to CEP/UNIFESP. Laboratory Analyzes: Venous blood was collected for basic laboratory test dosages, including the lipid profile (Enzymatic/Colorimetric method in Beckman-Coulter® AU5800 equipment) and Apo E gentrification (PCR method with equipment-7500 Real Time After blood collection the volunteers did Cognitive Tracking Instruments: The Rey Auditory Verbal Learning Test (RAVLT), Verbal Span of Digits, Stroop Test of Colors and Words, Trail Making Test, Verbal Fluency, and Categories, Codes and BECK Depression Scale

Results: In our study of ApoE and its alleles, we identified the following groups: Apo E2/E3 (n = 6, 10.7%), Apo E3/E3 (n = 34, 60.7%) , E3/E4 (n=15, 26.8%) and E4/E4 (n=1, 1.8%). In the logistic regression, the group of individuals with E2/E3 genotype (p = 0.014, 0.027) and the E3/E3 group (p=0.065 and 0.093) presented a slight significance in the Trail Making Test and Stroop Test tests respectively. The groups that had the E4 allele did not present significant difference between the cognitive tests until the moment. Regarding dyslipidemia, the groups E2/E3, E3 E3, E3/E4 and E4/E4 presented respectively; 33.3%, 23.5%, 13.3% e)% of dyslipidemias and 50%, 35.3%, 26.7% and 0% of family history.

Conclusion: Initially The authors concluded that in our sample, the E2/ E3 group had a higher number of dyslipidemics and a family history despite being a smaller group. The E3/E3 group presented 58.8% of cases with one of the two risk factors and the E4/E4 group, which despite being the most involved allele in AD, had neither dyslipidemias nor family history. Obviously, it will be necessary to increase our casuistry.

B-230

Performance characteristics study comparing Roche Cobas AmpliPrep/Cobas TaqMan and Cephied GeneXpert Real-Time PCR-based Hepatitis C Virus (HCV) Assays

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Background: Hepatitis C virus (HCV) RNA detection and quantification are the key diagnostic, prognostic and monitoring tools for the management of hepatitis C. The accurate and sensitive measurement of HCV RNA is essential and critical for the clinical management and treatment of infected patients and as a research tool for studying the biology of HCV infection. The aim of this study was to evaluate the performance characteristics and manufacture claims of the new Cepheid GeneXpert HCV assay in comparison with the FDA approved Roche Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) assay for HCV RNA quantification.

Method: Accuracy study between the two HCV assays CAP/CTM and the GeneXpert were performed on 167 available samples covering the 6 known HCV genotypes to determine whether the methods are equivalent within Allowable Total Error 20%, specimens were compared over a range of 0.00 to 6.75 Log IU/mL. Commercial HCV controls (genotypes 1, WHO fourth International standard) were used to evaluate linear range, Limit of detection (LOD), Limit of Quantification (LOQ) and precision, analytical specificity (Exclusivity) was evaluated by adding potentially cross reactive organisms with different concentrations into HCV negative EDTA plasma.

Results: The comparison (accuracy) study has been passed; difference between the two methods was within allowable error for 167 of 167 specimens (100%). The average Error Index (Y-X)/TEa was 0.00, with a range of -0.02 to 0.03. The largest Error Index occurred at a concentration of 5.27 Log IU/mL. The reportable range was verified near the manufacturer claimed range and found to be acceptable. The LOD evaluation was performed according to CLSI guideline E17-A2 and HCV RNA concentration that can be detected with a positivity rate of greater than 95% was determined to be 5.0 IU/mL. The LOQ analysis demonstrates that the HCV VL Assay can determine 10 IU/mL (1.0 log10) with an acceptable precision. The precession study passed (SD: 0.082, 95% confidence for SD: 0.057 to 0.150, CV: 1.5) within allowable total error of 20% and allowable random error of 10%. The linearity of the assay was accepted over a measured range of 0.0 to 6.9 log IU/mL within allowable systematic error (SEa) was 10.0%.

Conclusion: *Cepheid GeneXpert HCV assay shows excellent correlations and produces highly comparable results if compared to the FDA approved Roche CAP/CTM HCV assay. All performance characteristics of Cepheid GeneXpert HCV assay was verified and found to be within manufacturer claim. Cepheid assay is much easier and time saving than Roche assay. GeneXpert instrument modules should be considered as a separate Real-time PCR machines and the validation process should include all modules before testing patient samples.*

B-231

Incidence of inversion of Chromosome 1 as a cause of Infertility

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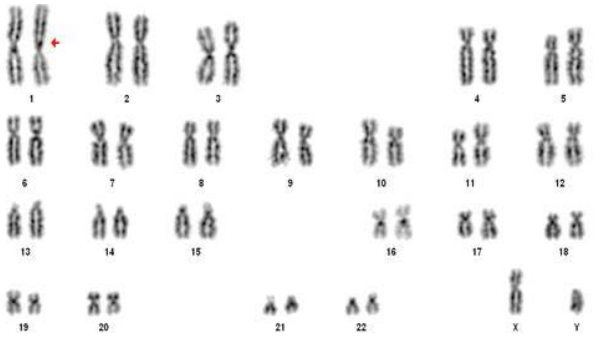
Background: Infertility can be defined as the inability to achieve a pregnancy within one year or the repeated failure to bring a pregnancy to term, most cases of infertility and abortion are not related to chromosomal abnormalities, but in absence of another causal factor, cytogenetic studies of the couple are indicated and may demonstrate infertility caused by changes in sex chromosomes not investigated and/or manifested during puberty or a balanced structural rearrangement.

