CASE REPORT: Molecular and Cyto genetic characterization of a 46,XX male

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background: The Y chromosome evolves from an auto chromosome and accumulates male-related genes including sex-determining region of Y-chromosome (SRY) and several spermatogenesis-related genes. 46,XX subjects carrying the determining SRY gene usually have a completely male phenotype.

Objective: To identify Y chromosome material in an azoospermic male with a 46, XX karyotype.

Method: A 39 years old male was tested to micro deletions of Y chromosome in order to investigate the origin of an infertility characterized by azoospermia. He did not report any previous familiar history of infertility and was never submitted to any infertility treatment before. The isolated DNA was submitted to a PCR reaction followed by electrophoresis on 2% agarose gel stained with ethidium bromide. Once the PCR reactions were made in the PCR reactions, the samples were labeled with different dyes and the settings in the analysis software GeneMapper ID-X v. 1.1.1 were modified to optimize the analysis of this genetic testing in different fluorescence channels in the 3730 DNA Analyzer.In order to evaluate such changes, the results of 35 patients who carried out the microdeletions of the Y chromosome were both analyzed on agarose gel and capillary electrophoresis. The Kappa statistic was used to compare the results.

RESULTS: Results accordance obtained between the two techniques from the 35 samples using Kappa statistics was perfect 1,0 (0,669 to 1,0 CI 95%). There were no statistically relevant difference (p<0,001) among compared methodologies.

Conclusions: We concluded that capillary electrophoresis could be used as an alternative method for the study of Y chromosome microdeletions, providing a more efficient and secure assay.

Validacion of molecular testing of the Y chromosome microdeletions in DNA analyser: A case of laboratory automation


BACKGROUND: Infertility is a medical condition that affects 10-15 % of couples seeking to have children_LARGE proportion of cases of idiopathic male infertility is due to the presence of microdeletions in the Y chromosome genes related to spermatogenesis. Located on the long arm of the chromosome Y, the 3 regions known as “azoospermia factors” (AZFa, AZFb and AZFc) are found fully or partially deleted in azoospermic patients or patients with severe oligozoospermia. Our laboratory offers the test for Y chromosome microdeletions by multiplex PCR followed by electrophoresis on 2% agarose gel stained with ethidium bromide. Once it is a manual method occasional errors may occur in prepare the gel or pipetting the samples. Furthermore, ethidium bromide is a mutagenic reagent and therefore the represents a risk to both the operator and the environment.

OBJECTIVE: The objective of this study is to standardize the diagnosis of Y chromosome microdeletions through a more efficient and secure methodology.

METHODS: For this study, the capillary electrophoresis was chosen to substitute the agarosegel electrophoresis. Modifications were made in the PCR reactions, the primers were labeled with different dyes and the settings in the analysis software GeneMapper ID-X v. 1.1.1 were modified to optimize the analysis of this genetic testing in different fluorescence channels in the 3730 DNA Analyzer. In order to evaluate such changes, the results of 35 patients who carried out the microdeletions of the Y chromosome were both analyzed on agarose gel and capillary electrophoresis. The Kappa statistic was used to compare the results.

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CONCLUSIONS: We concluded that capillary electrophoresis could be used as an alternative method for the study of Y chromosome microdeletions, providing a more efficient and secure assay.
genes in the <3Mb stretch of duplication have not been fully characterized. However, researchers believe that the duplication of one particular gene, known as TRX1, is responsible for many of the typical symptoms of the syndrome. aCGH is a molecular cytogenetics technique, able to identify unbalanced chromosomal changes (gains or losses of genomic material) through the general analysis of the entire genome in a single experiment. Objective: The objective of this study was to show the use of the technique Comparative Genomic Hybridization arrays (aCGH) as a molecular tool for the diagnosis of microduplication 22q11.2. Methods: 3 samples of peripheral blood of pediatric patients referred to the Institute for study of loss or gain of genomic material were used. The samples were collected in tubes 4ml EDTA and stored at 4 °C until processed. For DNA extraction was used QiagenDNA Blood Mini Kit (Qiagen). After extraction the samples were quantified and evaluated reasons for A260/A280 and A260/A230 in NanoDrop2000/2000c. The samples had concentrations above 50 ng/ul and A260/A280 (1.8 to 1.9) and A260/A230 (1.5 to 1.9) were among the reasons for standard values. gDNA was digested with restriction enzyme labeled with the cytinases 3 (Cy3-reference) and 5 (Cy5-patient) and purified by SureTag Complete DNA Labeling Kit (Agilent). After purification the samples were again quantified to measure the incorporation of Cyanine 5 (Cy5). All samples had A260/A280 reason to 1.8. DNA concentration above 420.0 ng/ul, the incorporation of Cy5 was above 10.3 pmol/ul, the specific activity was above 24 pmol Cy5/μg DNA and yield ug DNA was above 8.9. The OligoCGH/ChIP-on-chip Hybridization Kit (Agilent) and Human Cot-1 DNA (Agilent) kit was used to perform hybridization on the slide containing probes corresponding to 180,000 genes. After 24 hours of hybridization at 65 °C the slides were washed with AgilentOligoCGH/ChIP-on-Chip 1 and 2 Wash Buffer Kit (Agilent) and acetonitrile reagent (Sigma). After extraction of data through the Software ScanControls Software Feature Extraction, these were analyzed in Agilent CytoGenomics Edition Software 2.7.8.0. Results: All samples met the standards of quality required for the analysis. The technical benchmark DerivativeLR Spread (DLRS) was <0.30. Results for the same patient were compared between both labels. Conclusions: Concluding, almost half of the chromosome 7 long arm (7q36.3), also related with mental development delay; a duplication on the chromosome X long arm (Xq27.2), related with mental development delay and obesity; a deletion on the chromosome X long (9p24.3p24.2), related to developmental delay; a deletion on the chromosome X long (Xq27.2), related with mental development delay and obesity; a duplication on the chromosome 7 long arm (7q36.3), also related with mental development delay; and a deletion on the chromosome 15 long arm (15q11.2-q13.1), that overlaps Prader-Willi and Angelman syndromes region. Conclusions: Concluding, almost half of the patients presented a pathogenic alteration that was not detected by conventional karyotyping proving the importance of aCGH as a complementary diagnosis tool.

