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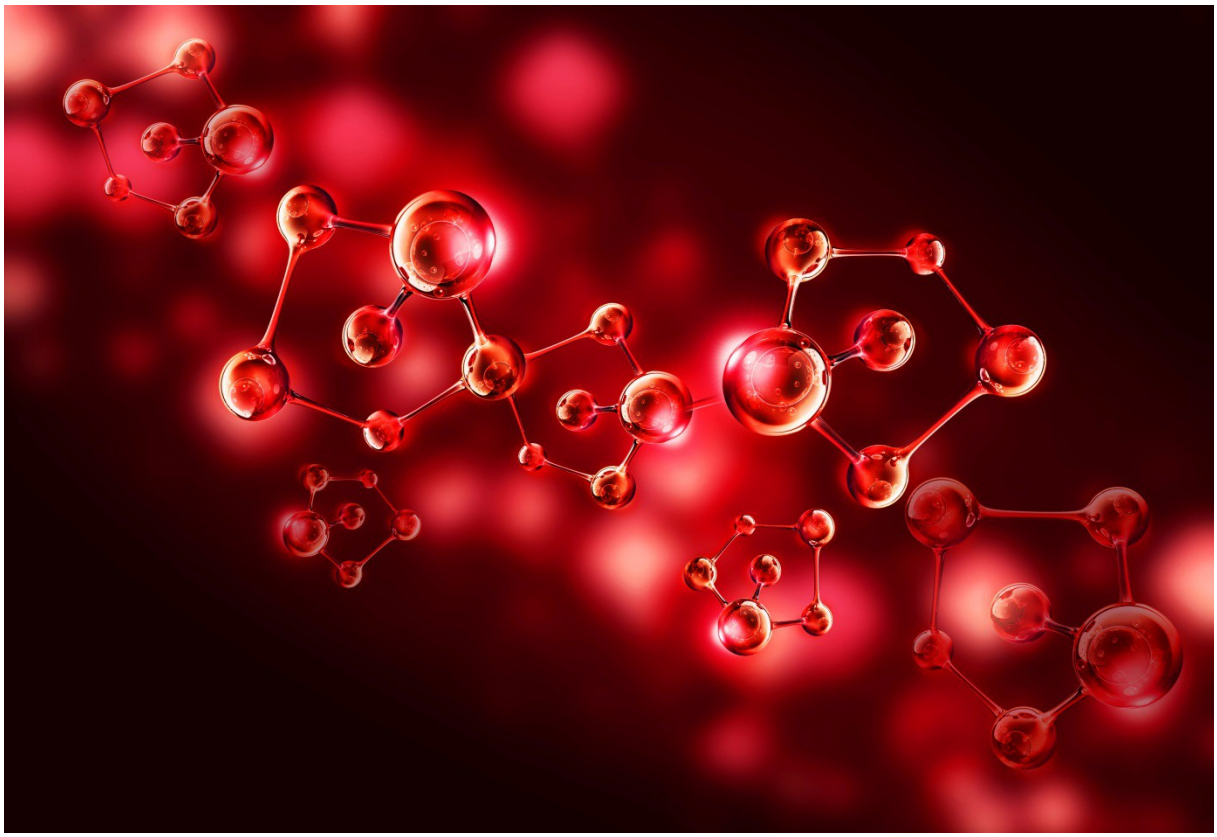

ABSTRACT BOOK 2018

Bachelor's and Master's Theses in Biomedical Laboratory Science

Table of Contents

Bachelor´s Theses:

Fondin, Erika
Regularly intake of resveratrol could improve the cardiovascular profile

Fridell, Ylva
Expansion and detection of antigen specific T cells from human blood

Hassanpour, Alan
Optimization of biochemical quantification method on HVA, VMA and 5-HIA in urine with LC-MS/MS

Jansson, Fredrik
Analysis of NF-κB activation by TNFα and TLR stimulation with bacterial ligands LPS, Pam3CSK4 and CpG-DNA

Johansson, Karoline
Isolation of circulating cell-free tumour DNA in plasma from patients with Non-Small Cell Lung Cancer - Prior to analysis with Next Generation Sequencing

Kaltak, Aida
Upregulation of two cellular glycosyltransferases in herpes simplex virus type 1 infected fibroblasts

Karlsson, Louise
Aggregates in whole blood bags gives a negative effect on platelet counts and platelet function but has no visible effect on Factor VIII

Larsson, Julia
No difference between accessory pathways antegrade electrical characteristics in asymptomatic and symptomatic patients

Lindahl, Malin
Lung function decreases as a consequence of smoking; A convenience study of students and staff at Al-Quds university

Ly, Lili
Circulating rotavirus genotypes in Västra Götaland between 2015-2017

Martinsson, Amanda
Quantification of inflammation and rejection estimation in transplanted human uterus

Mccormick, Anna
A Screening Assay to Measure the Sensitivity of Fanconi Anaemia Patients' Cells to Cancer Therapy

Moore, Julie
Genetic variability of hepatitis E virus ORF3 gene on the host immune response against the virus

Mrozek, Amilia
Risk-stratification of coronary artery disease with sound analysis of coronary artery blood flow. A comparative study against stress-ECG, stress-echocardiography and SPECT

Mårtensson, Frida
Differentiation between *Shigella* and Enteroinvasive *E. coli* (EIEC) with primers and probes aimed at sequences in *Shigella sonnei, Shigella flexneri, Shigella boydii* and the *lacY*- and *ipaH*-genes

Nehmé, Mohammad
Investigating the role of microparticles in type I Interferon gene regulation in Systemic Lupus Erythematosus

Niklasson, Sofia
Identified *Staphylococcus argenteus* among isolates identified as *S. aureus* in blood cultures

Norling, Henrietta
Fiber composition improves intestinal cell renewal, in mice after radiation

Pearson, Nicolina
Mitochondrial Lon protease degrade mitochondrial transcription factor A (TFAM) by binding within high mobility group 1 (HMG1)

Persson, Marie
Environmental bacteria from aquatic environments shows no indication for antibiotic resistance using phenotypical analysis and molecular methods

Pokorny, Anna Maria
Molecular diagnostics of fungi - Evaluation of specificity and sensitivity of PCR assay and preparation of Next Generation Sequencing for detection of fungal DNA directly in human whole blood performed with universal primers derived from the eukaryotic rRNA operon

Robakowska, Dorota
Analysis of ASNA-1 function in *C. elegans* – Study of interactions between ASNA-1, ENPL-1 and syntaxin

Schmidt, Patrik
Validation of biomarkers for clinical identification of *Streptococcus pseudopneumoniae*

Slupecki, Kamil
Validation of an LC-MS/MS method for detection of amphetamines and amphetamine-type stimulants in urine

So, Felix

Cloning, expression and characterization of disease-associated $POL_{\gamma}A$ mutants involved in mtDNA replication

Svensson, Charlotta
A comparative study between Modified Giemsa and alcian yellow-toluidine blue stains for the detection of *Helicobacter pylori* in digitalised vetnricle biopsy slides

Tekcan, Merve
Identification of *Haemophilus* and strict anaerobic bacterial isolates to the species level in premature infants compared to caesarean section and vaginal delivery

Tulius, Ingeborg Eugenia
Platelet count in the platelet concentrate for transfusion varies depending on analysis instruments, while the content of ABO antibodies is low

Vesik, Evelina
Intake of resveratrol improves cardiovascular health in healthy, young adults

Von Brömsen, Julia
No correlation between non-invasive strain parameters and the invasive PCWP in heart transplant recipients

P2Y12 RECEPTOR EXPRESSION DOES NOT CHANGE DURING STORAGE IN ASSOCIATION WITH TRANSFUSION.

By Anna Alehed

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2018

Supervisor: Camilla Hesse, Senior Lecturer

Introduction: Platelets are small anucleated discoid-shape blood cells which plays a key role in homeostasis. The normal number of platelets in the human body is between 150-400x10^9/L. Platelets originate from fragmenting megakaryocytes in the bone marrow and acquire RNA and translation machinery from it. The usages for platelets in transfusion medicine are numerous for example treatment against thrombocytopenia. Platelets can be stored up to seven days. During storage platelet storage lesion (PSL) occur, which damages the cell. The P2Y12 receptor present in the platelet is responsible for the irreversible last steps of aggregation and gets activated by ADP. Studies has shown a reduced responsiveness for ADP in stored platelets due to PSL.
Aim: The aim of this study is to analyze the changes in P2Y12 receptor expression in platelets throughout storage and try to establish a quantitative method with PCR and Western Blot.
Method and material: Concentrated platelets and freshly drawn blood where utilized for real-time PCR and Western Blot. A modified protocol for platelet rich plasma was used for preparation of the platelets followed by protein or RNA extraction. RT-PCR was conducted with the prepared RNA and a TaqMan based probe for the P2Y12 receptor. An endogenous control of GAPDH was also used. Platelets were prepared for protein extraction on day 1,2,3 and 6 of storage for protein analysis with Western Blot. For the detection of the P2Y12 receptor protein an anti-P2Y12 mono- and polyclonal antibody were used.
Result: The RT-PCR detected no P2Y12 in the prepared sample. The Western Blot for the monoclonal antibody showed an evenly colored band around ~60kDa in all wells except for the one prepared from freshly drawn blood. The polyclonal antibody detection failed.
Conclusion: The study shows that there are no changes in platelet receptor P2Y12 expression throughout storage. Further studies are needed to modify a working RT-PCR method and to confirm changes of platelet receptor P2Y12 expression.

Genotypic Characterization and Identification of Clinically-Relevant Strains of *Corynebacterium "genitalium"* and *Corynebacterium*"*pseudogenitalium*" by DNA Sequence Analyses

Shoroq Al-Shaer
Biomedical Laboratory Science Program

Supervisors:
Dr.Daniel Luchoro
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Prof. Dr.Edward Moore
Director and Curator at the CCUG

Institute of Biomedicine
Sahlgrenska Academy

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Abstract

Background :

Corynebacterium "genitalium" and *Corynebacterium "pseudogenitalium"* were defined as species of Corynebacterium for the first time by Furness and Evangelista in 1978 and Furness in 1979,respectively.These two species were included within the *Corynebacterium* genus and preliminarily differentiated from the other corynebacteria species, based on colony morphology and metabolic features.However, morphology and phenotypic tests were not enough to define and identify these species accurately. This study aims to perform sequence analysis of PCR-amplified 16S rRNA gene and *rpo*B to identify strains of *Corynebacterium* spp. that are expected to be *C. "genitalium"* and *C. "pseudognitalium"* by the phenotypic analyses.

Materials and methods:

PCR amplifications were carried out for partial 16S rRNA genes and partial RNA polymerase subunit B genes (*rpo*B) of 34 clinical and industrial isolates.PCR products were purified and sequenced, using Sanger DNA sequencing technology. Alignment analysis of the assembled sequences was done with reference sequences of *Corynebacterium* spp. The similarity percentages between the sequences of the strains and the species were calculated and phylogenetic trees were created for both of the genes, with all of the *Corynebacterium* spp. reference sequences. Antibiotic sensitivity tests also was done for most of the strains.

Results:

In cases of most of the strains ,the percent similarities based on the partial *rpo*B sequences were lower than those observed by the partial 16S rRNA gene sequences. Similarity percentages of both 16S rRNA gene and *rpo*B sequences confirmed that 10 strains are *C.*"*genitalium*" and 4 strains are *C.appendicis*. Some strainsclustered together and distant from the established *Corynebactrium* species in both 16S rRNA gene and *rpo*B phylogenetic trees ,which indicates that they have the same genotypic characteristics. One strain has high sequence similarities with strains representing novel species and also exhibits distinctive antibiotic sensitivity results.

Conclusion:

Diversity among *Corynebacterium* species is difficult to be observed by the conventional tests and phenotypic features. Therefore, the molecular-based, genotypic analytical approaches are more reliable for the classification and identification of the species. Phylogentic studies of species of *Corynebacterium* are better to be carried out, using 16S rRNA gene and *rpo*B sequencing in combination together.