Objective: To verify the incidence of cases with inversion of chromosome 1 in Brazilian patients with infertility and referred to perform a karyotype in a clinical laboratory.

Methods: Statistical survey of cases from 2014 to 2016 with diagnostic hypothesis of infertility and abortion.

Results: A total of 8,166 normal patients with only 165 altered were analyzed, and only 3 of them present the inversion of chromosome 1.Two male patients and one female patient with diagnostic hypothesis of infertility and repetitive abortion.

Conclusion: The inversion of chromosome 1 corresponds to a very low percentage of the alterations found, being less common than the inversion of chromosome 9. The importance of cytogenetics and the increase of the research on infertility help in the identification, for example, of the polymorphisms of the regions of constitutive heterochromatin, considered normal variations in the population, but that can be decisive for the determination of the cause of reproductive incapacity. The enormous variation of heterochromatin observed among the homologous pairs led several researchers to consider the idea that this difference could hinder the pairing or non-disjunction of the chromosomes, thus predisposing the carriers to alterations in the reproductive capacity.



B-232

Detection of an atypical 22q11.2 deletion not including the critical region related to the DiGeorge and Velocardiofacial syndrome

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Background: Deletions of chromosome 22q11.2 comprise the most common microdeletions in humans; it is associated with a highly variable phenotypic spectrum including Velocardiofacial and DiGeorge syndromes. The majority of patients (~97%) with the 22q11.2 microdeletion have either a recurrent 3 Mb deletion from LCR22-A to LCR22-D that contain at least 30 genes, or a less frequent smaller 1.5 Mb deletion that occurs between LCR22-A and LCR22-B. Both deletions include TUPLE1 and TBX1 as candidate genes to the major syndrome phenotypes. The presence of several highly homologous low copy repeats (LCRs) at the proximal end of the long arm of chromosome 22 predisposes to these rearrangements. However, a limited number of patients with atypical deletions have been described. **Objective:** To report a case of atypical distal 22q11.2 deletion detected by array-based comparative genomic hybridization (array-CGH) using the Agilent platform. **Case report:** We report a three-year-old girl with neuropsycomotor development delay, malformation of the central nervous system and typical dysmorphic features. The karyotype analysis at 450 band resolution shows 46,XX normal result. Fluorescence *in situ* hybridization (FISH) analysis was negative using the commercially available TUPLE1 probe. Array-CGH revealed a 369 Kb interstitial microdeletion: arr 22q11.2(19,425,275-19,794,119)x1, and a ~28 Kb deletion in 11q12.2 that could be classified as VOUS (Variant of Unknown Significance). In this study we reported an atypical deletion between LCR22-C and LCR22-D that includes 10 genes, this kind of deletion could be considered an atypical one. It is described in the literature as related to a variable phenotype that includes mild developmental delay (54%) behavioral disorders (30%), growth retardation and dysmorphic features. Among the present genes in this deletion, LZTR1 related to Noonan syndrome 10; AD. (OMIM 616564), and CRKL an oncogene that mediates Fgf8 interactions, responsible for part of the DGS and VCFS phenotype (OMIM 602007) are especially interesting. The haploinsufficiency of these genes, especially the LZTR1, must be the cause of the proband phenotype, furthermore, in contiguous gene deletions syndromes, the deletion composition is critical to the phenotypic presentation. The aCGH was capable to identify a critical deletion that could explain the proband phenotype, filling the lack of resolution of Karyotyping and the specificity of FISH probes. Distal deletions on 22q11.2 were described as inherited from healthy parents in 58% of the cases, which suggests an incomplete penetrance or variable expressivity so it is necessary to expand the investigation to the proband's parents. That investigation can be made using less expensive methodology like MLPA.

B-233

Assessment of six susceptibility variants of LRRK2 on the risk of Parkinson's disease: a case control study in China

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Background: Leucine-rich repeat kinase 2 (LRRK2) is identified as a candidate gene linked to both familial and sporadic Parkinson's disease (PD). A number of variants of LRRK2 have been reported to affect the risk of PD but the results were not always consistent in Chinese.

Methods: In this study, we conducted a case-control study comprised 296 PD patients and 297 matched controls to investigate the prevalence of three well-known pathogenic variants (R1441C/G/H, G2019S, I2020T) and three Asian-prevalent (R1398H, R2385R, R1628P) variants, and to assess their roles in the susceptibility to PD. All the patients were recruited from the Department of Neurology of Peking Union Medical College Hospital with standard diagnosis. Controls were age and gender-matched healthy people with no history of neurodegenerative diseases. DNA samples were extracted from peripheral blood and amplified for sequence analysis. Chi-square test was performed to compare frequency distribution of genotypes and alleles, and Hardy-Weinberg equilibrium was verified.