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Background: Recent studies have demonstrated the role of the interleukin 28B (IL28B) polymorphisms in predicting treatment response, spontaneous clearance and sustained virologic response (SVR) to pegylated interferon and ribavirin for subjects with homozygous T/T genotype than those with G/G genotype). To describe the frequency of the IL28B C/T SNP for rs12979860 and that

Table 1: Combined genotype frequencies of the interleukin (IL)28B single nucleotide polymorphisms rs1297860 and rs8099917

<table>
<thead>
<tr>
<th>SNP</th>
<th>GT (%)</th>
<th>GC (%)</th>
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<tr>
<td>rs1297860</td>
<td>6 (1%)</td>
<td>81 (1.6)</td>
<td>71 (12.5)</td>
<td>160 (25.2)</td>
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<tr>
<td>rs8099917</td>
<td>6 (1%)</td>
<td>81 (1.6)</td>
<td>71 (12.5)</td>
<td>160 (25.2)</td>
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Comparison of Invivoscribe’s T Cell Receptor Gamma Gene Rearrangement Assay 2.0 vs TCRG Gene Clonality Assay (developed by the Euroclonality, previously BIOMED-2 Group)

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Background Histology or cytology, supplemented with immunohistology or flow cytometric immunophenetyping has been used to discriminate between malignant and reactive lymphoproliferations. However, in some of the cases, the diagnosis is difficult. The diagnosis of lymphoid malignancies can be supported by clonality assessment as all cells of a malignancy have a common clonal origin. Gene rearrangement analysis used to be performed by Southern Blot-based techniques which is very reliable, but is increasingly replaced by PCR techniques because of
the greater efficiency and sensitivity of PCR. These gene rearrangements generate products that are unique in length and sequence for each cell. Therefore, PCR assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci. We evaluated the T cell receptor gamma gene clonality assay (Euroclonality’s primer) and T cell receptor gamma gene rearrangement assay 2.0, both from Invitrogen, for clonality assessment of T cells lymphoproliferative disorders.

Methods: 22 archived formalin-fixed, paraffin-embedded (FFPE) clinical samples were extracted with QIAamp DNA FFPE tissue kit, following manufacturer’s recommendation. 2 proficiency panel samples were included as well. The extracted genomic DNA was quantified and amplified using the specimen control size ladder master mix in the Invitrogen kits and AmpliTag Gold DNA polymerase. All samples were tested in duplicates of 50ng and 100ng of DNA to check for presence of inhibitors and DNA quality. Next, PCR was performed with TCRG tube A, TCRG tube B and TCRG 2.0 with samples in duplicate, polyclonal, monoclonal and negative control. The PCR products were denatured with Hi-Di formamide and GeneScan 600 Lize size standards and analyzed on ABI 3500 capillary electrophoresis instrument. Data are automatically displayed as size and color specific peaks.

Results: Majority of the samples were concordant with the in-house developed test for TCRG, TCRG clonality assay and TCRG 2.0. However, 2 of the samples showed discrepant results. TCRG 2.0 requires shorter hands-on time to perform and is easier to analyze as it is one tube instead of a single cell. TCRG clonality assay has 2 tubes (tube A and B) and each tube is dual-color (green and blue). The dual-color can be quite confusing to interpret. The clone size detected is different for both assays as the PCR primers are different. Hence, for different labs using these 2 assays, it is hard to correlate if the clone detected is the same.

Conclusion: The TCRG clonality assay and TCRG gene rearrangement assay 2.0 from Invitrogen generated similar results for clonality assessment of T cells lymphoproliferative disorders. We had 2 cases with discrepant results and will emphasize that the results of molecular clonality tests must always be interpreted in the context of clinical, histological and immunophenotypic data. TCRG clonality assay adopts the Euroclonality’s primers and is more widely used in laboratories. Even though TCRG gene rearrangement assay 2.0 is easier to perform and interpret, more data should be generated for the better comparison of these 2 assays.

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HPV E6/E7 mRNA RT-qPCR Assay for Detecting High Grade of Cervical Lesion with ThinPrep Pap Samples

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Background: Human cervical cancer is the second most common cancer among women worldwide. Several decades ago, human papillomaviruses (HPV) were found out to be a major factor of cervical cancer. HPV DNA genotyping assay has been the method of the choice, since it has shown high analytical sensitivity. The latest results show oncoviral HPV DNA appeared not only in cancerous tissues, but also in the normal tissues according to cytological diagnosis. For this reason, HPV test results show oncogenic HPV DNA appeared not only in cancerous tissues, but also in the normal tissues according to cytological diagnosis. For this reason, HPV test results show oncogenic HPV DNA appeared not only in cancerous tissues, but also in the normal tissues according to cytological diagnosis.