Abstract

Development of a cheap and reliable molecular tool set

By: Jesper Arnström

Bachelor thesis in Biomedical Laboratory Science performed at the School of Biological Sciences, Dublin Institute of Technology, 2018. Supervisor: Dr. Fergus Ryan

Background: Molecular tools, such as DNA polymerases and DNA molecular weight markers, or DNA ladders, are essential for both diagnostic and research purposes. Their applications include amplification of DNA in PCR, and molecular size estimation of DNA. Commercially available products are of high quality but come with great expenses because of the ubiquitous use of these molecular tools. In a world with increasingly limited funding, it could be economically viable for research laboratories to produce a home-made molecular tool set consisting of *taq* DNA polymerase and DNA ladders. To minimize non-specific amplification in PCR, commercially available *taq* polymerases often come with a monoclonal antibody to inhibit the enzyme's activity at room temperature. The use of oligonucleotides, known as aptamers, as inhibitors could have similar effect but be more cost efficient than the use of an antibody in "hot-start" PCR. **Objective:** To explore the possibilities of producing *taq* polymerase and DNA ladders, as cheap and reliable alternatives to commercially available products. Also, the inhibitory effects of aptamers on *taq* polymerase for use in "hot-start" PCR will be explored. **Methods:** Recombinant *taq* polymerase was produced, purified and used in standard PCR assays, in the presence and absence of aptamers. The pPSU plasmids were purified and cut with restriction enzyme to produce 100bp DNA ladders. **Results:** The recombinant *taq* polymerase produced showed high quality and specificity. The aptamers increased PCR products from low DNA concentration samples but no decrease in non-specific amplification was observed. The 100bp DNA ladders produced from the pPSU plasmids were of high enough quality to be used for molecular size estimation of DNA. **Conclusion:** The recombinant *taq* pol was successfully purified and the molecular size marker generated. Developing a reliable, home-made molecular tool set comes at negligible costs compared to buying commercially available products. Aptamers could potentially improve the yield of products in "hot-start" PCR.

Sample preparation method affects the outcome of quantifications of genetic aberrancies in myeloid leukemia

By Jennifer Backman

Bachelor thesis in Biomedical Laboratory Science performed at the Section of Gene Analysis, Department of Clinical Chemistry, Sahlgrenska University Hospital and the Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, 2018

Supervisors: Linda Fogelstrand, MD, Associate Professor. Erik Delsing Malmberg, MD. Anna Björquist, Molecular Biologist

Background. The assessment of Minimal Residual Disease (MRD) allows a detection of residual leukemic cells with high sensitivity. Clinical implication of MRD is of considerable interest and the development of useful techniques is highly warranted. **Aim.** The aim of this study was to compare results from reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) of AML/CML specific genetic aberrancies in samples prepared in two different ways; using lysed whole blood/bone marrow and enriched mononuclear cells from blood/bone marrow. **Method.**We used RT-qPCR assays to detect and quantify specific markers in AML/CML patients bearing myeloid leukemia markers. The samples were prepared in parallel using the following methods; whole blood/bone marrow after lysis of erythrocytes versus separated with density centrifugation for enrichment of mononuclear cells. TotalRNA was extracted following both methods whereupon the totalRNA was converted in to complementary DNA (cDNA). The aberrations of interest were amplified and subsequently compared to the amount of reference gene transcripts. The ratio of fusion transcripts and the MRD assignments for the lysed whole blood and the enriched mononuclear cells were compared. **Results.** In samples from patients with CML, there was a significant difference between the levels of MRD obtained from cells from lysed whole blood compared to isolated mononuclear cells. For samples from patients with AML, the number of positive samples was too low to allow for a reliable comparison, although there was no apparent difference between the two methods. **Conclusion.** In CML, usage of lysed whole blood allows for more sensitive quantification of MRD compared to enriched mononuclear cells.

Abstract:

Tick borne encephalitis virus infection increases expression of fucosyltransferase FUT6

By: Arman Bahari

Bachelor thesis in Biomedical Laboratory Science performed at the laboratory of Microbiology, Sahlgrenska Academy, University of Gothenburg, 2018.
Supervisor: Kristina Nyström

Tick borne enchephalitis virus (TBEV) is a flavivirus, encased in a lipid envelope with a diameter of about 40-60 nanometers. Throughout the 20th century and into the 21st, it has spread across europe and asia. The tick vectors spread the virus to human and animal hosts, and most cases are reported during tick season in the summer. In Sweden, the number of cases have increased exceptionally in the last three decades. Between years 2016 and 2017 the number of annual cases increased from 238 to 391, an increase of 64%. The aim of this study was to increase understanding of the viral patogenesis mechanisms, by infecting cell cultures of lung epithelial cells, T-cells, and monocytes, and analysing the expression of fucosyltransferases FUT1, FUT3, FUT5, FUT6, and FUT7. All three types of cells were successfully infected with TBEV, and it was found that infection increase expression of FUT6 in monocytes and lung epithelial cells.

Unraveling the role of peripheral dopaminergic signaling in the liver

By: Fanny Bodin

Bachelor thesis in Biomedical Laboratory Science at the faculty of medicine, University of Coimbra, Coimbra, Portugal, 2018.

Supervisor: Paulo Matafome

Introduction: The prevalence of obesity and its comorbidity, diabetes type 2, are reaching epidemic proportions worldwide leading to financial burdens, with high social impact. Therefore, it is urgent to find therapeutic strategies to overcome this health problem. Recent studies have shown that dopaminergic signaling plays an important role in metabolism, although the mechanism is yet to be fully understood. Liraglutide and Sleeve gastrectomy are two common therapeutic strategies in order to treat diabetes type 2. Liraglutide is an analogue to the incretin GLP-1 mimicking its effect by binding to its receptor. Sleeve gastrectomy is a surgical procedure that remodels the gastrointestinal tract. It has been observed not only to promote weight loss but also to improve insulin resistance and GLP-1 secretion in diabetic patients, although the full mechanisms involved are yet to be discovered.

Aim: We hypothesized that dopaminergic signaling in insulin-sensitive tissues could be modulated by the GLP-1, one of the most important incretins. Thus, the aim of this study was to discover if dopaminergic machinery signaling is altered in the liver, after submitting the animals to one of the therapeutic treatments including Liraglutide and sleeve gastrectomy.

Methods: Two different animal models were included in this study. The first animal model included to treat normal and type 2 diabetic Goto-Kakizaki (GK) rats with Liraglutide, two times a day during two weeks (200 μg/Kg). In the other animal model, high-fat diet-induced obese rats (6 months old) were submitted to sleeve gastrectomy. The animals were then sacrificed and the livers collected. To analyse dopamine signaling machinery Western Blot was performed, further, the protein bands were detected by using ECL substrate.

Results: In the animal model of Liraglutide a significant increase was observed in GK-control compared to the Wistar-control group (WC) ($p<0.001$) and GK-Liraglutide (GKL) ($p<0.05$) considering the expression of dopamine 2 receptors (D2R). In the rats of the sleeve gastrectomy model, a significant increased difference was observed in the GK-high fat diet group submitted to sleeve gastrectomy (GKHFD_Sl) compared to Wistar rats ($p<0.01$), the GK-High fat diet group (GKHFD) ($p<0.05$) and GKHFD submitted to sham surgery (GKHFD_Sh) ($p<0.01$) concerning DARP32-T expression levels.

Conclusion: This study indicates that treatment of diabetic rats with Liraglutide decreases the expression of D2R, promoting a balanced distribution between D1R and D2R, restoring its levels to the one in a normal state. Moreover, the animals submitted to sleeve gastrectomy revealed a high expression of DARP32-T levels suggesting a higher catabolic activity after the treatment that could be modulated through dopamine action.

PROTEOMICS AND qPCR ANALYSIS REVEAL *INS-35* AND *COPE-1* AS LIKELY MODULATORS OF ASNA-1/ENPL-1 INTERACTION IN *C. ELEGANS*

By Alexandra Boman

Bachelor thesis in Biomedical Laboratory Science performed at Sahlgrenska Cancer Center, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Gautam Kao, Researcher. Co-supervisor: Agnieszka Podraza, PhD-student

Analysis of *C. elegans* gene expression has been used for several decades as a powerful tool to study human diseases, such as diabetes, due to the homology between genes in humans and *C elegans*. Insulin, hormone expressed in pancreatic cells, is of great importance in the research involving diabetes, because of its function in regulation of metabolism. *C.elegans* genome encodes 40 insulins and two of them *daf-28* and *ins-4* have been widely studied. The aim of this project was to investigate if any other insulins were upregulated when *daf-28* and *ins-4* are mutated together, in order to find possible insulins which can substitute the function of mutated insulins. In addition, insulin gene expression in each single mutant *daf-28* and *ins-4* was studied to investigate if the upregulated expression was only specific to the double mutants. Genes associated with dense core vesicles like *unc-31* and *ida-1* were also studied in this project due to previous findings that both genes affect insulin secretion and ASNA-1/ENPL-1 interaction. ASNA-1 and ENPL-1 are two proteins important for insulin secretion. Earlier research has shown that these two proteins bind to each other in a *daf-28* dependent manner. One aim for this study was to investigate the potential interacting proteins that bind to ASNA-1. Further investigation was done with *mev-1* mutant, that has high oxidative stress level, to study the properties of ASNA-1 during oxidative stress conditions.

In this project, gene expression was investigated with qPCR technique using *f44* as reference gene. For *daf-28/ins-4* double mutant, results showed eight genes upregulated in expression: *ins-6, ins-10, ins-13, ins-16, ins-20, ins-22, ins-29* and *ins-35. ida-1* and *unc-31* showed no changes in expression. In *daf-28* single mutant, the results showed seven genes downregulated in expression: *ins-6, ins-10, ins-13, ins-16, ins-20, ins-29* and *ins-35.* In *ins-4* single mutant, the results were inconclusive. Analysis of ASNA-1 was performed using western blot and immunoprecipitation. Eluted protein sample was analyzed by Proteomics Core Facilities. Proteomic approach revealed presence of two proteins that strongly interact with ASNA-1 - TBH-1 and COPE-1. In conclusion, *ins-35* expression had upregulated expression in the *daf-28/ins-4* double mutant, but downregulated in the *daf-28* single mutant. To further understand the role of *ins-35* insulin regulation/secretion, additional investigations are now underway. *mev-1* single point mutant promotes oxidation of ASNA-1, and that is consisted with previous finding that ASNA-1 binds to ENPL-1 when oxidized. A proteomics approach revealed TBH-1 and COPE-1 as likely ASNA-1 interactors. This could imply that ASNA-1 interacts with ENPL-1 in the Golgi.

Enantiomeric separation and quantification of R/S-amphetamine in oral fluid using chiral liquid chromatography coupled with tandem mass spectrometry

By Angelica Borg

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Chemistry, Diagnostic Mass Spectrometry & Chromatography, Sahlgrenska Academy, University of Gothenburg, 2018

Supervisor: Moa Andresen Bergström (PhD), Magnus Axelsson (MD, PhD)

Background: Amphetamine (AM) is a central nervous system stimulant that is abused worldwide. It is also used for treatment of medical conditions such as ADHD. AM exists in two chiral forms, *R*- and *S*-AM. The majority of prescribed AM consists of primarily of 100% *S*-enantiomer and the illegal material consists of a racemic mixture of the two enantiomers. To distinguish between legal and illegal consumption, separation of the *R*- and *S*-enantiomers is required. This can be achieved by using a chiral analytical separation method. The aim of the project was to develop and validate a method based on chiral LC-MS/MS to determine the ratio and concentrations of the *R/S*-AM in oral fluid for fast detection of illegal use of AM.