Results: The results showed that all the three pathogenic variations were absent in our study, indicating they were not common pathogenic SNPs in Chinese. PD patients carried a higher frequency of variant R2385R than control subjects (10.8% vs. 5.7%; AA+AG vs. GG OR=2.0, 95%CI 1.08-3.68, P=0.027; A vs. G OR=1.89, 95%CI 1.05-3.39, P=0.033). However, no significant difference was found in the prevalence of variant R1398H (15.5% vs. 16.2%) and R1628P (2.4% vs. 1.0%) in PD patients and controls. In addition, we found a patient carrying both R1398H and R1628P variants.

Conclusion: Our study demonstrated that R2385R was a risk factor associated with increased PD susceptibility in Chinese, and called for larger samples or comprehensive systematical reviews for the other two Asian-prevalent variations for further confirmation.

Table 1. Genotype and allele distribution of Asian-prevalent variants and the association with PD.

Genetic variant	Geno-type	Dominant model				Al-lele	Allele model			
		Pa-tients n=296	Con-trols n=297	OR (95%CI)	P value		Pa-tients n=592	Con-trols n=594	OR (95%CI)	P value
R1398H	AA	2	2	0.95 (0.61-1.48)	0.836	A	48	50	0.96 (0.63-1.45)	0.847
	AG	44	46			G	544	544		
	GG	250	249			-	-	-	-	-
R2385R	AA	1	1	2.00 (1.08-3.68)	0.0268	A	33	18	1.89 (1.05-3.39)	0.0334
	AG	31	16			G	559	576		
	GG	264	280			-	-	-	-	-
R1628P	CC	0	0	2.37 (0.61-9.27)	0.214	C	7	3	2.37 (0.61-9.22)	0.212
	CG	7	3			G	585	591		
	GG	289	294			-	-	-	-	-

B-234

What is the yield of whole exome sequencing after unsuccessful standard-of-care genetic investigation?

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Rationale and Objective

In patients with a phenotype suspected to be due to a mendelian (single-gene) genetic disorder, after known single-gene candidates have been eliminated from consideration or when a multigene testing approach is prohibitively expensive, whole exome sequencing (WES) is currently used for the detection of rare variants. However, it is important to establish its diagnostic yield after an inconclusive initial standard-of-care diagnostic investigation. We thus performed a systematic review aiming to estimate the diagnostic yield of WES for suspected monogenic disorders when standard-of-care investigations have failed to provide a diagnosis.

Methods

We performed an electronic search of PubMed and Embase bibliographic databases from inception to February 1st 2015. We applied a comparable search strategy with adaptations for each database. The search strategy consisted of MeSH (medical subject heading) or Emtree terms and keywords related to "exome sequencing". To be eligible for inclusion in the systematic review, a genetic standard-of-care investigation must have been conducted in patients of the original studies. The different steps of the search and the selection process were in line with PRISMA recommendations. The diagnostic yield of exome sequencing was calculated as the percentage of patients with a suggestive molecular diagnosis after WES analysis following unsuccessful initial genetic standard-of-care molecular investigation.

Results

We retrieved 1104 potentially eligible original studies for detailed evaluation and inclusion in the review. Detailed evaluation led to the exclusion of 1003 publications where WES was performed on unaffected or affected parents, unaffected or affected relatives; or was performed on only one case. Overall, 101 studies that included at least two unrelated patients were considered relevant and were included in the review. Between 2 to 172 (median = 6) patients of all ages were enrolled in the selected studies, for a total of 1838 cases (77 diseases). Among the 77 diseases studied, the most common conditions were related to nonsyndromic or syndromic retinal dystrophies (n=6), neurologic disorders (intellectual disability, autism spectrum disorders, progressive cerebellar atrophy, primary microcephaly, brain atrophy and other malformations, and myopathy) (n=5), Noonan syndrome (n=4), and mitochodrial encephalomyopathy (n=3). A suggestive molecular diagnosis was found for 551 of cases (0 to 105/study; median: 3). When calculated within individual studies, the median diagnostic yield was 67%, while the overall molecular diagnostic yield calculated on pooled cases from all studies was 30% (95%CI 28-32%). WES did not provide a molecular diagnosis in a total of 1112 patients (61%), while 174 patients (9%) had a molecular diagnosis of uncertain or partial significance.

Conclusion

We observed an overall diagnostic yield of 30% after unsuccessful initial standard-of-care molecular investigation, suggesting that additional molecular investigation by WES is relevant for identifying pathogenic mutations linked to rare genetic conditions after failure of standard-of-care investigation.

B-235

Disease targeted NGS coupled with homozygosity mapping improves theefficiency of mutation identification in patients with epidermolysis bullosa

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Background: Epidermolysis bullosa (EB) comprises a phenotypically and genotypically heterogeneous group of blistering disorders with the clinical hallmark of skin and mucosal fragility. The lack of definitive clinical findings suggestive of a candidate gene for molecular diagnosis of EB makes patient skin biopsies for electron microscopy and/or immune-epitope mapping necessary as a screening method before mutational analysis. However, antigen mapping is a complicated and demanding technique in terms of expertise and cost and is restricted to a few referral EB centers.