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condition, with approximately 150 mutations and 400 variants identified. Molecular studies seek to define the origin of the enzymopathy in a determined population and correlate the G6PD variants with the clinical course of the disease, as well as identifying the G6PD deficiency in heterozygous females. Previous reports have shown that the prevalence of G6PD deficiency, in several regions of Brazil, is around 10% among males of African origin and between 1-6% on euro-descendent males. The data regarding the G6PD variants in Brazilian population are fragmentary and scarce. The capital of Brazil, which lies in the Federal District, has a mixed population representing the different regions of Brazil. The Neonatal Screening Program (NSP) in the Federal District indicates a prevalence of 4.5% for G6PD deficiency. The objective of the current study was to identify the types of variants in the G6PD gene in a group of children screened through the NSP in the Federal District, and correlate these data with the presence of neonatal jaundice. Methods: Oral mucosa samples were collected from eighty boys and four girls diagnosed with G6PD deficiency through the NSP in January and February of 2014, whose parents signed an informed consent form. The majority of the newborns presented with residual enzyme activity of around 50% (moderate deficiency). All representatives of the children filled out a questionnaire with relevant details regarding family history, history of neonatal jaundice and therapy. Molecular analysis was carried out using real-time PCR (allelic discrimination). The G202A and C563T mutations in the G6PD gene were analyzed using specific primers and probes. Results: Seventy of the 84 families were unable to provide information regarding ethnic origin of the child, 13 claimed indigenous descent, and one claimed Portuguese and Spanish descent. 60.7% of the children presented with neonatal jaundice, 76.5% presented at 48 hours post-natal, and 29% required phototherapy. Molecular analysis identified a high proportion (98.8%) of neonates positive for the G202A mutation (variant G6PD A-): 79 boys were hemizygous and 4 girls were homozygous for this mutation. Only one boy presented the Mediterranean C563T mutation. Analysis of the correlation between genotype and presence of neonatal jaundice was compromised by the intense predominance of the G202A mutation in the sample group. Conclusions: This is the first study carried out in the population of individuals with G6PD deficiency in the Federal District of Brazil. Although the sample group studied was relatively small, the high prevalence of a single mutation suggests that G6PD deficiency in the population of the Federal District is principally due to the G202A mutation. Neonatal jaundice was frequent among G6PD deficient children. The absence of cases of heterozygous females in the sample group may reflect the inability of neonatal enzyme screening to detect G6PD deficiency in these cases.

Validation of a quantitative CMV Simplexa kit for clinical use in a private hospital in Sao Paulo, Brazil

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Background: Human Cytomegalovirus (CMV) is a member of the Herpesviridae family. Primary CMV infection in healthy individuals is asymptomatic or results in a mild, non-specific illness. After acute infection, CMV establishes latent infection. Reactivation can occur in immunocompromised patients with an important morbidity and mortality in this group. Early diagnosis and CMV viral load monitoring in high risk patients are critical for an efficient infection management. To improve molecular diagnostic of CMV in our Clinical Laboratory, we validated the quantitative FOCUS CMV Simplexa kit in plasma samples.

Methods: Nucleic acids from plasma samples were extracted using automated EasyMag system and submitted to Real Time PCR with Focus Simplexa CMV kit, using the 3M Integrated Cycler equipment which is a nucleic acid amplification system based on a centrifugal micro fluidic platform. Validation was conducted according to CAP guidelines. We evaluated accuracy, linearity, precision (intra- and inter-assay) and sensitivity. Accuracy was tested comparing obtained results with previous data. Linearity was determined using dilution series of a high viral load sample, while intra and inter-assay variations were determined using 4 pools of samples (high, medium, low-medium and low) and a negative plasma obtained from the blood bank. Sensitivity was established using samples with viral loads close to the detection limit of the kit.

Results: For accuracy, we compared the results of 53 samples and obtained a mean log difference of 0.21 Log copies/mL. Analytical linearity was evaluated in quadruplicates using the calibration CMV standard from FOCUS, ranging from 2,010,000,000 copies/mL(9.3 Log copies/mL) to 750 copies/mL (2.8 Log copies/mL). The correlation coefficient obtained was R=0.9993. Intra and inter-assay variation of all 4 pools of samples were lower than 10%, as expected. Finally, Detection limit was tested with samples with 330, 153 and 78 copies/mL. The test was able to detect viral load of 330 copies/mL with 100% confidence and 153 copies/mL with 90% confidence.

Conclusion: The results of validation demonstrated that the kit is reliable and useful for quantification of CMV in plasma samples, and combined with its fast turnaround time and decreased hands-on time, make this assay highly suitable for the rapid diagnostics of CMV infections in the clinical laboratory.

Sodium citrate at 8% is equivalent to EDTA as anticoagulant of choice for circulating cell-free DNA analysis: low contamination by blood cells genomic DNA and inhibition of blood nuclelease activity.