Method: Authentic oral fluid samples were obtained from six patients. The sample preparation method is based on the salting-out liquid-liquid extraction (SALLE) principle. The chromatographic method used a Lux® AMP column with a mobile phase consisting of 0.1 M Ammonia in H_2O pH 11 (A) and Methanol (B). The injection volume was 1 µL and the total run-time was 7 min. Analyte detection was accomplished using positive electrospray ionization and multiple reaction monitoring transitions (m/z 136.1 > 91.0 and m/z 136.1 > 119.0). The method was validated according to the Clinical Chemistry department guidelines at Sahlgrenska University Hospital.

Results: The calibration range was 2.5-2500 ng/mL for each enantiomer. Concentrations of the calibration samples were within ±5 % of the target concentrations (±7% at LLOQ). The within-assay precision and between-assay precision were ≤1% and ≤1.3% respectively. The method accuracy was determined by analysis of external controls samples. Unknown samples obtained from drug abusers and patients receiving prescribed *S*-AM were also tested. The mean *R/S*-ratio for illicit racemic AM intake and patients medicating with AM was 1.14 and 0.02 respectively.

Conclusion: A robust, precise and fast LC-MS/MS method for enantioselective separation and quantification of *R*- and *S*-AM in oral fluid was developed and validated. The method showed excellent accuracy and a very low variation. The measured *R/S*-AM ratios proved very useful for discrimination between subjects using illicit racemic AM and those undergoing *S*-AM treatment. The method has a simple sample preparation that does not involve time-consuming derivatization step.

Routines for specimen handling of tumor tissues
-Quantitative and qualitative effects regarding results of molecular pathology analysis

By Linnéa Brauer

Bachelor thesis in Biomedical Laboratory Science performed at the department of Pathology and Genetics, Sahlgrenska Academy, University of Gothenburg, 2018.
Supervisor: Katarina Junevik, PhD

Background: Nucleic acids (DNA and RNA) from tumors can generate supportive information about cancer diagnosis and choice of cancer treatment. The nucleic acids are analyzed with techniques used by the "Molecular pathology" laboratory, such as Polymerase Chain Reaction (PCR), realtime-PCR (qPCR), sequencing by Sanger or Next Generation Sequencing (NGS). However, it is well known that the nucleic acids are very unstable, especially RNA, and are easily degraded and fragmented if not handled properly. One critical point is the preserving step of the tissue. The two most common preserving and fixation methods are "formalin-fixed and paraffin-embedded" (FFPE) and "fresh-frozen" method in -80°C. The FFPE-method is easier to manage but does not generate as high quality and quantity on the nucleic acids as the "fresh-frozen" method. There are other methods for preparing and fixation of tissues but they are not as commonly used in clinical laboratories, such as fixation in RNA*later*™ Stabilization Reagent. This method could be an alternative to the fresh-frozen if it generates high quality and quantity nucleic acid.
Aim: The aim of the study was to investigate how the three different methods for sample preservation and fixation, "formalin-fixed and paraffin-embedded", fresh-frozen and tissues fixed in RNA*later*, affected the obtained RNA-concentration and quality of molecular pathology analysis.
Method: Tumors from three patients were prepared with three different fixation methods 1) FFPE, 2) fresh-frozen and 3) in RNA*later*. RNA was extracted and the quantity and purity was measured by NanoDrop (spectrophotometry) and Qubit (fluorescense). The quality of the extracted RNA was analyzed by Reverse Transcriptase-qPCR and the obtained Cq-values gave a measure on RNA integrity and fragmentation.
Result: The mean concentration of extracted RNA from FFPE was 81,9 ng/µl, 166,1 ng/µl from fresh-frozen and 339,3 ng/µl from RNA*later*. The mean Cq-value for GAPDHv2(89 bp) was 28,5 for FFPE samples, 18,7 for fresh-frozen and 17,9 for RNA*later*.
The Cq-value for RPs10v2 (177 bp) was 33,5 for FFPE, 18,5 for fresh frozen and 17,0 for RNA*later*.
Conclusion: There was no statistic significant difference between the three methods due to the low number of samples, however a trend could be seen between the three methods. The trend indicates that the FFPE method is not as suitable as fresh-frozen and the RNA*later* in obtaining high RNA yield or quality. RNA*later* seem to provide higher yields than the fresh-frozen method but gives just as good quality on the RNA.

Myocardial perfusion imaging with D-SPECT in reconstruction with 8 and 16 frames and echocardiography gives equivalent assessment of left ventricular volumes and ejection fraction

By Hanna Carlsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2018.

Supervisors: Jehangir Khan (M.Sc) and Tobias Rydén (PhD)

Background. Accurate measurement of left ventricular ejection fraction (LVEF) is important in the diagnosis and determination of coronary heart diseases. Many times, cardiac magnetic resonance is considered as gold standard for measurement of LVEF but there are more techniques that can measure and validate LVEF. Gated myocardial perfusion, dynamic single photon emission computed tomography (D-SPECT) is recommended in the diagnosis of ischemic heart disease, and assessment of LVEF can improve diagnostic accuracy in these patients. Previously comparative studies have been made to evaluate left ventricular systolic performance in the different imaging techniques. This is important to know how the methods are reliable. **Aim.** The aim of the study was to compare quantitative measurements of left ventricular volumes and ejection fraction in images from echo and D-SPECT. Additionally, the same comparisons were made between myocardial perfusion imaging (MPI) in reconstruction with 8 and 16 frames. **Method.** 24 patients underwent echo and MPI within one month. Reconstruction of the images from MPI was made in Processing Station (Spectrum Dynamics Medical) and left ventricular volumes assessed using the quantitative perfusion SPECT software (QPS, Cedars-Sinai Medical Center). For measurement of end diastolic and systolic volume and left ventricular ejection fraction (LVEF) from echo Simpson method was used. To compare all the different values Pearson correlation, p-values and Bland-Altman plots were made. **Result.** The result showed significant concordance between the techniques. For LVEF, both MPI with 8 frames vs TTE and MPI with16 frames vs TTE Pearson correlation were 0.73 (p-value <0.001) Additionally, comparison between reconstruction of MPI data divided in 8 and 16 frames represents correlation between 0.9 to 1 (p-value <0.001 to 0.03). **Conclusion.** There is a strong correlation between the techniques which shows that MPI with D-SPECT in the different reconstructions and echo gives equivalent values for left ventricular volumes and LVEF.

REGULARLY INTAKE OF RESVERATROL COULD IMPROVE THE CARDIOVASCULAR PROFILE

By: Erika Fondin

Bachelor thesis in Biomedical Laboratory Science performed at Escola Superior de Tecnologia da Saúde de Coimbra - Instituto Politécnico de Coimbra, Portugal 2018
Supervisor: Telmo Pereira and Bente Gruener Sveälv

Background: Previous studies shows that rich resveratrol-diet decreases the risk of getting cardiovascular diseases. The aim of this study was to evaluate if a long-term intake of resveratrol could have positive effects on the heart´s structural and functional parameters.

Methods: The study was a randomized, single blinded and controlled clinical trial. Comparing two groups, one group which recived resveratrol and one group reciving placebo pills. Baseline evaluations were performed by echocardiography and ventricle-arterial coupling exams. Furthermore, the participants started an intake of 100 mg resveratrol per day and after 30 days the evaluations was repeated.

Results: Evaluations of baseline measurements showed no statistical significance between the groups and the values were within a normal range. No significant structural or functional changes of the heart were observed from baseline to post-intervention in neither of the groups. However, a positive trend could be seen for the parameters of Doppler and tissue Doppler imaging. There were significant changes within the group of intervention for the VAC parameter compare to the control group. The VAC mean difference were 0.15 with a $p<0.001$ for the intervention group.

Conclusion: This study shows an improvement of the vascular parameters that could be connected to improvement of the heart after intake of resveratrol. Therefore, regularly intake of resveratrol could be a good preventive approach for cardiovascular diseases, but further research is needed.

Expansion and detection of antigen specific T cells from human blood

By Ylva Fridell

Bachelor thesis in Biomedical Laboratory Sicence performed at Sahlgrenska Cancer Centre, Institute of Biomedicine, University of Gothenburg, 2018
Supervisor: Anna Martner PhD, Annica Andersson PhD

Background: Antigen-specific cytotoxic T cells are a critical component of the adaptive immune system, that contribute to the elimination of virus infected cells and cancer cells. In general, the number of antigen-specific T cells in blood is very low, and they are therefore difficult to detect in the laboratory.

Aim: The aim of this project is to optimize protocols for the expansion and detection of virus-specific cytotoxic T cells from human blood. The protocols may in the future also be utilized for the expansion and detection of rare populations of tumor-specific cytotoxic T cells.

Method: To stimulate an expansion of virus-specific T cells, peripheral blood mononuclear cells (PBMC) from healthy donors were cultured with viral peptide pools and IL-2 for 2-3 weeks. The expanded T cells were then stimulated by PBMCs presenting the same peptides, followed by flow cytometry quantification of IFN-γ-producing $CD8^+$ T cells. To enable the generation of antigen specific T cells also from the naïve T cell pool, a method utilizing dendritic cells (DCs) as antigen-presenters has been developed. Hence, DCs generated from human monocytes were pulsed with viral peptides and cocultured with T cells in the presence of IL-2, after which the cells were restimulated and the fraction of antigen-specific T cells determined.

Results: Culture of PBMCs in the presence of viral peptides and IL-2 resulted in a pronounced expansion of virus-specific cytotoxic T cells. Also, the presentation of viral peptides by DCs generated from fresh monocytes led to an expansion of virus-specific T cells.

Conclusion: The protocols that have been developed can be successfully employed to expand and detect virus-specific cytotoxic T cells from PBMC. Further studies are needed to verify if the DC protocol also triggers a generation of antigen-specific T cells from the naïve T cell pool. These protocols may be used as a basis for further studies of expansion and detection of antigen-specific T cells from human blood, including studies to detect T cells specific to tumor antigens.

OPTIMIZATION OF BIOCHEMICAL QUANTIFICATION METHOD ON HVA, VMA AND 5-HIA IN URINE WITH LC-MS/MS

By: Alan Hassanpour

Kromatografiska Specialanalyser, klinisk kemi, Sahlgrenska Universitetssjukhus

Uppsats/Examensarbete:	15 hp Vt/2018
Program och/eller kurs:	Biomedicinska analytikerprogrammet, Bachelor thesis
Handledare:	Henrik Ryberg, 1:e Kemist

ABSTRACT

Elevated concentrations of urinary VMA, HVA and 5-HIAA can be the cause of a neuroendocrine tumor, such as pheocromocytoma, paraganglioma and neouroblastomas, or carcinoid tumors. Neuroendocrine tumors can affect the body production and metabolism of catecholamines, such as dopamine, Epinephrine and Norepinephrine. Which can result in the increase of urinary VMA and HVA. Carcinoid tumors can affect the tryptophan metabolism in the body leading to an increase in serotonin and eventually it's metabolite 5-HIA. At Sahlgrenska University Hospital the current method for quantifying these metabolites in urine is done by HPLC coupled with electrochemical/fluorescent-detection.
The aim of this study was to optimize a method enabling simultaneous extraction and quantification of the metabolites HVA, VMA and 5-HIA, and then compare the new method results with patient-samples and quality control-levels already analyzed by the routine method.

In this study we describe an extraction method for urine using Supported-Liquid-Extraction (SLE+ ISOLUTE) that we later analyze with LC-MS/MS. The chromatographic separation is done on a biphenyl-column. The detection and quantification is done by a Waters Xevo triple quadropole-system using negative electrospray ionization and multiple reaction monitoring (MRM).

The injection-to-injection time was 8 minutes with a good linearity for VMA and 5-HIA (r =0,999, $r^{\wedge 2}$ = 0,998 and $r^{\wedge 2}$=0,995) between the ranges 2 – 250 µmole/L. Comparison of patient-and external control-samples between the different methods showed good reliability. The external controls showed better comparability between the methods compared to the patient samples. The within-run precision showed good repeatability for the metabolites, while the between-day precision showed higher values than expected.