Methods: To circumvent these problems, we developed an EB-targeted next generation sequencing (NGS) panel consisting of 18 genes reported to be causative of EB (*COL17A1, COL7A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KRT5, KRT14, LAMA3, LAMB3, LAMC2, PKP1, PLEC1 TGM)* and 3 (*CD151, CDSN, CHST8)* causing a skin fragility disorder in the differential diagnosis. In addition, to find the appropriate candidate genes for sequencing, in consanguineous pedigrees we applied homozygosity mapping with genome-wide single nucleotide polymorphism (SNP) arrays consisting of 550,000 markers.

Results: A total of 94 patients with clinical diagnosis of EB (and two controls) were sequenced. In 50 patients, pathogenic variants were found in one candidate gene and for the others several variants of unknown significance were found in more than one gene. SNP-based homozygosity mapping identified runs of homozygosity of ≥2 Mb, and in

43 probands there was at least one candidate gene identified by co-alignment of the gene with a homozygosity block. Using this approach pathogenic variants were found in 86 out of 93 families (detection rate of 94.6%) in 17 different EB-associated genes. All mutations were confirmed by Sanger sequencing. The most common EB mutated genes were *COL7A1, COL17A1*, *PLEC* and *LAMB3* (23%, 16%, 13% and 10% of all EB cases, respectively); and, collectively they comprise about 62% of mutated genes. A previously unreported splice junction mutation, the second pathogenic variant in *CD151*, was found in a patient with pretibial EB and nephropathy. The pathogenic splice mutation leads to exon skipping and was confirmed by RT-PCR from whole skin and Sanger sequencing. The other 5 mutations were located in *LAMA3* (5%), *LAMC* (4%), *EXPH5* (2%) and *FERMT1* (1%). Collectively, genome-wide SNP-based homozygosity mapping facilitates identification of candidate genes in EB families.

Conclusion: The specific mutation information forms the platform for prenatal testing and preimplantation genetic diagnosis, as well as for development of allele-specific therapies in the realm of precision medicine for this group of currently intractable disorders. We developed a disease-targeted next generation sequencing approach which is rapid, minimally invasive and cost-effective in identifying mutations in patients with EB.

B-236

Clinical Utility of Genetic Testing for Mitochondrial Disorders in Adult Patients and the Importance of Tissue-Specific Analyses

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Background and Objectives: Mitochondrial diseases are a group of clinically heterogeneous disorders, typically of childhood onset, caused by mutations in mitochondrial DNA (mtDNA) or in nuclear genes encoding mitochondrial proteins. DNA sequencing is considered the gold standard for diagnosis and, historically, the preferred specimen was a muscle biopsy. Over time, however, practitioners have come to favor less invasive screening. Whole mitochondrial genome analysis by next generation sequencing (NGS) was launched one year ago by the Mayo Clinic Molecular Genetics Laboratory and, to date, 110 unique patient samples have been received for clinical testing. Surprisingly, nearly 70% of our specimens were received from adults (≥18 years old). Here we present our data on the clinical utility of mtDNA testing in adult patients, and highlight two unique cases in which a diagnosis could only be made when tissue biopsies were analyzed rather than blood.

Methods: The mitochondrial genome was amplified by long-range PCR, and NGS was performed on the PCR products using a TruSeq Nano library preparation sequenced on an Illumina MiSeq (primary) and an Ion Plus Fragment library preparation sequenced on an Ion Torrent PGM (confirmatory). Large deletions detected by the MiSeq were further confirmed by gel electrophoresis. A retrospective study of all patient results and clinical information, when provided, was then performed. Samples received for verification or proficiency testing were excluded from our analyses. All variants detected were classified according to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines. A positive diagnosis was defined as having one or more likely pathogenic or pathogenic mtDNA mutations as per ACMG guidelines.

Validation: Accuracy was demonstrated for our NGS assay by testing 127 DNA samples extracted from blood, cultured cells, and muscle biopsies that had been previously genotyped using established methods. Results were 100% concordant. Precision was assessed using eight samples run in triplicate both on the same run and across three separate runs. All results were 100% concordant among replicates down to the limit of detection (LOD). The LOD was determined to be 1.875ng input of DNA for the long-range PCR and 25ng input PCR product for library preparation. The minimum detectable variant frequency was 6% for single nucleotide variants and insertions/deletions, and 20% for large deletions.

Results: Our diagnostic yield was approximately 15% (16/110). Eighty-eight percent (14/16) of these positive cases were adults and, interestingly, 43% (6/14) of these were above age 60. Of note, two patients who were negative for mtDNA mutations in blood were found to be homoplasmic and 62% heteroplasmic for pathogenic variants in their muscle and renal biopsies, respectively. One case has the oldest known onset of Kearns-Sayre syndrome, and the other expands the phenotypic spectrum of MT-ND5 mutations beyond Leigh syndrome, MELAS, and optic atrophy.

Conclusions: Next generation sequencing of the whole mitochondrial genome may be especially useful in adult patients suspected of having a mitochondrial disease. Due to heteroplasmy, patients with negative testing on blood may benefit from additional testing of other tissues.