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Background: Despite the intensive research, few circulating cell-free DNA (cfDNA) analysis have been translated to clinical practice. The lack of preanalytical consensus is a major obstacle. Traditionally, the EDTA is the anticoagulant of choice for studding cfDNA. Moreover, because of the lack of cell protection, the cfDNA is susceptible to blood nucleases, but the impact of these enzymes has long been neglected. Here, we studied the initial amount of cfDNA, it’s stability and the blood nucleases activity in plasma (EDTA, citrate, heparin) and serum samples.

Methods: Fresh blood from 20 health donors was collected simultaneously in K3EDTA, sodium citrate 3.2%, sodium heparin, and Z serum clot activator tubes (all from Greiner-bio-one). The citrate 8% samples were obtained by transferring fresh blood sequentially to 3 citrate 3.2% tubes. Serum or plasmas were generated within 10-15 minutes after the venipuncture. DNA extraction was performed by using Nuclens easyMAG (Biomerieux). RNAse P was the target used for cfDNA quantification in a StepOne qPCR System (Life technologies) by using hydrolysis probe chemistry and absolute quantification. The results were shown as median in Genomic Equivalents/mL. Statistical analysis was Friedman’s test. The cfDNA stability was evaluated treating (or not) the samples with 25U of DNAs I for 1h at 37°C before RNAse P assay. To investigate sample’s nucleace activity a hydrolyze probe and a passive reference (ROX) were added to the crude samples and the fluorescence increase were measured for 24h at 37°C in the qPCR system. For nucleases inhibition assay a serial dilution of citrate (0.4 to 14%) was used.

Results: The cfDNA amounts in EDTA (158.7 GE/mL) and in citrate (130 GE/mL) were similar (p=0.27) and lower than the levels found in heparin (413 GE/mL; p<0.001-EDTA, p<0.001-citrate) and in serum (815 GE/mL; p=0.0012-EDTA, p>0.001-citrate). The nucleace activity was higher in heparin (arbitrary considered 100%), 90% in serum, 66% in citrate and not detected in EDTA. The nucleace activity in citrate was different from serum and heparin suggesting an inhibitory effect. The treatment with DNAs I reduced the cfDNA amount in EDTA by 1.1-fold, in serum by 1300-fold, in heparin by 242-fold and in citrate by 1.3-fold. In the citrate serial dilution experiment, no nucleases activity was detected from 7%. Increasing the citrate concentration to 8% did not change the initial cfDNA amount (96.86 GE/mL) compared to EDTA (1.29 GE/mL; p=0.12) and citrate (89.57 GE/mL; p=0.99). The nucleace activity was not detected in citrate 8% and treatment with DNAs I did not alter its cfDNA amount, reduction of 1.01-fold.

Conclusion: The citrate 3.2%, citrate 8% and EDTA have similar initial cfDNA, although lower when compared to heparin and serum. The nucleace activity was higher in heparin and serum, partially inhibited in citrate 3.2% and completely blocked in EDTA and citrate 8%. The diveral ions chelators citrate 8% and EDTA share a common mechanism of both avoid blood cells genomic DNA contamination to cfDNA and inhibit blood nucleases. The high-levels of cfDNA in serum and heparin should be attributed to the coagulation and direct lyses of blood-nucleated cells respectively.

Development of a real-time PCR genotyping assay to detect HLA-B*5701 allele associated with abacavir hypersensitivity reaction

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Background: Abacavir sulfate is a nucleoside reverse-transcriptase inhibitor with potent antiviral activity against HIV. 5-10% of individuals being treated with abacavir develop a potentially life-threatening hypersensitivity reaction (ABC-HSR). Human leukocyte antigen (HLA) B*5701 allele strongly predicts ABC-HSR. Therefore, Pharmacogenetic screening for the HLA-B*5701 allele is recommended prior to initiation of abacavir therapy. In San Francisco General Hospital, a two-color B57-specific immunofluorescence assay with FacsCanto flow cytometry was utilized to detect all HLA-B57 subgroups. Preliminary positive samples are sent to a reference
lab to identify true HLA-B*5701 positive patients. Recent studies have shown that a HCP5 single-nucleotide polymorphism (SNP), rs2395029, is in perfect linkage disequilibrium with the HLA-B*5701 allele: the sensitivity of the HCP5 SNP for carriage of the HLA-B*5701 allele was 100% and specificity was 99%. **Objective:** Develop an accurate in-house assay utilizing real-time polymerase chain reaction (PCR) and fluorescence monitoring. **Methods:** DNA extraction from blood samples was performed with a QIAamp DNA mini kit, and DNA concentration was determined by using a Nanodrop ND2000. A rapid-cycle PCR was developed using the Rotor-Gene Q 2plex HRM system. Forward primer: GAGTGCCCATTGAACTACACA, reverse primer: GTCTGGTCTCTGGACACATACTGT, wild-type probe: FAM-AGTCGCCCACAGG-BHQ1 plus, mutant probe: CAL Fluor Orange 560 - AGTGGCCCAAGG-BHQ1 plus. Thermocycling conditions were 20 sec at 95 °C, followed by 40 cycles at 95 °C for 3 sec and 60 °C for 30 sec. PCR was performed in a 25-μl volume in the presence of 1X Taqman GxTaq master mix, 900 nmol/L of each primer, 250 nmol/L of each probe, 2 ul DNA, and DEPC H2O. 2-fold serial dilutions of a wild-type (T/T) sample and a mutant sample (G/G), with each dilution amplified in triplicates, were tested to evaluate the linearity and repeatability, as well as the limit of detection of the genotyping assay. A standard curve was constructed for each sample on the basis of DNA serial dilution, on which Ct values were plotted against the log value of the target DNA amount. Blood samples of 49 patients who were diagnosed of HIV were included in the patient comparison study between the new RT-PCR assay and PCR-SSOP method of the reference lab. **Results:** Ct values were obtained from amplification of serial dilutions of a wild-type sample from 100 pg/ul to 3.125 pg/ul and a homozygous mutant samples from 10 pg/ul to 0.625 pg/ul, respectively. The regression equation of the wild-type sample was y = -3.4317x + 30.912, with a R2 of 0.9985. The intra-assay coefficient of variation (CV) for all dilutions ranged from 0.037% to 0.73%. The regression equation of the homozygous mutant was y = -3.2123x + 31.021 with a R2 of 0.9907. CV for all dilutions ranged from 0.13% to 0.31%. Patient comparison study revealed that this real-time PCR assay demonstrated 100% sensitivity and 100% specificity when validated with 10 positive and 39 negative samples previously confirmed by the reference lab. **Conclusion:** A real-time genotyping assay was developed to identify positive and negative HLA-B*5701 alleles. This approach offers a sensitive, rapid and cost-effective screening assay prior to abacavir prescription. The genotyping assay has a wide dynamic range of reliable amplification linearity.
Combi or Combi PT immunoassay (Roche Diagnostics, Switzerland) were sent for western blot confirmation and also tested for viral load using the improved Roche Cobas Taqman HIV1 Monitor version 2 (Roche Diagnostics, Switzerland).