To ensure the reliability of the new method more patient-and external control-samples need to be measured and compared with existing routine-methods. Further improvements must also be made to the extraction method to ensure that the between-day precision reaches satisfying levels.Further studies are also needed to ensure that extraction and quantification of HVA in patient urine samples is possible and incorporated in the final method.

Analysis of NF-κB activation by TNFα and TLR stimulation with bacterial ligands LPS, Pam3CSK4 and CpG-DNA

Analys av NF-κB-aktivering med TNFα samt TLR-stimulering med de bakteriella liganderna LPS, Pam3CSK4 och CpG-DNA

By: Fredrik Jansson

Kurs: BMA062 Examensarbete 15 hp
Program: Biomedicinska analytikerprogrammet 180 hp
Nivå: Grundnivå
Termin, år: Vårterminen, 2018
Handledare: Frida Svensson, forskningstekniker, Malin Johansson, universitetslektor

Abstract

NF-κB is a central part of the innate immune system which upon activation leads to expression of a broad spectrum of pro-inflammatory genes. It is a family of transcription factors activated by at least two intracellular pathways. One of these begins with stimulation from TNFα or interaction between so called Toll-like receptors and microbial ligands. Therefore, bacterial stimuli and TNFα secretion leads to NF-κB activation and an inflammatory response is initiated.

The aim of this study was to develop a novel experiment for the course "BMA043 Molecular biology methods" for the students of the bachelor programme in biomedical science at Sahlgrenska academy, Gothenburg University. The experiment had to contain cell culturing and transfection of a reporter plasmid expressing the bioluminescent enzyme luciferase, linked with a promotor sequence that binds NF-κB. These cells would then be exposed to TLR stimulation which was supposed to yield an interesting and educational experiment for the course.

Cell lines HEK293T, Caco-2, SW1116 and LoVo were transfected with the reporter plasmid pNF-κB-Luc and stimulated with TNFα, lipopolysackaride, lipopeptide Pam3CSK4 and doublestranded bacterial CpG-DNA. Cells where then lysed and assayed for luciferase activity. These experiments where part of an optimization to eventually yield an experiment which would be robust and reproducable. Our goal was to have an optimized experiment complete with written instructions for the students at the end of this bachelor thesis.

Conclusion: Caco-2 cells responded to TNFα stimulation which lead to higher luciferase activity than in non-stimulated cells after transfection of pNF-κB-Luc. TLR stimulation gave inconsistent results and further optimization is needed to yield a robust final experiment which is reproducible. Cell based models contain several elements which can give rise to errors and difficulties to reproduce results. Consistent results had still not been achieved after ten weeks and, unfortunately, my work had to be discontinued.

Isolation of circulating cell-free tumour DNA in plasma from patients with Non-Small Cell Lung Cancer -

Prior to analysis with Next Generation Sequencing

By: Karoline Johansson

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Molecular Pathology laboratory, Sahlgrenska University Hospital.
Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Carola Andersson, Biologist

Background: Patients with metastatic non-small cell lung cancer with driver mutations in genes coding for *EGFR, ROS1* and *ALK* are currently offered treatment with targeted gene therapy with tyrosine kinase inhibitors that inhibit cell signaling pathways and therefore induce apoptosis of tumour cells. In tumour cell growth, acquired resistance against the drug is suspected. A molecular analysis is then performed to detect possible resistance mutations. Circulating cell-free tumour DNA that is released to the blood from tumour cells is the target analyte. Circulating cell-free tumour DNA has a fragment length of about 180-200 bp and occur in low blood concentrations. Therefore, an isolation method of high specificity for short ctDNA is required. **Purpose:** The purpose of this study was to compare three different methods for isolation of circulating cell-free tumour cell DNA. The aim is to asses which kit provides the highest concentration of circulating cell-free tumour cell DNA in eluate and generate good quality sequencing with Next Generation Sequencing with a high coverage and low limit of detection for detection of the lowest frequency mutations. **Methods:** Circulating cell-free tumour cell DNA was isolated from pooled patient plasma from lung cancer patients in the volumes of 1, 2 and 4 mL with three different kits, MagMAX ™, QIAamp® and MagDEA®. DNA concentration was measured on the eluate and fragment length analysis was performed. The circulating cell-free tumour DNA isolated with the various methods was eventually sequenced with Next Generation Sequencing. **Results:** The kit that generated the desired concentration of circulating cell-free tumour DNA was QIAamp® isolated from 4 ml plasma. The fragment length analysis showed that QIAamp® and MagMAX™ isolated shorter fragments than MagDEA®. The result of the Next Generation Sequencing analysis showed that the samples isolated with QIAamp gave a high coverage and low limit of detection, while the results varied for samples isolated with MagDEA® and MagMAX™.
Conclusions: For the MagDEA® kit, DNA concentration in the eluate and sequencing result did not reach the desirable level. Neither did the fragment length analysis that showed that this kit isolated DNA fragment lengths longer than ctDNA. As something went wrong during the isolation with MagMAX™ no conclusions regarding this kit could be drawn.
QIAamp® was easy to work with and generated the desired concentration of DNA in eluate upon isolation from 4 mL plasma and isolated DNA fragments in the length of ctDNA. This kit generated consistent sequencing results with high coverage and low limit of detection.
This method could therefore be assessed as the most reliable for isolation of circulating cell-free tumour DNA for detection of low-frequency mutations with Next Generation Sequencing.

Upregulation of two cellular glycosyltransferases in herpes simplex virus type 1 infected fibroblasts

By Aida Kaltak

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Infectious Medicine, Sahlgrenska Academy, University of Gothenburg, 2018.

Supervisior: Rickard Nordén (PhD microbiologist), Ebba Samuelsson (postgraduate)

Introduction. Herpes simplex virus type 1 (HSV-1) is a common virus that causes lytic infections in the oral cavity or genitally, primarily in epithelial cells, but also in different types of underlying cells, such as fibroblasts. HSV-1 has the ability to change the expression of cellular genes during an infection. For example, glycosyltransferases, which are a group of approximately 150 different enzymes located in the golgi apparatus, each performing a specific catalyzation of glycosidic bonds in glycosylation. In glycosylation, carbohydrate structures (glycans) are synthesized on proteins (cellular and viral), that passes through the golgi apparatus after translation. Human viruses have no genes encoding glycosyltransferases, hence they are dependent on cellular glycosyltransferases for expression of glycans on their surface proteins. The purpose of our study was to measure the gene expression of glycosyltransferases in HSV-1 infected fibroblasts with ten new developed real-time primer/probe-systems.

Method. The functionality of ten commercial gene expression systems was tested with one-step reverse transcription (RT) -real-time PCR. The product from the PCR reactions was ligated into a plasmid vector containing selection genes, such as ampicillin resistance and beta-galactosidase.
A transformation of the bacterium E.coli with the plasmid vector was carried out on plates containing ampicillin and X-gal, to select bacterial colonies that have taken up the plasmid.
The colonies that became positive were used to purify and harvest plasmids to make a standard curve which served as a positive control of the ten primer/probe-systems. Fibroblasts were infected with HSV-1 and RNA was harvested. The gene expression of the glycosyltransferases was then measured in HSV-1 infected and uninfected fibroblasts using RT real-time PCR.

Results. We managed to produce five functional plasmids of the ten systems tested. Five different infections were generated and the gene expression was analyzed. Glycosyltransferases EGOT, B4GALT5, ST3GAL1 were downregulated in HSV-1 infected fibroblasts while B3GNT4 and GCNT3 were upregulated, compared to uninfected fibroblasts.

Conclusion. It has previously been shown that herpesvirus causes a downregulation of cellular gene expression during infection, but some genes are instead upregulated including certain glycosyltransferases. In this study we have found two new glycosyltransferases upregulated by HSV-1, when the virus infects human fibroblasts. Additional studies will be required to define the type of carbohydrate structures that are formed as a consequence of the altered expression pattern, if it leads to altered carbohydrates on viral proteins, and if they have any biological activity that affects the life cycle of the virus.

Aggregates in whole blood bags gives a negative effect on platelet counts and platelet function but has no visible effect on Factor VIII

By: Louise Karlsson

Bachelor thesis in Biomedical Laboratory Science performed at transfusion medicine, Sahlgrenska Academy, University of Gothenburg, 2018 Supervisor: Lisa Kylbring and Camilla Hesse

Introduction: Whole blood is divided into its components (erythrocytes, plasma and platelets) for transfusion. The laboratory divides whole blood from givers with Reveos semiautomatic system and sometimes aggregates appears in the bags. It is unknown what the aggregates consist of and how it affects the quality of the blood bags.

Aim: The aim with the study was to investigate if aggregates in whole blood bags affects the concentration of factor VIII (FVIII) in plasma or platelet counts and function.

Method: Fifty plasma samples was taken from units belonging to the whole blood with aggregate. When sampling a visual assessment was made of the aggregates with a degree 1-4. Platelet yield index (PYI) was noted for all the samples. Eighteen miniplatelet bags from the whole blood bags with aggregate was analyzed with Multiplate® and agonists TRAP, ADP, ASPI was used. Ten miniplatelet bags from units without aggregates analyzed as a comparison. The Plasma samples was analyzed with one step clotting method for measures of FVIII concentration.

Results: PYI and degree of aggregates showed a significant correlation. The miniplatelet bags from units with aggregates in whole blood (group 1) had significant lower PYI (median 17) than the miniplatelet bags without aggregates in whole blood (group 2) (median 90). The platelet function test showed that group 1 had significant lower values when it comes to ASPI and TRAP meanwhile ADP was more comparable between the groups. Low PYI values correlates with lower function with the agonists ASPI and TRAP. In this study it was no correlation between degree of aggregates in the unit and FVIII.

Conclusion: Aggregates in the whole blood units are most likely platelet aggregates which are affecting the miniplatelet units. The plasma from the whole blood aggregate units wasn't affecting the concentration of FVIII and by that the plasma units keep a good quality.

No difference between accessory pathways antegrade electrical characteristics in asymptomatic and symptomatic patients.

By: Julia Larsson

Bachelor thesis in Biomedical Laboratory Science performed at the intervention 3, Sahlgrenska university hospital, University of Gothenburg 2018.

Supervisors: Lennart Bergfeldt, Monika Dahlin, Aigars Rubulis och Britt-Marie Jinhage Åberg.

Background: The Wolf-Parkinson-White (WPW) syndrome is present when the patient has at least one accessory pathway (AP) for electrical conduction between atrium and ventricle in addition to the AV-node/His bundle. In addition, the patients should have symptoms related to atrioventricular reentry tachycardia. Preexcitation is detected on the ECG as a delta-wave when the pathway leads impulses in an antegrade direction (from atrium to ventricle). Asymptomatic patients with preexcitation have presently very low priority according to national guidelines when it comes to ablation treatment. It is first after they have had their first symptom they get prioritized. The first symptoms of WPW can be sudden death. There are until now no studies that investigate if there is any difference in risk of sudden death between symptomatic and asymptomatic patients by comparing the antegrade AP conduction capabilities.

Objective: To compare the antegrade AP conduction capabilities in symptomatic versus asymptomatic patients with preexcitation.

Method: The patients (n=775) with overt preexcitation were identified in the clinical Ablacure registry and grouped based on if they were symptomatic or asymptomatic, if they were children (< 18 years old) or adults and depending on their AP locations. The Wenckebach cycle length and effective refractory period of the AP for each asymptomatic patient were compared to the range of these measures in the symptomatic group with the same AP-location.

Result: The Wenckebach cycle length and effective refractory period of the measures for the asymptomatic patients were either within or below (more severe) the range for these measures in symptomatic patients with one exception.

Conclusion: The symptomatic and asymptomatic patients had similar antegrade electrical AP-properties and presumably similar risk of sudden death related to preexcitation. All patients diagnosed with preexcitation should therefore be offered electrophysiological risk assessment and ablation.