B-237

Pathogenic genomic findings revealed by aCGH in a boy with Klinefelter Syndrome (47, XXY) and loss of Xq28: Rett Syndrome or inactive X chromosome?

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Background: Klinefelter Syndrome is a common chromosomal disorder affecting 1/500 live birth males affecting physical and cognitive development. Rett Syndrome (RTT) is a X-linked condition that affects almost exclusively females showing learn and motor skills delay. This condition is mainly caused by loss of functional copy of *MECP*2 gene (Xq28). The probability for the simultaneous occurrence of Klinefelter and RTT events is very low (about 1/10 to 15,000,000 live births). Here, we described a boy (9y) previously diagnosed by karyotyping with Klinefelter Syndrome, showing chromosome 1 long-arm duplication (19.54Mb) and an extra chromosome X with Xq28 deletion (3.26Mb). Familial investigation showed that grandmother and mother had apparently balanced translocation between chromosomes 1 and X. In addition, a younger sister (4y) has chromosomes 1 partially duplicated and Xq28 deletion. This girl show neuropsychomotor development delayed, intellectual deficiency and strabismus. **Objective:** The aim of this study was to investigate genomic alterations at the proband and his sister using a higher resolution methodology microarray-based comparative genomic hybridization (aCGH) to improve the clinical diagnosis and management in a putative RTT syndrome. **Methodologies:** DNA was extracted from both proband and his sister after informed consent term. The aCGH was performed using Cytochip ISCA 180K V.2. Data were analyzed using the BlueFuse Multi v3.3 software and Cytochip v2 algorithm. The classification of each change followed the recommendations of the American College of Medical Genetics. **Results and Discussion:** aCGH findings for proband were: arr[hg19]1q42.13q44 (229,676,306-249,212,666)x3; arr[hg19] Xp22.33q28(0-151,906,593)x2; arr[hg19] Xq28(151,974,942-155,232,894)x1. Sister genomic findings were: arr[hg19] 1q42 .13q44(229,676,306-249,212,666)x3; arr[hg19] Xq28(151,974,942-155,232,894) x1. Few cases of duplications in 1q are known but poorly characterized. Almost all patients with 1q42-qter duplications present craniofacial dysmorphisms but a quite normal psychomotor development. However, Xq28 deletions including *MECP2* gene is lethal in men and clinically relevant in women. The present case has an extra X chromosome that allows its compatibility with life. To our knowledge, this event is uncommon in the literature. All these findings lead us to hypothesize that proband must present RTT phenotypic characteristics. If not, is possible to assume that extra X chromosome with Xq28 deletion must be preferentially inactivated. **Conclusion:** Based on these results it is necessary follow-up proband´s endophenotype and family members to investigate eventual familial common alteration. The next step is to investigate X inactivation to confirm our hypothesis that suggests a preferential X inactivation as important modulator factor of the severity of the RTT phenotype in these patient. The use of aCGH allows the identification of new genomic findings and helps clinicians at diagnosis of rare genetic syndromes. The familial study is essential for genetic counseling and a better understanding of neurological disorders.

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Validation of a Quantitative Digital Droplet PCR Assay for Assessment of EGFR T790M Mutation Status using Plasma cfDNA

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Background: The release of tumor-derived cell-free DNA (cfDNA) into circulation allows for rapid and non-invasive plasma-based tumor genotyping. Plasma-based genotyping provides a solution to some of the limitations of traditional tissue-based genotyping, which include slow turnaround time (TAT), limited tissue biopsy material, and the risk of failure due to sampling bias. In the case of non-small-cell lung cancer (NSCLC), determining the EGFR p.T790M mutation status is critical for treatment decision-making. Two-thirds of NSCLC patients who carry activating EGFR mutations and experience disease progression after being treated with an EGFR-TKI have developed the T790M resistance mutation. Early detection of the T790M mutation may provide benefit by allowing an earlier switch to an alternative therapy without necessity of a repeat biopsy. The objective of this study was to evaluate the analytical performance of the Bio-Rad EGFR T790M digital droplet PCR (ddPCR) assay for the identification and quantification of EGFR p.T790M mutations in plasma cfDNA. **Methods:** A high-throughput semi-automated ddPCR assay was developed using commercial reagents from Bio-Rad. Plasma cfDNA was extracted