Samples are determined to be western blot positive if any 2 bands for p24, gp41, gp120/160 or 2 of 3 envelope bands with or without Group Antigen and/or polymerase bands are present, as defined by Centers for Disease Control and Prevention and World Health Organisation criteria respectively. Indeterminate results are defined as the presence of bands that do not meet positive criteria while inconclusive findings are those that do not fit negative, positive or indeterminate criteria.

Methods: A total of 84 anonymised samples were screened for infection by the Human Immunodeficiency Virus, using the Roche Diagnostics immunoassay HIV Combi or Combi PT. Seventy samples were determined to be at the grey zone cut-off index of 0.9 or greater, indicating presumptive positivity. These samples were subsequently sent for western blot analysis at the National Reference HIV Laboratory with split samples tested using the HIV Monitor v2 on the Roche Taqman 48.

Results: Of 84 samples tested, 14 samples were determined to be screening-negative with no viral RNA detected by nucleic acid testing. Thirty-four samples were screening-positive and subsequently confirmed by western with viral loads of log 1.53 to 6.00 copies per milliliter. Two samples were defined as western blot indeterminate, with no virus detected, while the remaining 7 indeterminate samples and 1 inconclusive sample had viral loads of log 5.33 to 6.48 copies per milliliter. The mean and standard deviation of viral loads for western blot non-positive and non-negative samples were 6.17 and 0.44 respectively. Western blot positive samples yielded a lower average value of log 4.62 copies per milliliter with a standard deviation of 0.73.

Conclusion: While there is a robust concordance between de-infection and viral RNA copies in samples. To evaluate the performance and concordance between HER2 RT-PCR and FISH test, breast invasive ductal carcinoma (IDC) or adjacent normal specimen was collected and processed into FFPE or OCT sample immediately after biopsy or surgery. RNA samples were determined to obtain optimal final concentration of 25µg/mL. HER2 positive cutoff value was established by conducting a statistical population study on tumor versus adjacent normal samples.

Results: The intra- and inter-run coefficient of variations of the assay were <5% (in terms of concentration in log_10). The dynamic range of the assay was between 5.4x10^10 and 3.5x10^12 copies/mL (R=0.99). Moreover, around 85% overall percentage agreement (OPA) was obtained when compared with FISH test with FFPE or OCT samples.

Conclusion: A standardized RT-PCR assay and user procedure presented here can produce consistent test results regardless of the types of patient samples and has high potential to be included in the ASCO/CAP HER2 testing guideline.

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Real-time NASBA Targeting HPV E6/E7 mRNA Overcomes Low Specificity of HPV DNA Test

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Background: Cervical cancer is one of the most common cancers in women and ranks second by female cancer mortality rate. About 40 of human papillomaviruses (HPV) can infect the cervix and are divided into high- and low-risk groups based on their frequent detection in carcinoma or low-grade lesions, respectively. Persistent infection with these types is the major risk factor for the development of cervical cancer. So it was recommended HPV genotyping test as complementary diagnosis. But, HPV DNA was detected both in cancerous tissues and in normal tissues according to cyoto logical diagnosis. Several studies have suggested that detection of E6/E7 oncogene transcripts of high-risk HPV types would provide higher specificity in screening for the risk of development of high-grade of cervical samples. The synergistic effects of E6 and E7 proteins results in disturbance of cell cycle regulation, prevention of apoptosis, and, ultimately, transformation and survival of neoplastic and dysplastic cells.

Methods: This study aims to evaluate the clinical performance of the commercial kit targeting HPV E6/E7 mRNA and compare it with HPV DNA genotyping for the detection of high-grade squamous intraepithelial lesions (HSIL) and cancer in a Korean population. NucliSENS EasyQ HPV kit (bioMérieux, Marcy, France) using a nucleic acid sequence-based amplification (NASBA) technique is chosen. This assay utilizes molecular beacon probes for real-time detection and typing of E6/E7 mRNA from HPV genotype 16, 18, 31, 33 and 45.