LUNG FUNCTION DECREASES AS AN CONSEQUENCE OF SMOKING; A CONVENIENCE STUDY OF STUDENTS AND STAFF AT AL-QUDS UNIVERSITY

By: Malin Lindahl

Essay/Thesis:	15 hp
Program and/or course:	Biomedical Scientist program
Level:	First Cycle
Semester/year:	VT/2018
Supervisor:	Akram Amro, Arthur Saniotis, Mohammad Hjouj and Bente Grüner Sveälv

Abstract

Background

Smoking of cigarettes and water pipe are a chief public health problem for young and older individuals in Palestine. In this study we examine lung function between smokers and non-smokers in a Palestinian population that was divided into two age categories: students (18-30yrs) and staff (31-60yrs). The aim of this study is to increase knowledge on the association between smoking, stress and exercise habits in relation to lung function in a selected population in Palestine. Additionally, this study may make it possible to detect respiratory diseases in an early stage, also in an asymptomatic population.

Methods

There were 76 participants; students (N=51) and staff (N=25), from Al-Quds University. Most participants were selected from the Al-Quds Medical, Health and English literature departments. Participants were chosen from a healthy male only population between 18-60 years of age. Questionnaire was used. Blood pressure (BP), heart rate and O^2 saturation were measured using a pulse oximeter. Lung functional tests were also conducted.

Results

Lung function data between smokers and non-smokers showed significant differences with respect to forced vital capacity (FVC), forced expiratory volume for 1 second (FEV1) and FEV1/FVC ratio ($p < 0.05$).

In relation to the smoking groups (18-30yrs and 31-60yrs), there were observed differences and variations between them. These differences and variations were statistically significant.

84% of the total sample reported stress. Of them 47.6% reported severe stress, 33.3% moderate stress and 19% mild stress.

We found that stress shows a significant negative correlation with lung function of FVC, FEV1/FVC ratio and FEV1 ($p < 0.01$).

We did not find any statistical significant difference in exercise, systolic and diastolic blood pressure or O^2 saturation.

Conclusion

In a selected population in Palestine we found that there was no significant findings in terms of lung function and smoking in the group of young smokers. Alternatively, we found a statistically significant difference between smokers in the two age groups, with respect of the difference between predicted and measured values.

Reduced lung function in the young smokers group was not apparent, probably because of the relatively short amount of time which they had been smoking. Longitudinal studies are required in order to optimally evaluate the effect of smoking in young Palestinians.

Circulating rotavirus genotypes in Västra Götaland between 2015-2017

By: Lili Ly

Bachelor Thesis in Biomedical Laboratory Science performed at the Clinical Microbiology unit of Sahlgrenska University Hospital in Gothenburg, 2018
Supervisor: Maria Andersson, Phd and biomedical laboratory scientist

Background. Rotavirus is one of the pathogens that causes acute gastroenteritis. It is estimated that 215 000 children under the age of five die each year because of complications from the rotavirus gastroenteritis. Two vaccines, Rotarix® and RotaTeq® have been recommended for use by the WHO since 2006. The Rotarix vaccine was introduced in april 2016 in Västra Götaland. In preparation of a national introduction of the vaccine in Sweden a continuous monitoring of the most common circulating rotavirus genotypes before and after the vaccine introduction is necessary to determine which genotypes the vaccine is effective against.
Aim. The aim of the study was to identify the most common circulating rotavirus genotypes in Västra Götaland during the period of the vaccine introduction. The different genotypes were also determined in the age groups infants <5 years and the elderly >70 years of age since the virus causes the most severe symptoms in those ages.
Methods. A total of 309 faecal samples collected from patients with acute gastroenteritis between 2015-2017 where rotavirus was determined to be the main cause were used. A multiplex real-time RT-PCR which detected the most common G- and P-types (G1, G2, G3, G4, G9, G12, P[4], P[6] and P[8]) was used. Samples that could not be genotyped with the multiplex real-time RT-PCR method were sequenced with the Sanger method.
Results. The results showed a significant distribution of two different genotypes between the age groups <5 years and >70 years of age. G2P[4] was most common among the elderly and G9P[8] is mostly found in infants. In 2015 the most common genotype was the G9P[8] and in 2016-2017 an outbreak of G3P[8] occurred which made it the most common genotype in total over the 2015-2017 period that this study was conducted over.
Conclusion. During the period of vaccine introduction a few cases of G12P[8] was detected which had not been detected in Sweden before. The G3P[8] outbreak in 2016-2017 was also of a type that had not been detected in Sweden before. Sanger sequencing showed an equestrian-like G3 that before had only been detected in a few countries that used the Rotarix vaccine. This implies that the Rotarix vaccine has some connection with the emergance of the G3P[8] in Västra Götaland. The number of rotavirus infected elderly decreased when G2P[4] no longer circulated to the same extent as previous years. This suggests that different age groups are more affected by specific genotypes.

Quantification of inflammation and rejection estimation in transplanted human uterus.

By Amanda Martinsson

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Pathology and genetics laboratory, Sahlgrenska Academy, University of Gothenburg, 2018

Supervisor: Johan Mölne, Verena Bröcker

Background: Absolute Infertility Factor Infertility (AUFI) is a form of infertility that is due to a malformation of the uterus, causing women not to be able to have their own children. Uterus transplant is the only way these women can become pregnant and in 2014 the first child was born from a transplanted uterus. The woman who gave birth to the child was a participant in a research study with 8 other women, that was performed at Sahlgrenska University Hospital. The transplants are then removed after the first or second birth, and are sent to the pathologist for morphological examination.

The transplants were followed by regular protocol biopsies from the cervix / portio and a morphological rejection assessment system was prepared based on the pathologist's experiences. By using these methods, the immunosuppressive treatment can be controlled.

The purpose of the study was to investigate whether the transplanted uterine tissue differs in number of inflammatory cells compared to normal uterine tissue. The study also aims to show whether it is possible to retain the uterus after birth and use it for new pregnancies.

Method: The material in the study consisted of tissue samples from 5 patients transplanted and surgically removed uterus. This material was compared to tissues from 5 patients with normal uterus and 5 postpartial uterine patients. In the study, three areas were selected from the uterus tissue; parametrium with blood vessels, myometrium and cervix. The tissues were stained with the histochemical staining Mayer's hematoxylin eosin and Elastin van Gieson. The tissues were also stained with immunohistochemical staining for T cells (CD3), B cells (CD20), macrophages (CD68) and C4d to see if antibody mediated rejection was ongoing. In the parameter, 10 arteries were counted and an average of each patient was calculated. In the myometrium, cells were counted in 10 high power fields. In the cervix, inflammation of the tissue was evaluated first qualitatively and then quantitatively. For the quantification an analogue scale was used.

Result: The result showed a statistically significant difference in the number of T-cells, B-cells and macrophages in arterial walls, in the parametrium tissues and for macrophages in the cervix. In the remaining samples, a trend was observed that there were more inflammatory cells in the transplanted tissue. However, there was no statistically significant difference between them.

Conclusion: The conclusion is that there is a larger amount of inflammatory cells in the transplanted tissue, but no clear rejection is seen in the uterine uterus transplant. The result also indicates that the transplant can be used for more births than two.

A Screening Assay to Measure the Sensitivity of Fanconi Anaemia Patients' Cells to Cancer Therapy

By: **Anna McCormick**

Gothenburg University
Dublin Institute of Technology
2018
BSc (Hons) in Biomedical Science

Department of Clinical Chemistry at Sahlgrenska University Hospital,GU

GU Supervisor; Dr. Pegah Johansson and prof Ola Hammarsten

DIT supervisor, Dr. Fergus Ryan

Abstract

Fanconi Anaemia (FA) is a rare, inherited disorder associated with a DNA repair deficiency and an increased predisposition to cancer. Complications arise, as often patients exhibit a hypersensitivity to DNA cross linking agents e.g. chemotherapies, causing severe side-effects. The cell division assay (CDA) has been suggested as a tool by our group to predict patients' sensitivity to cancer treatments. The CDA measures the *in vitro* sensitivity of patients' blood cells by assaying their proliferative ability after treatment. FA cell lines from different individuals along with control individuals, were used as a model system to validate the CDA's detection of chemotherapy sensitivity. CDA measurements were also compared to other methods, namely, the apoptosis assay and the γ-H2AX assay.

Cells from FA patients were found to be significantly more sensitive to cyclophosphamide than the controls. FA cell lines as a group were not more sensitive to etoposide or ionising radiation, however, an individual variation in sensitivity within the group to all cancer therapies was seen. The CDA data correlated with both the apoptosis assay and the γ-H2AX assay.

The employment of the CDA in routine oncology in the future could help guide radio- and chemotherapy dosing and increase cancer patient survival rates.

Genetic variability of hepatitis E virus ORF3 gene on the host immune response against the virus

By Julie Moore

Abstract

Hepatitis E (HEV) is a small, single stranded RNA virus encoding for three open reading frames (ORF1-3). It is non-enveloped in faeces but enveloped in blood. There are five genotypes infecting humans, HEV1-4 and 7, all zoonotic. HEV3 is global with pigs and wild boar as the main animal reservoirs. ORF3 encodes a multi-functional protein that is involved in cell survival, proliferation and transport. It is present in the viral envelope in serum.

Strains from infected individuals with and without immune response against HEV were analyzed. Three polymerase chain reaction methods were used amplify and sequence HEV RNA, reverse transcriptase (RT) PCR, semi-nested PCR and cycle sequencing. Several PCRs were tested before amplicons of correct size could be obtained. The amplified products were visualized by agarose gel electrophoresis. The sequences were generated by a Sanger sequencing technique.

The sequenced fragment covered the end of the ORF1 region, a junction region between ORF1 and 3 and the beginning of the ORF3 region. Nucleotide divergence was observed between the strains, especially in the junction region. No specific mutation in the sequenced region was found related to the host´s immune response against HEV.

The difficulty in amplifying full ORF3 may be due to complex stem loops in this region, which could make the sequence complementary to the primers inaccessible for binding. Another reason could be sequence mismatches between the primers and target causing inability of the primers to stay attached to the target. Further analysis must be carried out on the ORF3 region.

Risk-stratification of coronary artery disease with sound analysis of coronary artery blood flow. A comparative study against stress-ECG, stress-echocardiography and SPECT.

By: Amilia Mrozek

Bachelor thesis in Biomedical Laboratory Science performed at Sahlgrenska Academy, University of Gothenburg, 2018

Supervisor: Kambiz Shahgaldi

The coronary arteries provide the heart muscle with blood and oxygen. When the coronary arteries are compromised by a disease, blood flow through the heart decreases and the heart muscle does not get the adequate amount of blood and oxygen. Since coronary artery disease (CAD) is a common cause of death, recent technology has developed an acoustic system for risk stratification of CAD to simplify and speed up the evaluation process. The system uses sound analysis of turbulent blood flow post-stenosis and produces a so called CAD Score. A low CAD Score (≤20) indicates a low probability of disease and a high CAD Score (>20) indicates a high risk for disease. The aim of this study is to validate and confirm the CAD Score system as a rule-out method for CAD. In this study, we sought to compare the CAD Score system with standard tests for evaluating CAD. The tests including exercise ECG, exercise echocardiography and single-photon emission computed tomography (SPECT) are performed at Clinical Physiology department at Sunderby Hospital in Luleå, Sweden. Hundred fourteen patients were admitted for these tests and were asked to participate in the study. By placing the CAD Score in the fourth intercostal space the system makes an analysis and provides a CAD Score. The results of the CAD Score and the tests are then compared. The data shows a sensitivity of 81.2%, a specificity of 28.8%, positive predictive value of 20.0% and a negative predictive value of 87.5%. The high sensitivity and high negative predictive value indicates for the system to be a trustworthy method for ruling out CAD and confirming CAD in patients.