using Qiagen's QIAmp Circulating Nucleic Acid kit. ddPCR was performed in a 96-well plate format using an automated droplet generator (AutoDG™, Bio-Rad), a standard thermocycler and a fluorescence droplet reader (QX200™, Bio-Rad). A Bio-Rad multiplexed TaqMan probe-based assay, designed to detect both the *EGFR* c.2369C>T (p.T790M) mutation and the corresponding wild-type nucleotide, was utilized. Analytical performance was assessed using reference standards (Horizon Discovery), cell-line derived EGFR p.T790M nucleosomal DNA (ATCC #H1975), tissue-derived tumor DNA, and cfDNA from patients and healthy donors. Using these materials, the following parameters were measured: specimen stability, intra- and inter-assay imprecision, linearity, accuracy, carryover, analytical specificity and cross reactivity, LOD and LOQ, analytical turnaround time and reference intervals. **Results:** Plasma specimens were stable up to 21 days stored at -80C. Intra- and inter-assay imprecision of twenty replicates were measured at five different concentrations of mutant DNA (EGFR T790M) spanning the anticipated analytical measurement range. Probit regression analysis of this data determined that the LOD was five mutant droplets (15 copies/mL of plasma). CVs were <20% at concentrations as low as 40 mutant DNA copies/mL of plasma. Dilution linearity was demonstrated from 3750 to 30 copies/mL of plasma. The lowest reportable copy number (LOQ) was determined to be 40 mutant DNA copies/mL of plasma. Increasing wild-type DNA (over two orders of magnitude) had no impact on the accuracy of mutant DNA concentrations. A reference interval study failed to find any detectable mutant DNA copies in 60 healthy individuals. A blinded method comparison study was performed using tumor-derived DNA and demonstrated 100% concordance between the Bio-Rad ddPCR assay and the FDA-approved Roche Cobas EGFR v2 plasma cfDNA assay (n=20). **Conclusion:** The Bio-Rad EGFR T790M ddPCR assay performed using the AutoDG/QX200 system is a robust, economical and rapid method for determining EGFR T790M status from plasma cfDNA.

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Polymorphisms on *MTOR* and *FOXP3* genes are associated with impaired renal function at one-year post-transplant in kidney recipients

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Background: Monitoring of immunosuppressive drugs, such as calcineurin and mTOR inhibitors, is essential to avoid undesirable kidney transplant outcomes. Polymorphisms in pharmacokinetics-related genes have been associated with variability in blood levels of immunosuppressive drugs and adverse effects, but influence of pharmacodynamics-related genes remains to be elucidated. The aim of this study was to investigate the relationship of polymorphisms in *MTOR* e *FOXP3* genes with renal function in kidney transplant recipients within the first-year post-transplant.

Methods: Two-hundred seventy-five kidney transplant recipients were recruited at a kidney transplant center in São Paulo city, Brazil. The patients were randomized in three groups of immunosuppressive regimens containing tacrolimus (TAC), everolimus (EVR) and mycophenolate sodium (clinical trial NCT01354301). Clinical and laboratory data, including renal function parameters and TAC and EVR blood levels, were recorded. Genomic DNA was extracted from blood samples to analyze gene polymorphisms (*MTOR* rs1057079, rs1135172, rs1064261; *PPP3CA* rs3730251; *FKBP1A* rs6033557; *FBKP2* rs2159370 and *FOXP3* rs3761548, rs2232365) by real-time PCR.

Results: *MTOR* rs1057079 (c. 1437T>C), rs1064261 (c.2997C>T) and *FOXP3* rs3761548 (c.-23+2882A>C) were associated with variability on serum creatinine and estimated glomerular filtration rate (eGFR), but not with cellular rejection episodes at month 12. *MTOR* c.1437CC and c.2997TT, and *FOXP3* c.-23+2882GG genotypes were associated with high levels (upper tercile) of serum creatinine (OR=2.44, 95%CI=1.27-4.69, p=0.008; OR=1.97, 95%CI=1.06-3.65, p=0.032; OR=2.35, 95%CI=1.19-4.63, p=0.013, respectively), at month 12. *MTOR* c.1437CC and c.2997TT, but not *FOXP3* c.-23+2882GG genotypes, were also associated with low values of eGFR (lower tercile) (OR=2.47, 95%CI=1.28-4.77, p=0.007; OR=2.06, 95%CI=1.10-3.85, p=0.023). Patients carrying high genetic score (three risk genotypes) of *MTOR* and *FOXP3* variants showed higher serum creatinine than carriers of lower genetic score (up to two risk genotypes) (p=0.018) at month 12. *MTOR* and *FOXP3* polymorphisms were not associated with rejection episodes within the first year post-transplant (p>0.05).

Conclusions: Polymorphisms in *MTOR* and *FOXP3* genes are associated with increased serum creatinine and reduced eGFR, suggesting that individuals carrying risk genotypes are more susceptible to graft dysfunction within the first-year post-transplant.

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Performance of a multi-level, multi-analyte external assayed quality control developed for the detection of Healthcare-associated infections (HAIs).

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Background: Bio-Rad recently obtained FDA clearance for Amplichek II as an external assayed quality control material to monitor the performance of the Cepheid GeneXpert assays targeted for HAI detection. The Amplichek II quality control product consists of 4 levels targeted for HAI assays manufactured by Cepheid: Level Negative containing Methicillin sensitive *S. epidermis* (MSSE), Level 1 containing Methicillin Sensitive *S. aureus* (MSSA), Level 2 containing low concentrations of Methicillin Resistant *S. aureus (MRSA)*, *C. difficile (Cdiff)*, and Vancomycin resistant *Enterococci* (VRE), and Level 3 containing high concentrations of MRSA, Cdiff, and VRE. The performance of the product was tested on the appropriate Cepheid assays over 24 months, on multiple control and reagent kit lots. The Amplichek II product was also tested on four additional HAI testing platforms.