Results: HPV DNA tests were positive in 100% and 53% of abnormal (SCC, HSIL, ASC-H, LSIL, and ASC-US) and normal cytology cases, respectively. Positive rates of HPV E6/E7 mRNA assay were 75%, 74%, 60%, 56%, 29% and 9% for SCC, HSIL, ASC-H, LSIL, ASC-US, and normal cases, respectively. Positivity rates of HPV E6/E7 mRNA assay were 75%, 74%, 60%, 56%, 29% and 9% for SCC, HSIL, ASC-H, LSIL, ASC-US, and normal cases, respectively. The data from this study seems clearly show that the real-time NASBA for diagnosis of cervical cancer has higher clinical specificity than HPV DNA genotyping test, since the positivity rate detected by the real-time NASBA in normal samples was significantly lower than that detected by the HPV DNA genotyping test. However, the positive rate detected by the real-time NASBA in cancerous samples was much lower than that detected by the HPV DNA genotyping test. In this respect, the sensitivity of the real-time NASBA for cervical cancer diagnosis seems to be lower than current HPV genotyping test. The main reason of this low sensitivity was primarily due to the different prevalence of the high-risk cervical cancer causing HPV genotypes of Korea.

Conclusion: Data from the current study suggests that the NucliSENS EasyQ HPV E6/E7 assay (bioMérieux) has higher specificity than DNA assays, and can overcome DNA assays' shortcoming of low specificity in clinical detection of high-grade cervical lesions and ability to predict the risk of their development in HPV-infected women. Expanding the types of HPV targeted by mRNA assays may increase sensitivity as well as specificity of detection of high-grade cervical lesions.
Bioinformatics analysis to determine prognostic mutations of 72 de novo acute
myeloid leukemia cases from The Cancer Genome Atlas (TCGA) with 23 most
common mutations and no abnormal cytogenetics

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Objective: Acute myeloid leukemia (AML) is a heterogeneous malignancy with
many described karyotypic and molecular abnormalities. Recurrent karyotypic
abnormalities in AML have been well established for treatment. However,
approximately half of AML patients have no karyotype abnormality (CN-AML).
An important issue in the treatment of AML is how gene mutation patterns may help
physicians guide the management of patients in daily practice. The Cancer Genome
Atlas (TCGA) database has available data from 200 cases of de novo AML including
cytogenetics, 260 gene mutations, and survival duration for each case. As previously
reported, in this database a total of 23 genes were significantly mutated and another
237 were mutated in two or more samples. We utilize clustering analysis on this
database to correlate the presence of 23 common mutations with the prognosis of de
 novo CN-AML cases.

Methods: Cases with positivity for the most common 23 mutations and no cytogenetic
abnormalities were selected from the TCGA. Unsupervised neural network analysis
with NeuroXL Clusterizer (OLSOFT LLC, Moscow) was performed on these cases to
group them into clusters according to their pattern of mutations and survival duration.
The next step was to find interaction between wild-type genes, gene mutations (with
their associated proteins) and common chemotherapy to gain more insight into
response of CN-AML patients to treatment, which was achieved with Ingenuity IPA
software (Ingenuity Systems, Inc., Redwood City, CA).

Results: 121 cases with positivity for the 23 most common mutations were obtained
from the original set of 200 AML cases. Subsequently, 72 cases with no cytogenetics
abnormalities (CN-AML) were obtained from these 121 cases. Within the 72 CN-
AML cases, the following mutations were not present: TP53, NRAS, KIT, EZH2,
and HNRNPK, leaving 18 mutations in this subset of patients. Using appropriate
threshold for mutation frequency (75%), clustering was found to be based on only
4 mutations: NPM1, FLT3-ITD, RUNX, and DNMT3A. The following prognostic
groups were found: (a) good: NPM1, CEBPA, or TET2, (b) intermediate: NPM1/
DNMT3A, or other mutations, (c) poor: RUNX1, FLT3-ITD/NPM1, FLT3-ITD/
CEBPA, or FLT3-ITD. Pathway analysis revealed significant causality between the
mutations FLT3-ITD, NPM1, DNMT3A, IDH2, RUNX1, TP53, KIT, and CEBPA
and the chemotherapy agents cytarabine, irinotecan, and etoposide, hydroxyurea,
dexamethasone, methotrexate, and decitabine.

Conclusions: Combinations of mutations appear to dictate the clinical behavior of
AML in terms of prognosis. This study provides further molecular characterization
and prognostic data for the heterogeneous group of CN-AML patients.

Validation of a genetic test for lactose tolerance in a Brazilian hospital

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Background: Lactose, the predominant carbohydrate in breast milk, is hydrolyzed in
the intestinal mucosa by lactase-phlorizin hydrolase (LPH). Lactase is produced
during the weaning and undergoes a physiological decline in adult, which leads to
lactose intolerance symptoms. In some populations LPH activity persists throughout
life, a condition known as adult lactase persistence. The conventional diagnostic
test is performed challenging the patient with lactose, which can cause diarrhea
and abdominal pain in intolerant patients. Several studies have shown that two single
nucleotide polymorphisms in the introns of a helicase (MCM6) upstream the lactase
promoter. Intolerance genotype is a recessive trait in both SNPs: rs13910 and
found a frequency of 51.86% CC, 41.49% CT and 6.65% TT for LCT-13910 and
51.66% GG, 40.70% GA and 7.63% AA for LCT-22018.