Differentiation between *Shigella* and Enteroinvasive *E. coli* (EIEC) with primers and probes aimed at sequences in *Shigella sonnei, Shigella flexneri, Shigella boydii* and the *lacY*- and *ipaH*-genes.

By: Frida Mårtensson

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology, Hallands hospital, Halmstad, 2018.
Supervisors: Peter Nilsson, molecular biologist and Arne Kötz, attending physician, PhD.

Shigella, a gram negative bacteria causing dysentery, has been showed to have a highly genetical relationship with *Escherichia coli (E. coli)* especially Enteroinvasive *Escherichia coli* (EIEC). Differentiation between *Shigella* and EIEC with conventional methods such as biochemical and serological test requires a bacterial isolate. But *Shigella* is hard to culture and today's realtime-PCR-methods is aimed at the *ipaH*-gene with is present in both *Shigella* and EIEC. *Shigella* lack the *lacY*-gene whereas this gene is present in all *E. coli* and most EIEC.

The aim of the study was to develop realtime-PCR-methods to differentiate between *Shigella* and EIEC in stool samples as well as bacterial isolate with primers and probes aimed at sequences in *S. sonnei, S. flexneri, S. boydii* and the *lacY*- and *ipaH*-gene.

A total of 68 isolate from *S. sonnei* (n=25), *S. flexneri* (n=23), *S. boydii* (n=6), EIEC (n=7), and other pathogenic *E. coli* (n=7) was examined with the realtime-PCR-methods. Also 100 stool samples that had been negative and 21 stool samples positive for the *ipaH*-gene where examined. The *ipaH*-positive stool samples had been cultivated and typed with conventional methods but 14 of the sample had been negative.

The *S. sonnei*-PCR manage to identify all 25 *S. sonnei*-isolates and all 5 of the *ipaH*-positive stool samples typed to *S. sonnei.* Of the 14 culture negative samples two were positive in the *S. sonnei*-PCR. Of the 23 isolates of *S. flexneri* 12 were identified with the *S. flexneri*-PCR, five isolates didn't give any readings in the *Shigella* specific PCR's and six isolates were positive in the *S. boydii*-PCR. Two of the *ipaH*-positive stool samples typed to *S. flexneri* gave readings in the *S. flexneri*- and *S. boydii*-PCR respectively and of the 14 culture negative two gave readings in the *S. flexneri*-PCR. The six isolates typed to *S. boydii* where all positive in the *S. boydii*- PCR. All *Shigella* isolates where positive for *ipaH* and negative for *lacY.* The seven isolates typed to EIEC gave no reading in any of the *Shigella* specific PCR's and were positive for the *ipaH*- and *lacY*-gene. Of the seven isolates of pathogenic *E. coli* other than *Shigella* and EIEC, no one gave any reading in any of the *Shigella* specific PCR's nor for the *ipaH*-gene, only the gene for *lacY.* Neither one of the 100 stool samples negative for the *ipaH*-gene had any readings in the *Shigella* specific PCR's; 97 of them were positive for *lacY.*

The three realtime-PCR's targeting *S. sonnei, S. flexneri* and *S. boydii* showed potential in being of use as a diagnostic tool for differentiation of *Shigella* and EIEC with the *S. sonnei*-PCR having the best performance. Additional primers for the *S. flexneri*- and *S. boydii*-PCR is of interest to optimize the identification of these strains of *Shigella.*
The realtime-PCR targeting the *lacY*- and *ipaH*-gene was more suited for bacterial isolates than stool samples due to the present of other *lacY*-positive bacteria in these samples. *lacY* had other limitations since not all EIEC are *lacY*-positive make. A *lacY*-positive result rules out *Shigella* whereas a *lacY*-negative result can indicate either Shigella or EIEC.

UNIVERSITY OF GOTHENBURG

SAHLGRENSKA ACADEMY

Investigating the role of microparticles in type I Interferon gene regulation in Systemic Lupus Erythematosus

Mohammad.T.Nehmé

Course: Bachelor thesis 15 hp
Program: Biomedical Laboratory Science 180 hp
Semester/year: 6-semester spring 2018
Supervisor: Dr. Claire Wynne
School of Biological Sciences, Dublin Institute of Technology,Kevin Street, Dublin 8

Abstract

Systemic lupus erythematosus (SLE) is a chronic, inflammatory autoimmune disease which results in the dysregulated, overproduction of type I interferons (IFNs). SLE patients produce antibodies against a wide spectrum of self-antigens that forms immune complexes which deposit in vascular tissues and activate cells for the immune system. Autoantibodies from SLE patients mediate in organ damage. MPs in SLE patients serve as an extracellular nuclear molecule that can form pathogenic immune complexes. MPs can therefore initiate autoimmunity by releasing their content of bioactive molecules which also has the ability to be involved in multiple receptor systems simultaneously.

Due to increased apoptotic pathways, SLE patients have increased amounts of MPs in circulation which we propose contributes to the pathology (increased type I IFN production) associated with the disease. In this study, we aim to assess the role of MPs in Toll-like receptor (TLR) signalling and downstream type I IFN production using differentiated THP1 cells as a model.

Moreover, PCR was employed to look at the specific genomic materials, immunoblotting for specific proteins and ELISA for pro-inflammatory cytokines. In conclusion after optimisation of protocols, the results demonstrate that MP plays a major role in the production of Type I IFN.

Identified *Staphylococcus argenteus* among isolates identified as *S. aureus* in blood cultures

By Sofia Niklasson

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology, Sahlgrenska University hospital, Gothenburg, 2018
Supervisor: Liselott Svensson Stadler, PhD

In 2015 Tong *et al*. described a novel species called *Staphylococcus argenteus* that previously was described as the non-pigmented *Staphylococcus aureus* Clonal Complex 75. The occurrence of *S. argenteus* in Europe is not well studied, but in recent studies there seems to be a wider spread of the bacteria in eastern Asia and Australia. A previous study has been made in western Sweden where they found that 16 Methicillin-resistant *Staphylococcus aureus* isolates where Methicillin-resistant *S. argenteus.*

In this project, we investigated *S. argenteus* occurrence in antibiotic sensitive blood culture isolated from sepsis patients, that had been identified as *S. aureus* in 2017 (n=354). Chocolate agar plates where used to identify colour. Non-pigmented isolates (n=136) where picked for further analysis. The non-pigmented isolates where sequenced using sanger sequencing (3130/3500 Applied Biosystems 8x sequencing-capillary). The gene that was sequenced was the SodA gene, which is a housekeeping gene. The sequences where analysed and edited using the BioNumerics software.

Out of the 345 blood isolates that was identified as *S. aureus* in 2017, we could identify that one out of the isolates was in fact a *S. argenteus.*

In conclusion, one *S. argenteus* isolate was found. Compared to previous researched conducted, there was significantly less *S. argenteus* isolates in Gothenburg then in eastern Asia and Australia. Since the characteristics of *S. argenteus* and the *S. aureus* that are non-pigmented is a lot alike, the fact that they have been misidentified is not surprising. Therefore, the standard identification routines need an upgrade, either with a more sensitive nuc hydrolysis probe, or an update of the Matrix Assisted Laser Desorption Ionization Time-of-Flight VITEK MS (BioMérieux) software.

FIBER COMPOSITION IMPROVES INTESTINAL CELL RENEWAL, IN MICE AFTER RADIATION

KOSTFIBERSAMMANSÄTTNINGEN PÅVERKAR TJOCKTARMENS CELLFÖRNYELSE HOS MUS EFTER STRÅLBEHANDLING

By Henrietta Norling

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, 2018.

Supervisor: Cecilia Bull, PhD.

Cancer can be a terribly aggressive illness, which is one reason to use quite aggressive means to combat it, one example being radiation therapy. Unfortunately, because of the aggressive nature of irradiation therapy, normal tissue cells around the cancer cells also get wounded in the process. We seek to define the injury and repair processes in the colon of mice, after irradiation designed to mimic the therapy given to patients suffering from for example cervix cancer or prostate cancer. Here, we determined if the content and/or ratio of fermentable to non-fermentable fibers have an effect on the injury and repair processes in the irradiated colon tissue. In this specific experiment, we tested various immunohistochemically approaches for visualizing Ki67 positive cells in the crypts of methacarn-fixed colon. Ki67 is produced in dividing cells, thus showing the rate of tissue regeneration in the colon after the irradiation. After establishing a working protocol, we quantified cell proliferation six weeks after irradiation in combination with the fiber diets. The experiment showed that the group of mice given high amounts of fermentable fibers had the most active cell proliferation. The knowledge gained in this experiment add to the growing evidence of the importance of dietary fiber in intestinal health. Hopefully, our data and others will promote the development of evidence-based dietary guidelines for patients who have, or will be subjected to pelvic radiation therapy.

MITOCHONDRIAL LON PROTEASE DEGRADE MITOCHONDRIAL TRANSCRIPTION FACTOR A (TFAM) BY BINDING WITHIN HIGH MOBILITY GROUP 1 (HMG1)

Av: Nikolina Pearson

Bachelor thesis in Biomedical Laboratory Science performed at the Institution o Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Jennifer Uhler, PhD

Lon is one of three ATP-dependent proteases located in the mitochondrial matrix. Lon contributes to quality control by degrading defective, damaged and oxidatively modified proteins. One of the known substrates to Lon is the mitochondrial transcription factor A (TFAM). Lon regulates TFAM:mtDNA ratio by selective degradation of TFAM. The TFAM protein consists of two high mobility group (HMG) domains. Previous studies have shown that Lon recognizes TFAM within the HMG1 box.

The aim of this study was to create mutants with different deletions within the HMG1 box to investigate whether Lon can degrade these mutants over time,

Mutants were obtained by PCR-mediated deletion of the TFAM-MBP plasmid. These were transformed into One Shot® TOP 10 competent *E.Coli* and cleaved in three restriction sites before they were sent for sequencing. After sequencing, the plasmids were transformed and expressed in Rosetta ™ competent E. coli and purified by nickel and heparin purification. Protein concentration determination and degradation analysis were then performed. Degradation analyses were performed with and without ATP and the reactions were stoped at six different time points after Lon was added. By visualizing the protein on gel, the relative amount of protein broken down since time 0 could be calculated.

The result shows a complete breakdown of TFAM wild-type after 60 minutes with ATP. TFAM-HMG2 showed a breakdown of ATP to 5.5% and without ATP to 47.5% and TFAM-HMG1 a breakdown of ATP to 64.5% and without ATP to 85% after 60 minutes. All mutants showed incomplete degradation after 60 minutes with ATP. The mutant that showed least degradation was TFAMΔ43-90 which showed a breakdown of ATP to 53.5% and without ATP to 66% after 60 minutes. These results suggest that the amino acid sequence between amino acid 43 and 90 within HMG1 of TFAM is of great importance when Lon recognizes and degrades the TFAM.

ENVIRONMENTAL BACTERIA FROM AQUATIC ENVIRONMENTS SHOWS NO INDICATION FOR ANTIBIOTIC RESISTANCE USING PHENOTYPICAL ANALYSIS AND MOLECULAR METHODS

By Marie Persson

Bachelor thesis in Biomedical Laboratory Science performed at the Dublin Institute of Technology during spring 2018.