Although the Cepheid assays provide qualitative assessments for the presence of HAIs, we used the data from a precision study to assess whether the semi-quantitative C_t values could be used to track assay performance. Here, we show an example of the potential use of C_t values to track performance over a period of 24 months on the Cepheid Cdiff assay.

Methods: The Amplichek II product, stored at 2-8°C for the length of the study, was tested on the appropriate Cepheid assays at the following time points: 4, 8, 12, 16, 20, and 24 months. The qualitative data results were analyzed as % agreement with the expected results for each of the time points. Additional platform testing of Amplichek II was assessed for Level Negative and Level 3 only. The additional platforms in the study were the BD GeneOhmTM/Max (VRE and MRSA), Nanosphere Verigene (Cdiff), Luminex xTAG (Cdiff), and Meridian Illumigene (Cdiff), for their respective analytes. The results were analyzed for % agreement with the expected results. A precision analysis was performed by monitoring C_t values on the Cepheid assays according to recommendations provided by CLSI document EP5 (2 replicates tested twice a day, over a period of 10 days, 40 total replicates). The data was analyzed using Microsoft excel's Analyze-IT software package. The Cepheid assays' %CV analyzed using semi-quantitative Ct values is <6%. Based on this finding, the semi-quantitative C_t data from the Real Time stability study (for a representative analyte, Van A, with 6 %CV), was assessed using a Levey Jennings plot.

Results: Amplichek II was in 100% agreement of expected results throughout the 24 month shelf life of the product for all levels and all analytes. Additional platform testing shows that Level Negative and Level 3 were in 100% agreement for all analytes, on all platforms.

Conclusion: Bio-Rad's Amplichek II control provides an independent assessment of the performance of in vitro laboratory nucleic acid testing procedures developed for the detection of HAIs.

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Performance of a bi-level, multi-analyte external unassayed quality control across multiple sample type configurations, for molecular diagnostic platforms detecting sexually transmitted infections.

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Background: The CDC estimates that the incidence of sexually transmitted infections (STIs) is 20 million new cases every year in the United States, accounting for almost $16 billion in health care costs. Part of the prevention and treatment plan includes the surveillance of STIs through the use of numerous FDA cleared molecular diagnostic tests. Unlike molecular diagnostic tests of other diseases, tests targeted for STIs utilize multiple patient specimen types ranging from swab to cytology specimens, which require additional precaution during validation and quality assurance. Bio-Rad's Amplichek STI external unassayed quality control was developed to monitor the performance of these assays in the various specimen or sample types, to add confidence in the reliability of test results. The use of quality control materials is

indicated as an objective assessment of the performance of methods and techniques in use and is an integral part of good laboratory practices. Amplichek STI consists of a negative level (negative for CT, NG, and HPV, while containing HPV negative human cells) and a positive level (positive for CT, NG, and HPV), developed for use across multiple STI molecular diagnostic assays and various specimen type configurations (e.g. swab, urine, PreservCyt).

Methods: A comprehensive performance evaluation study was conducted on the Amplichek STI product, in order to assess its performance across the various specimen types validated for use by the assay manufacturer, for 8 commonly tested STI assays for the detection of CT, NG, and HPV. The study was conducted using 3 separate testing sites, with 2 replicates per day, over a period of 5 days (n=10 replicates per site, for a total of 30 replicates).

Results: The results were within 100% agreement for both the Level negative and Level positive, on all assays, across all sample type configurations.

Conclusion: Bio-Rad's Amplichek STI is the first molecular control formatted for use across multiple sample type configurations on common molecular assays used in the detection of STIs, providing an independent assessment for laboratories to maintain good laboratory practice.

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Precision of a multi-level, multi-analyte external assayed quality control for molecular diagnostic platforms monitoring viral load of blood borne pathogens.

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Background: Monitoring the viral load for the blood borne pathogens, HIV, HCV, HBV, facilitates in assessing the prognosis and viral response to treatment. The Abbott RealTime and Roche COBAS AmpliPrep/COBAS TaqMan are FDA cleared to monitor the viral load of these blood borne pathogens. Bio-Rad Laboratories' Amplichek I is an external independent assayed quality control developed to monitor the performance of these assays to add confidence in the reliability of test results obtained for unknown specimens. The routine use of assay agnostic quality control materials is indicated as an objective assessment of the precision of methods and techniques in use and is an integral part of good laboratory practices.

Methods: Precision tests were performed for Amplichek I based on the guidelines recommended by CLSI document EP5, in which two replicates were tested twice a day, over a period of ten days (n=40 total). Amplichek I Level Negative (negative for HIV, HCV, and HBV), Level 1 (low positive for HIV, HCV, and HBV, targeted for Roche) and Level 3 (high positive for HIV, HCV, and HBV, targeted for both platforms), were tested on the Roche assays for the respective analytes. Amplichek I Level Negative (negative for HIV, HCV, and HBV), Level 2 (low positive for HIV, HCV, and HBV, targeted for Abbott) and Level 3 (high positive for HIV, HCV, and HBV, targeted for both platforms), were tested on the Abbott assays for the respective analytes. Precision analysis was performed using the Microsoft Excel "Analyze-it" tool package for precision measurements.