Conclusion: Molecular test was implemented in our Clinical Laboratory in
November, 2012 and since then has been an excellent option for patients instead of
the conventional test. Approximately, half of tested patients were intolerant to lactose.
Those data are in accordance with previous Brazilian studies, and may reflect the great
admixture in Brazilian population.

Genotyping of drug-metabolizing enzymes CYP2D6, CYP2C19, CYP2C9,
CYP3A4 and CYP3A5 in patients prescribed pain medications

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Background: The cytochrome P450 (CYP450) superfamily represents a group of
enzymes which are associated with the metabolism of more than 90% of human
drugs. To date, a large number of allelic variants as well as copy number variations
(CNV) have been reported and the number of alleles is still growing. Many genetic
polymorphisms within the CYP450 superfamily result in altered enzyme expression
or activities, significantly effecting drug metabolism. Based on the genotypes of the
CYP450 enzymes, patients may be categorized as ultra-rapid metabolizer (UM),
extensive metabolizer/normal metabolizer (EM), intermediate metabolizer (IM), and
poor metabolizer (PM). Even though the value of routine pharmacogenetic testing
is still debated, accumulated evidence indicates that genotyping drug-metabolism
enzymes will help physicians to tailor pain treatment to individual patients.

Methods and Materials: DNAs were extracted from buccal swabs collected from 200
patients prescribed pain medications. Five genes in CYP450 superfamily: CYP2D6,
CYP2C19, CYP2C9, CYP3A4, and CYP3A5, were genotyped. Genotypes of the 5
genes were reported using the star (*) allele nomenclature. 16 alleles (*2, *3, *4,
of CYP2C9, 6 alleles (*1B, *2, *3, *12, *17, *22) of CYP3A4, and 8 alleles (*1D,
*2, *3, *1B, *6, *7, *8, *9) of CYP3A5 were tested. Allele *1 is assigned by default
when no other listed alleles are detected. Phenotypes were assigned based on the
guidelines from the clinical pharmacogenetics implementation consortium (CPCIC)
and/or published general rules: 1) UM: more than 2 copies of functional alleles;
2) EM: 2 copies of functional allele, or 1 copy of functional allele and 1 copy of
decreased functional allele; 3) PM: 2 copies of non-functional allele, or 1 copy of
non-functional and 1 copy of decreased functional alleles; 4) PM: 2 copies of non-functional alleles; 5) PM: 2 copies of non-functional alleles.

*11, *17, *41) in CYP2D6, 3 allelic variants (*2, *9, *17) in CYP2C9, 2 allelic variants
(*2, *3) in CYP2C9, 3 allelic variants (*1B, *3, *22) in CYP3A4, and 4 allelic
variants (*1D, *2, *3, *6) in CYP3A5 were detected. The most common
polymorphisms and phenotypes are: 1) for CYP2D6: *2 (*2, *12) (EM, 20%), *1D (EM,
13.5%), and *1D/A1 (EM, 10.5%); 2) for CYP2C9: *1 (*1, *21) (EM, 38.5%), *1/*2
(EM, 27%), and *1/*2 (EM, 19%); 3) for CYP2C9: *1/*1 (EM, 67.5%), *1/*2 (EM, 22.5%)
and *1/*3 (EM, 8%); 4) for CYP3A4: *1/*1 (EM, 80.5%), *1/*1B (EM, 10%); and
*1/*22 (EM, 4.5%); and 5) for CYP3A5: *3/*3 (PM, 76.5%), *1/*3 (IM, 16%), and
*3/*6 (PM, 1.5%).

Conclusions: The frequencies of allelic variants and genotypes we found in the five
genes are in accordance with previously published results. In our targeted population,
the most common allelic variant found was the non-functional *3 allele in CYP3A5,
with a frequency of 86%.

Development of an LNA-Blocker enhanced, allele-specific, loop-mediated
isothermal amplification (AS-LAMP) method for detection of single base
mutations in beta-thalassaemia patients

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Background: It was found that five mutations, namely 654M, 41/42M, 28M, 17M
and 27/28M, are the most commonly occurring single base mutations in beta-thalassaemia
patients in China. Being able to detect and distinguish these mutations is important
for patient screening and for diagnosis of the disease. The loop-mediated isothermal
amplification (LAMP) method is known to be a rapid (complete in less than 45 min)
and simple way for detection of gene deletion and insertion events, but is not specific

enough to distinguish single base difference. This study was aimed to improve the sensitivity of LAMP method on single base mutation detection in beta-thalassemia patients by using Locked Nucleic Acid (LNA) modified primers in the reaction.

**Methods:** The LNA-Blocker enhanced, allele-specific, loop-mediated isothermal amplification (AS-LAMP) was carried out under isothermal condition. To enhance the specificity of AS-LAMP, an LNA modified allele-specific primer and an LNA modified wild type blocking primer were used in the reaction. LNA is a high affinity DNA analogue that has increased target specificity and high melting temperature. This method was used to detect and distinguish single base mutations in beta-thalassemia patients. This method was validated with positive and negative controls and with 145 clinical samples of patient genomic DNA (46 positive samples containing all 5 mutations and 99 negative samples).