Supervisor: Dr Julie Ann Naughton and Dr Shane Dillon

Introduction: The spread of antibiotic resistance is seen as a major global threat to the overall human health. Bacterial species can gain genes coding for antibiotic resistance either naturally or acquired through horizontal gene transfer of genetic material i.e. plasmids. As pathogenic and antibiotic resistant bacteria from humans and animals interact with environmental bacteria, opportunities for exchanging genetic material coding for antibiotic resistance increases. The presence of antibiotic resistant bacteria in Irish water sources is an understudied area and might act as a potential pathway for the spread of antibiotic resistant bacteria to humans and animals. **Aim:** Investigate the presence and the prevalence for antibiotic resistance genes in bacterial species isolated from constructed wetlands in Ireland as well as examining karstic spring water using phenotypical analysis and molecular screening methods. **Material and methods:** Water samples from karstic springs in Ireland were filtered through a membrane (0,45 µm pore size) and cultivated on selective agar. Microbial contamination was evaluated as cfu/ml. Isolated species from constructed wetlands in Ireland were tested for antibiotic susceptibility using disc diffusion. Colonies from *Enterobacteriaceae* and *Pseudomonas* spp. were screened for plasmids were colonies containing plasmids were screened for twelve different antibiotic resistance genes by PCR. **Results:** Filtration and counting the cfu/100 ml confirmed water samples were contaminated with *E. coli, Enterococci* and *Pseudomonas* spp. Disc diffusion showed all species were sensitive to the antibiotic agent applied. Out of 84 screened *Pseudomonas* and *Enterobacteriaceae* colonies, 31 *Pseudomonas* spp. contained plasmids. No plasmids were detected for *Enterobacteriaceae* spp. No antibiotic resistance genes were detected in *Pseudomonas* spp. throughout the PCR screening. **Discussion:** Water from all nine karstic springs are heavily contaminated and not suitable for human consumption. The disc diffusion was performed on mixed populations and should be repeated on pure cultures. As 31 *Pseudomonas* colonies were carrying plasmids it highlights a possible scenario for horizontal gene transfer. Results from a recent study on conjugative gene transfer showed 58% of *Pseudomonas* spp. (n=36) were able to transfer antibiotic resistance genes to a recipient *E. coli.* As antibiotic resistant phenotypes within *Pseudomonas* spp. and *Enterobacteriaceae* spp. are increasing and common as human pathogens, the constant release of these species in aquatic environments highlights the need for further research as both species were isolated from constructed wetland and karstic springs.

Conclusion: Antibiotic resistance is not present in *Pseudomonas* spp. isolated from constructed wetlands, however a larger sample should be screened as well as other species should be examined. Many karstic spring water samples are contaminated with *E. coli, Pseudomonas* spp. and *Enterococci* spp. and further research should be made to evaluate the prevalence of antibiotic resistance genes.

Molecular diagnostics of fungi

Evaluation of specificity and sensitivity of PCR assay and preparation of Next Generation Sequencing for detection of fungal DNA directly in human whole blood performed with universal primers derived from the eukaryotic rRNA operon

By: Anna Maria Pokorny

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Microbiology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Hedvig Engström Jakobsson

Introduction: Fungi are known for being an opportunistic pathogen and invasive fungal infections (IFI) often caused by *Aspergillus* or *Candida* species represent an important threat to a constant growing population of immunocompromised patients. Conventional methods as histopathology and culture, still considered the gold standards, both represent limitations in sensitivity and specificity and are time consuming. New molecular methods as *panfungal* polymerase chain reaction (PCR) which enables detection of fungi direct from a blood sample is evaluated as the solution for both quicker and improved diagnostics. PCR can detect practically any species of fungi using universal primers usually derived from the 18S rRNA gene or the internal transcribed spacer (ITS) region who are both widely used for fungal identification in molecular biology. To identify which species causing the IFI, PCR must be complemented with sequencing and the possibility to do so with High-throughput Next Generation Sequencing (NGS) has started to be investigated. The aim of this study was to investigate the possibility to detect fungal DNA direct from whole blood samples with panfungal PCR using universal primers with sequences derived ether from the 18S rRNA gene or the ITS-region or both. The PCR-reaction was optimised for sensitivity and specificity and is supposed to provide the basis (library construction) for future high-throughput NGS for even better detection of fungi and therefor NGS-primers was included in the PCR.
Material and method: The specificity of four different primer sets targeting the 18S rRNA gene or the ITS region was first examined on DNA extracted from pure reference strains of *A. fumigatus, C. glabrata, C. albicans* and *C parapsilosis.* Second, pre-treatment and method for extraction of fungal DNA from whole blood spiked with *C. albicans* was optimised. PCR was preformed and optimized for each primer set together with NGS-primers. Last a dilution series of whole blood spiked with either *C. albicans* or *A. fumigatus* was performed for evaluation of the sensitivity of the primers.
Results: All primer sets showed specificity to fungi when performed in a PCR reaction with 20 cycles, but all primers targeting the 18S rRNA gene showed false positive results on pure human DNA when preformed in higher cycle conditions. However, none of the primer sets showed false positive result on un-spiked whole blood samples except for the 18S Hugerth primers when preformed on the dilution series. The ITS primes showed high specificity and sensitivity in all cycling conditions that was executed up to 30 cycles. All primer pairs worked well with the ABC-primer.
Conclusion: It's possible to detect fungal DNA directly from whole blood samples with panfungal PCR using universal primers targeting both the 18S rRNA gene and the ITS region. The ITS primers showed highest specificity and sensitivity to fungal DNA. It needs evaluation in higher cycle condition for final statement on sensitivity and specificity. All primers functioned well with the NGS-primers but the 18S Hugerth primers which are designed to perform well in sequencing analysis needs further optimisation to be correctly evaluated.

Analysis of ASNA-1 function in C. elegans – Study of interactions between ASNA-1, ENPL-1 and syntaxin

By Dorota Robakowska

Bachelor thesis in Biomedical Laboratory Science performed at the Sahlgrenska Cancer Center, University of Gothenburg, 2014
Supervisor: Gautam Kao, Researcher

ASNA-1 protein is important in controlling insulin secretion and insulin signaling and response to the anti-cancer drug cisplatin in C.elegans and mammalian cells. How it does that is of great importance for diseases like diabetes and cancer. To understand how ASNA-1 works, it is important to study other proteins that might work with it. In C.elegans, the protein ENPL-1 plays roles similar to ASNA-1 in controlling insulin secretion and cisplatin response. But it is not known if the two proteins work together to control this process. If ASNA-1 and ENPL-1 proteins can be shown to bind to each other in a protein complex, this finding will help us to say that the two proteins work together. Worms expressing GFP tagged ASNA-1 and FLAG tagged ENPL-1 were used to perform co-immunoprecipitation studies. These experiments showed that pull-down of Asna-1::GFP could precipitate Enpl-1::FLAG protein and pull-down of Enpl-1::FLAG could precipitate Asna-1::GFP in levels above the non-specific levels. The activity of ENPL-1 might be controlled by phosphorylated tyrosines. Using an antibody specific for phosphotyrosines it was shown that worm extracts contain proteins at the size range of ENPL-1 that have phosphotyrosines. Lastly, the syntaxin protein (Unc-64 in C.elegans) belongs to a class of transmembrane proteins that also bind to ASNA-1 and is needed for insulin secretion. To see if Unc-64 works with ASNA-1, we made a GFP tagged Unc-64 to be expressed by a C.elegans promoter. This cloning was successfully done. A collaborator in Umeå University showed that the Unc-64 protein locates to the endoplasmic reticulum and also perhaps to vesicle like structures in the cells. We can conclude from this study that ASNA-1 acts in a protein complex with ENPL-1 and may together stimulate activities like insulin secretion and response to cisplatin.

Validation of biomarkers for clinical identification of *Streptococcus pseudopneumoniae*

By Patrik Schmidt

Bachelor thesis in Biomedical Laboratory Science performed at the Culture Collection
University of Gothenburg, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Lucia Gonzales Siles, PhD

Streptococcus pseudopneumoniae is a bacteria which has been proved to be a pathogen both in a murine model and in humans, mostly being involved in respiratory infections and which has a pathogenic potential linked to its ability to form biofilms and colonise the respiratory tract. Since its discovery in 2004 as a very closed related species to S. pneumoniae, one of the main pathogens worldwide, its differentiation from *Streptococcus pneumoniae* has become a challenge. The traditional methods for identification of S. pneumoniae and *S. pseudopneumoniae* have been shown to not always deliver accurate results leading to misidentification of both pathogens. This study sought out to find more effective markers for a specific identification of *S. pseudopneumoniae* which could be applied into diagnostics.

The DNA of 30 *S. pseudopneumoniae* strains from the Culture Collection of Gothenburg (CCUG) were extracted, purified and analysed for quality and quantity. Passing the quality control, the samples were analysed for the presence of the "*Xisco*" gene, showing that all strains were non-pneumoniae strains. Further, samples were tested by multiplex and singleplex PCR:s, for the detection of the proposed unique markers for identification of *S. pseudopneumoniae* and also for the presence of *lytA, ply* and *cpsA* virulence genes. Finally, the results were compared to the ones obtains by optochin test and MALDI-ToF analysis, which are the methods currently used widely in clinics. Two of the six markers (899-*S. pseudopneumoniae* and 228-*S. pseudopneumoniae*) stood out as effective potential markers since they were identified in 26 out of 30 samples. Optochin-test and MALDI-TOF showed that they can lead to misidentification with *S. pneumoniae* and other species of the *Streptoccus Mitis*-group. An interesting result is that the *S. pseudopneumoniae* type strain (CCUG 49455T), which is a reference strain, was incorrectly identified as *S. pneumoniae* by MALDI-TOF. In conclusion, we propose the detection of the two biomarkers as a method for identification of *S. pseudopneumoniae* on the clinics.

Validation of an LC-MS/MS method for detection of amphetamines and amphetamine-type stimulants in urine.

By Kamil Slupecki

Bachelors thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Norra Älvsborgs Länssjukhus, 2018

Supervisor: Patrick Hardenklo

Background: Amphetamines and amphetamine-type stimulants such as methamphetamine or methylphenidate are widely used worldwide as medicines to treat such conditions as ADHD, narcolepsy or obesity. What the substances have in common is the ability to stimulate the central nervous system and achieve various emotional and cognitive effects in humans such as euphoria, increased wakefulness and improved cognitive control. Most of these substances are excreted unmetabolized in the urine and in analytical chemistry are usually detected with various immunochemical screening methods and later in case of positive findings verified with more powerful liquid chromatography-masspectrometry methods. **Aim:** Aim of this study was to validate a new method for detection of amphetamine, methamphetamine, MDMA, MDA, mephedrone, cathinone, ephedrine, 4-fluoroamphetamine, MDPV, methylphenidate (MPH) and MPH's main metabolite, ritalinic acid in urine with liquid chromatography-masspectrometry. The new method would later be compared to already existing method on another liquid chromatography-masspectrometry instrument to be able to use it as a routine method. **Methods:** Urine samples containing amphetamines and amphetamine-type stimulants (n=75 per substance) were analyzed on both instruments to ensure new methods reliability. Lower limit of quantification was identified with positive urine samples with drug concentrations varying from 200µg/L to 3µg/L (n=10 per concentration). Within-run precision was obtained by the analysis of 24 samples directly after each other consisting of three controls (conc. 200µg/L, 1000µg/L, approx.750µg/L). Between-day precision was obtained by the daily analysis of three calibrators (conc. 200µg/L, 1000µg/L and 12500µg/L) in 11 different days. **Results**: The new method has shown good reliability compared to the existing method on Waters Xevo TQ-MS for all the substances except for MPH and ritalinic acid. Those two methods are known to generate unreliable results with the old method. Lower limit of quantification was identified as low as 25µg/L for MDPV, as high as 150µg/L for MDMA, MDA, ephedrine, mephedrone, 4-fluoroamphetamine and ritalinic acid and 100µg/L for the rest of the substances. Within-run and between-day precision test have shown good repeatability and robustness for all the substances except for MPH and ritalinic acid. The latter two substances did show too much of a difference in mean values in both between-day and within-run precision tests (mean for ritalinic acid for between-day precision test for 1000µg/L calibrator was 751,57±46,63 for example). Otherwise biggest mean difference for 200µg/L in between-day precision analysis was shown by MDPV, mean=193,78±6,69, and biggest mean difference for 200µg/L in within-day precision analysis was shown by MPH, mean=207,87±6,11. **Conclusion:** The new method can successfully be implemented in routine analysis for detection of amphetamine, methamphetamine, MDMA, MDA, mephedrone, cathinone, ephedrine, 4-fluoroamphetamine and MDPV in urine on liquid chromatography-masspectrometry instrument Agilent 6460, whereas implementation of MPH and ritalinic acid as routine analysis were not successful and will need further studies.