Results: The precision results were within the expected assay performance documented in the respective manufacturers' package inserts, for all the analytes tests.

Conclusion: Bio-Rad's Amplichek I provides an independent assessment of the performance of in vitro laboratory nucleic acid testing procedures for the quantitative detection HIV-1, HBV and HCV on the Abbott RealTime and Roche COBAS molecular diagnostic platforms.

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Electrochemically Modified Sensitive Nitric Oxide Sensors for Detecting Nitric Oxide at the Level of Single Cells.

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Background: Nitric oxide (NO) is an important biological molecule that has diverse functions in human physiology. The concentration of NO in tissues and cells are of vital importance and the presence of too low NO concentration is the source of a variety of diseases. Measurement of NO in biological samples is a challenging task because of its rapid chemical reactions with a wide range of biomolecules, its nano-molar concentrations in tissues and its very short half-life of approximately a few seconds. In general, NO measurement techniques can be classified as direct (measuring NO itself) and indirect methods. Analytical techniques for direct measurement of NO include electrochemistry; fluorometry and electron paramagnetic resonance (EPR). Amongst them electrochemical tools are most promising because they allow fabrication of miniaturized probes with electro-catalytic surfaces which greatly enhances the sensitivity and selectivity for direct, real time and accurate measurement of NO in cell lines and tissue samples along with enabling very low limit of detection.

Methods: In this work, we fabricated combined reference/working carbon fiber electrodes with 7-micron diameter tips for direct placement near the cell lines. The exposed surface of the fiber tip was electrochemically modified with ruthenium oxide and Poly(3,4-ethylenedioxythiophene) (PEDOT). To improve the selectivity of our sensors we coated the surface with an ionic liquid composite and measured the response to NO using Differential Pulse Amperometry to differentiate NO response from other interferents present in biological samples. Madin-Darby Canine kidney (MDCK) epithelial cell lines were used to detect NO release with our sensor. To get a detailed analysis of the temporal and spatial resolution of the NO release, simultaneous monitoring by fluorescence and electrochemical method was performed.

Results: With our method of sensor modification we attained a normalized sensitivity of 2.82E-4 pA/nM/μm^2 and 4.31E-4 pA/nM/μm^2 towards NO in the linear range of 0.1 -3.2 μM and 2-16 nM (biologically relevant range) respectively, with a R^2 value of 0.993. The limit of detection (LOD) of our modified sensor to NO with a signal to noise ratio of 3 was as low as 250 pM. Such an enhanced LOD is unique to our method of sensor modification in comparison to other work done in this field. In terms of selectivity our sensors could effectively discriminate amongst the major biological interferents that are present near NO producing systems. The interference (%) was 0.0003 for 1.0 mM L-Arginine; 0.0002 for 1.0 mM NO_3^-; 0.11 for 0.1 mM NO_2^- and 0.015 for 0.1mM ascorbic acid.

Conclusion: The relevance of our work to clinical laboratory medicine is the development of a highly sensitive and selective NO sensor capable of direct and accurate measurement of very low concentration of NO at the level of single cells and biological samples due to its excellent linearity in a relatively wide concentration range as well as selectivity towards NO in the presence of interferents. Thus, our sensors can be used to study clinical pathology of diseases where NO release is significantly decreased.

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Microparticles derived from tissue factor, leukocyte, endothelium and neuron are associated with Alzheimer's Disease

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Background: In the last few years, microparticles (MPs) have been studied as new specialized structures for intercellular communication. MPs are important messengers in cell-cell communication and contribute to the induction of endothelial damage, inflammation, and angiogenesis, carrying signaling molecules, such as chemokines, cytokines, enzymes, growth factors, receptors, adhesion molecules, mRNAs and microRNA. This study aimed to evaluate the levels of MPs derived from platelets, leukocytes, endothelium, tissue factor and neuron in Alzheimer's Disease (AD) patients compared with cognitively healthy individuals.

Methods: 54 individuals were recruited and classified as probable AD (29 patients - 15 men and 14 women, age 72.9±7.0 years) and cognitively healthy individuals (25 controls - 8 men and 17 women, age 73.2±7.7 years). Blood samples (citrate plasma) were collected and MPs were isolated by ultracentrifugation and measured by flow cytometry. Trucount control tubes were included as a quality control. Statistical analyses were performed using Mann-Whitney test on SPSS program version 13.0. Values of $p < 0.05$ were considered significant.

Results: The median (interquartile range) levels of MPs (MPs/μL) derived from tissue factor [78.8 (82.2)], leukocytes [109.9 (86.9)], endothelium [40.9 (76.5)] and neuron [200.3 (362.4)] are significant higher in AD group than in control group [37.4 (13.4); 39.0 (27.3); 21.8 (20.8) and 41.4 (72.4), respectively; all $p<0.05$]. MPs derived from platelet did not differ between the groups ($p = 0.167$).**Conclusion:** The results suggests that MPs derived from tissue factor, leukocytes, endothelium and neuron could be associated with the physiopathology of AD and, in the future, the MPs may be included as diagnostic biomarker for AD in the clinical routine.