**Results:** The LNA-Blocker enhanced AS-LAMP method showed high specificity with either plasmid or genomic DNA targets in reactions. The assay had a detection limit of approximately 100 copies of the target sequence (95.7% in sensitivity, 44/46), comparable to the traditional AS-LAMP. However, the specificity of LNA-Blocker enhanced AS-LAMP (96.0%, 95/99) was much higher than the traditional AS-LAMP (75.8%, 75/99). Multiplex detection of all 5 targets was also achieved with LNA-Blocker enhanced AS-LAMP.

**Conclusion:** LNA-blocker enhanced AS-LAMP provides a highly specific isothermal method for detection of single mutations and for screening of single mutations in beta-thalassemia patients.

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**Detection of fetal aneuploidies by quantitative fluorescent polymerase chain reaction in the Brazilian population**

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**Background:** Aneuploidy occurs from non-disjunction during gametogenesis and results in an abnormal number of chromosomes in the gametes. The gamete joined in fertilization contains an extra copy of one chromosome or one of the chromosomes is missing. These alterations can result in trisomy or monosomy in the fetus or embryo which are frequently associated with severe congenital abnormalities, mental retardation and shortened life expectancy. The majority of first trimester abortion cases is caused for aneuploidies. Quantitative fluorescent polymerase chain reaction (QF-PCR) is a method which has been regularly used for the diagnosis of common chromosomal abnormalities in recent years with low error rates. Objective: To determine the frequency of the most common chromosomal aneuploidies causing abortion in Brazilian population. Methods: The study is the retrospective statistical analyses of data registered on 70 women submitted to molecular studies of chromosome disorders at the Hermes Pardini Institute, Belo Horizonte, Minas Gerais state, Brazil in 2013. All patients had spontaneous abortion and the ovular remains, embryo tissue were analyzed after the DNA extraction. Was compared the genetic profile of the sample with the maternal blood, a differential that allows to detect maternal contamination in the sample, improving the reliability of the results. Contaminated samples were excluded from the study. After that all samples were tested using quantitative fluorescent polymerase chain reaction (QF-PCR) for detection or exclusion of aneuploidy in chromosomes 13, 15, 18, 21, 22, X and Y (Chomo Quant® QF-PCR Kits). Results: The age average of the patients was 34.4 ± 4.7 years old. We detected 44 subjects (62.9%) with aneuploidies or euploidies.

Nine cases of trisomy 15 (12.9%), nine cases of trisomy 22 (12.9%), seven cases of monosomy X (10.0%), four cases of trisomy 13 (5.7%), four cases of trisomy 16 (5.7%), four cases of trisomy 21 (5.7%), five cases of trisomy 21 (7.1%), a case of trisomy 18 (1.4%) and a case of paternal isodisomy (1.4%) were detected by QF-PCR. Conclusions: Quantitative Fluorescent PCR is a robust and accurate method for rapid aneuploidy detection. One the first suspicions of spontaneous abortions is numerical abnormalities in 13, 18, 21 and sex chromosomes, representing 22.8% of our cases of aneuploidy. But the results show the importance of also being analyzed changes in chromosomes 15, 16 and 22 which concentrated 31.5% of the aneuploidies detected. We are going to analyze a larger number of samples in order to confirm the importance of study chromosomes 15, 16 and 22 disorders.
Background: OpenArray® is a new PCR technology that uses a microscope plate and allows the detection of many SNPs at the same time.

Objective: We have used this technology to describe the genotypic frequencies of some SNPs related to venous thrombosis (G1691A and G20210A), to hyperhemocistinemia conditions (C677T, A1298C), and to hereditary hemochromatosis (C282Y, H63D and S65C), among Brazilian blood donors.

Methods: We tested 400 blood samples from Fundação Pró-Sangue Hemocentro of São Paulo, Brazil. The DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA concentration and quality was determined by spectrophotometry method using NanoDrop 2000 (Thermo Scientific, Wilmington, DE). The OpenArray platform (Life Technologies, Foster City, CA) was used to genotype the samples through TaqMan Genotyping detection system. The reproducibility of this method was evaluated running nine samples in triplicate in three different assays. The DNA samples were also tested using TibMol (TibMol, Berlim, Germany) reagents at Light Cycler 2.0 (Roche) plataform, with FRET as detection system.

Results: Of 400 samples, 392 showed valid results. The results showed agreement of 100% for all assays tested, exception to HFE C282Y (95.5% agreement). The only discordant sample result for HFE C282Y was confirmed by direct sequencing which showed that OpenArray result were correct. The calculated frequencies of each SNP discordant sample result for HFE C282Y was 60.3% (A/A), 33.6% (A/C), 6.1% (C/C); HFE C282Y 96.0% (G/G), 4.0% (G/A), HFE H63D 78.1% (C/C), 20.3% (C/G), 1.6% (G/G); HFE S65C 98.1% (A/A), 1.9% (A/T).

Conclusion: The Open Array genotyping method was used to detect simultaneously 7 different mutations in thrombophilia, folate and hemochromatosis related genes. Compared to other genotyping methods such as PCR-RFLP and sequencing, this method is, easy to perform and useful for high-throughput routines. The results found describe the frequency of SNPs related to diseases not well established by previous literature for Brazilian population. They are important to highlight the genetic profile of Brazilian blood donors.