Cloning, expression and characterization of disease-associated POLγA mutants involved in mtDNA replication

By Felix So

Bachelor thesis in Biomedical Laboratory Science performed at Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2018.
Supervisor: Bertil Macao, Associate Professor

Human mitochondrial DNA (mtDNA) carries genes coding for proteins involved in the respiratory chain. DNA polymerase POLγA coded by DNA polymerase γ gene (POLGl) has the function of repairing and replicating mtDNA. Mutations in POLGl affect the replication of new mtDNA and may lead to diseases. A 16 months old patient was diagnosed with a mitochondrial disease, Alper's syndrome, and carried A and B amino acid substitutions in POLGl. The aim of this study was to investigate if A and B mutations could explain the disease phenotype of the patient, and to describe the methods of cloning, expression and characterization of the POLγA mutants. POLG1 was cloned into a baculovirus vector and mutated to create a A mutant and a B mutant. The sequence was verified and correct plasmid was purified. The mutated POLγA was recombinantly expressed in insect cells. The protein was purified and detected both with SDS-PAGE and Western blot. The protein was characterized by electroforetic mobility shift assay (EMSA) and coupled 3´-5´-exonuclease-polymerase assay. The result showed that a small amount of POLγA B was purified and detected. The result of EMSA showed that POLγA B could bind to DNA and the DNA affinity did not differ much from wild type POLγA. The result of coupled 3´-5´-exonuclease-polymerase assay showed that POLγA B had a significantly lower exonuclease and polymerase activity than the wild type. POLγA A has been characterized earlier and proved to have poor DNA affinity and dNTP affinity. In conclusion, cloning, expression and characterization of the POLγA mutants was described and the B mutation in POLG1 did not affect the interaction between DNA and polymerase, but affected the dNTP affinity and polymerization, which is a reasonable explanation for the disease phenotype of the patient.

A COMPARATIVE STUDY BETWEEN MODIFIED GIEMSA AND ALCIAN YELLOW-TOLUIDINE BLUE STAINS FOR THE DETECTION OF *HELICOBACTER PYLORI* IN DIGITALISED VENTRICLE BIOPSY SLIDES

By Charlotta Svensson

Bachelor's thesis in Biomedical Laboratory Science performed at the Department of Clinical Pathology and Genetics, Sahlgrenska Academy, University of Gothenburg, 2018

Supervisors: Mats Wolving, Katarina Junevik, Karin Blomqvist

Background: Developments in scanning techniques are offering an intriguing alternative to regular light microscopy. The digitalization of slides opens up new possibilities and can dramatically change the way histopathological diagnosis are achieved. Through the use of digitalized whole slide images, slides can be viewed by pathologists at their desk workstation. This makes for a better work environment, easier sharing of workload and sharing of expertise. With the introduction of this new technique validation studies have been undertaken in laboratories both internationally and here in Sweden. During these validation studies some problems have been brought to light. The detection of microorganisms such as *Helicobacter pylori* are often difficult to detect in digitalized slides, and this makes diagnosis hard to achieve. **Aim:** This study compares two stains for the detection of *H. pylori* to investigate whether a better color contrast between tissue, mucin and bacteria increases the detectability of *H. pylori* in digital slides. **Method:** New 4µ sections were taken from existing paraffin embedded tissue blocks that had previously been diagnosed positive and negative for *H. pylori*, and which already had slides stained with Modified Giemsa and Hematoxylin and Eosin stains. The new sections were put on slides and stained with Alcian yellow-toluidine blue. The old and new slides were first scanned at 40x with 1 focal plane and later at 20x with 9 focal planes and at 40x with 5 focal planes. The whole slide images at 40x and with 5 focal planes were then viewed in an anonymized, randomized manner by an experienced pathologist. The pathologist rated the digital slides on *H. pylori* presence, if there was a need for immunohistochemistry and the experience compared to regular slides in light microscopy. The results were compared statistically. **Results:** Initial scanning at 40x did not allow for a confident detection of *H. pylori* in neither stain. Continuous scanning in 20x with 9 focal planes and later in 40x with 5 focal planes presented a more satisfactory result. **Conclusion:** The study showed that Modified Giemsa gave a better result than Alcian yellow-toluidine blue, for the detection of *H. pylori* in digital ventricle biopsy slides, but for confident detection in whole slide images the choice of magnification and number of focal planes seems to be the key for success.

Identification of *Haemophilus* and strict anaerobic bacterial isolates to the species level in premature infants compared to caesarean section and vaginal delivery.

By: Merve Tekcan

Bachelor thesis in Biomedical Laboratory Science performed at department of Infectious diseases, Institute of Biomedicine, University of Gothenburg, 2018.

Supervisor: Fei Sjöberg (PhD)

Background: The bacterial colonization during infancy is important for the human health. During pregnancy, the infant is in a sterile milieu. Immediately after birth the colonization begins with microbiota from the mother's vaginal and fecal flora for infants born vaginally. Infants born via caesarean section get their first colonizers from skin and surrounding milieu. The first colonizers on skin is species from the family Firmicutes and Actinobacteria. The oral microbiota has many species, but the dominant is species from the *Veillonella* genus. In the gut the dominant species are from the Firmicutes phylum. In the vaginal flora, the most dominant bacteria is species from the *Lactobacillus* genus.
It is still not entirely well understood how the etablishment of microbiota is between infants born via vaginal canal and caesarean section and how the early colonization patterns for each delivery affects the risk of developing diseases. Therefore, the aim of this study is to identify *Haemophilus* and anaerobic bacteria to species on skin, faeces, rectal and oral flora of 42 premature infants in comparison with vaginal and caesarean section birth.

Method: To determine skin, oral, intestinal and rectal microbiota of 42 premature infants, previous isolated bacterial samples recultured and analysed using Matrix assisted laser desorption ionization time of flight masspectrofotometry (Maldi-TOF). The ribosomal proteins for each bacteria separated by their time-of-flight and a database identified the *Haemophilus* and anaerobic bacterias to species.

Fisher's exact test was used to compare the bacterial colonization pattern of infants born via vaginal canal to infants born via caesarean section.

Results The *Haemophilus* samples showed a dominance of *Haemophilus influenzae* and *Haemophilus parainfluenzae* whether the infants was born via vaginal canal or caesarean section.
The anaerobic samples from rectal and faeces microbiome showed a dominance of *Bifidobacterium* spp, *Clostridium perfringens*, and *Lactobacillus* spp.
No statistical significance was found regarding the colonization pattern between the different delivery modes.

Conclusion Difference in colonization pattern was not detected for *Haemophilus* and anaerobic bacteria in oral, skin, rectal and fecal samples in relation to birth mode for premature infants. More studies are needed to understand how birth mode affects the colonization pattern and prematurity.

Platelet count in the platelet concentrate for transfusion varies depending on analysis instruments, while the content of ABO antibodies is low

by Ingeborg Eugenia Tulius

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Immunology and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg 2018.
Supervisor: Camilla Hesse, Senior lecture and Lisa Kylberg BMA

When Transfusion Medicine at Sahlgrenska University Hospital introduced a new method for blood component production, it was noted that TPK in the platelet concentrate was lower than the estimated PYI value. The aim of the study was to measure TPK in different platelet concentrates using different methods and to study the content of isoagglutinins after adjusting plasma levels. The study was performed by first preparing the platelet units and than measuring the TPK value with ABX Micros ES 60, Advia® 2120i and Cell-Dyn Sapphire™. Titration of isoagglutinins anti-A and anti-B was performed in the platelets apheresis of blood group O which was compared to blood donor plasma tubes. Titration of isoagglutinins was also performed in pooled platelet concentrates of blood group O and blood group A. The results of the study showed that platelet count in platelet concentrates for transfusion may vary depending on the instrument and method used and when using a factor, there was a better agreement. The PYI value from Reveos® was higher by comparing with the routine method Cell-Dyn Sapphire ™. The isoagglutinins anti-A and anti-B in the platelets apheresis with blood group O, resulted in 2 titer steps lower concentrations than in the blood donor plasma tube. The isoagglutinins concentrations in pooled platelets of blood groups O and A was lower. When CD61 histogram was analyzed the result showed that in 13 out of 50 cases there were too many events outside the CD61 limits. The study's conclusion is that the platelet count varies depending on the method and instrument used, but with the use of a factor there is a better agreement between the methods. Isoagglutinins anti-A and anti-B was low in all the units included in this study and because of that, this can make the basis to stop performing isoagglutinin titration in platelet concentrate but future research needs to be done.
The events that occurred when examining the CD61 histogram are unclear and this could be studied in the future.

Intake of resveratrol improves cardiovascular health in healthy, young adults.

A randomized clinical trial

By: Evelina Vesik

Bachelor thesis in Biomedical Laboratory Science performed at the Polytechnic Institute of Coimbra, Portugal, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Telmo Pereira, Senior lecturer, Ph.D.

Introduction

Cardiovascular disease is a worldwide problem and was the cause of death for 17,7 million people in 2015. One of the risk factors for dying prematurely by cardiovascular disease is arterial stiffness. Increased stiffness of the vessels leads to haemodynamic changes as a result of insufficient adaption to the changes of blood pressure and flow. This can later develop into left ventricle hypertrophy and serious cardiovascular disease. Natural bioactive substances such as the polyphenol resveratrol have interested scientists lately and promising results have been found regarding the effects on the cardiovascular system. However, few studies have been performed in humans and the effect on young, healthy people have not been investigated. Hence, we aimed to examine the effect of an ingestion of resveratrol on pulse wave velocity and carotid pulse wave in young, healthy adults.

Methods

A 30-days, randomized, controlled, single blinded, clinical study was performed to assess the effects of resveratrol supplementation by pulse wave velocity and pulse wave analysis, in 29 healthy, young adults. The participants were randomized into two groups; intervention and placebo, consisting of 14 and 15 individuals. They were administered 100mg resveratrol or placebo, per day, with evaluation before and after intervention.

Results

Resveratrol supplementation showed several significant improvements in parameters such as carotid-femoral pulse wave velocity, central and brachial diastolic blood pressure, central mean arterial pressure, heart rate, augmentation index and end-systolic pressure.

Conclusions

A long-term intake of a resveratrol improved cardiovascular health in young, healthy adults.

No correlation between non-invasive strain parameters and the invasive PCWP in heart transplant recipients

By Julia von Brömsen

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Physiology, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Entela Bollano MD, PhD, Bente Grüner Sveälv PhD

ABSTRACT

Background: Heart transplant (HTx) recipients are at risk of diastolic dysfunction and increased mortality. A reliable non-invasive marker like the left ventricular global longitudinal strain (LVGLS) could help identifying elevated filling pressures in this population and simplify long term follow-up.

Aim: The purpose of this study was to assess agreement between deformation imaging by 2D speckle-tracking echocardiography (STE) and cardiac catheterization (PCWP) in heart transplant (Htx) recipients.

Method: We evaluated 50 HTx patients who underwent 2D speckle-tracking echocardiographic imaging (STE) and right-sided heart catheterization at one year post transplantation at Sahlgrenska University Hospital. Left ventricular filling pressure (LVFP) was estimated invasively by measuring the pulmonary capillary wedge pressure (PCWP).

Results: We did not observe any significant relation between non-invasive STE-derived parameters and cardiac catheterization. The coefficient of variation (CV%) within two observers were: EF 7,22%, and GLS 22,5%.

Conclusion: This study indicates that non-invasive STE-derived parameters are lacking the predictability in estimating filling pressure and diastolic function in a HTx population and should be interpreted with caution.