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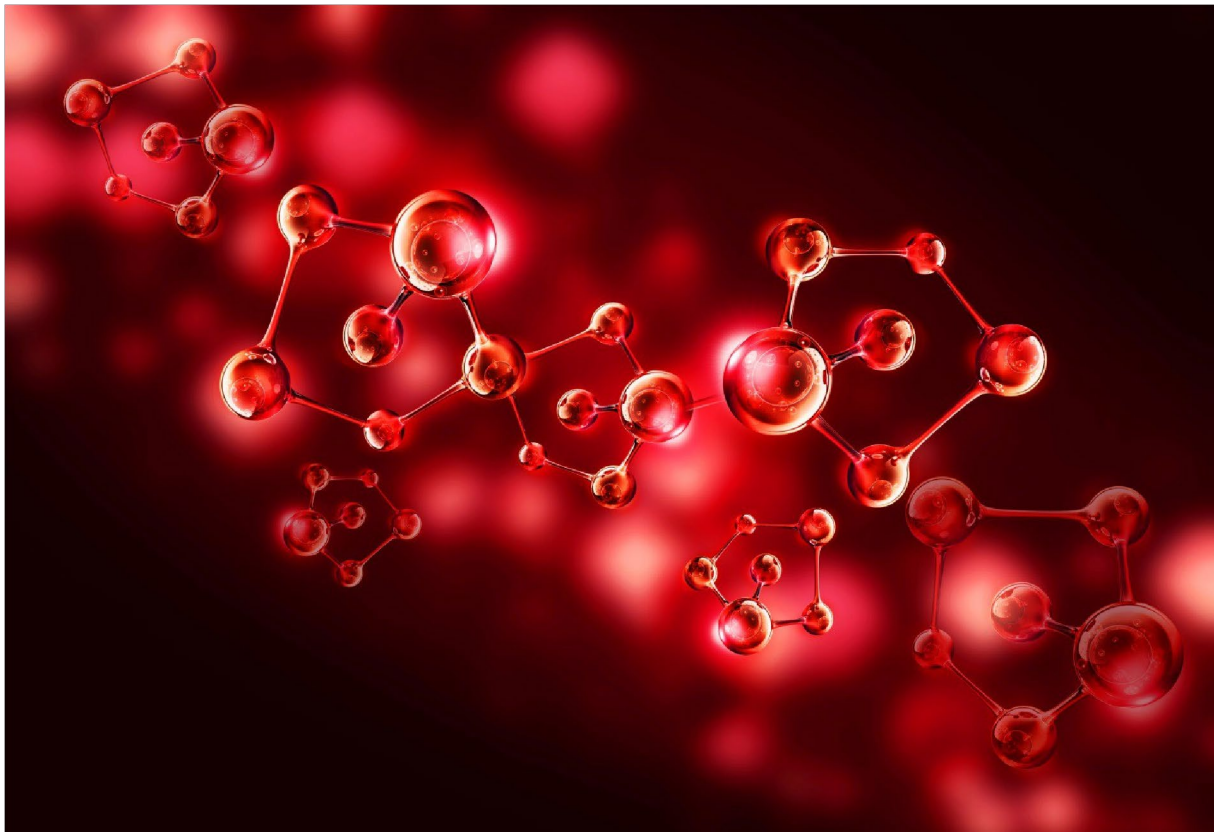

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Bachelor's and Master's Theses in Biomedical Laboratory Science

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Development and improvement of the enzymatic analysis of adenosine deaminase

By: Jihaan Abdirashid Farah
Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska Academy, University of Gothenburg, 2021.
Supervisors: Carlos Rodriguez Gonzalez and Thu Trinh

Background:
Adenosine deaminase is an enzyme that is part of the metabolism of purine and catalyzes the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine. If high levels of ADA are found in pleural fluid or ascites fluid, it can be linked to liver cirrhosis, hepatitis or mononucleosis. In the case of mutations in the ADA gene, the activity decreases or it is completely eliminated, which leads to the amount of deoxyadenosine increasing to levels that are toxic to the lymphocytes. This results in a sharp reduction in the amount of immune cells and you get symptoms that indicate severe combined immunodeficiency (SCID).

Aim:
The aim of the project is to improve and modernize the current method for analyzing the ADA activity by scaling down the amount of reagents and completing the reading in a new automated plate reader. And recalculate the reference interval of activity in erythrocytes with normalization per gram of hemoglobin

Method:
It was deemed necessary to examine whether the decrease in the total volume of the reaction affected the volatile product ammonia which evaporated in the ADA reaction. For this reason, the validation parameters dilution linearity, detection limit and precision were tested. In this study, 20 samples from healthy individuals were used to calculate the reference interval in erythrocytes. For the method comparison, the samples were run on the current instrument Ultrospec 8000 and then compared with the runs on the plate reader.

Results:
The method comparison for Ultrospec 8000 and Spectramax i3x on the 20 samples analyzed showed according to the statistical analysis Passing-Bablok regression analysis test a p-value> 0.2%.

Conclusion:
The conclusion was that one must supplement the study with another 120 samples to be able to say with certainty that there is no difference between the two methods as it results in a narrower 95% confidence interval.

Children with allergies at the age of 8 have lower number of bacteria in feces one week after birth

By Neda Abdollahi

Bachelor thesis in Biomedical Laboratory Science preformed at the Department of Infectious Diseases,
Sahlgrenska Academy, University of Gothenburg, 2021
Supervisor: Hardis Rabe (PhD), Monica Gio-Batta (MSc, MPH)

Background: Allergy is a major problem in society. Children living on farms have lower rates of allergy, but no one really knows why. However, it may be related to a more abundant microbiota in farm children that has an altered interaction with the immune system.

Purpose: The purpose of this study was to count the number of bacteria in the feces of children who either grew up on farms or not, over the children's first 6 months, and to identify how many bacteria were bound to IgA or IgE antibodies. A second purpose was to find out if these factors were linked to allergies later in childhood**.**

Method: The Farmflora study includes children and their families who either live in farms with cows (n = 24) or who live in the same area but no farms (n = 33). Fecal samples collected from 57 children at 1 week, 1 month and 6 months were analyzed by flow cytometry and the number of bacteria in the faeces was calculated, as well as the proportion of bacteria in the faeces bound by IgA or IgE antibodies.

Result: The number of bacteria in the faeces was constant between 1 week and 6 months of age among all children in the cohort and did not vary between farm children and non-farm children. However, the number of bacteria in feces was lower at 1 week of age in children with eczema at 8 years of age and tended to be lower during the first 6 months in children with asthma at 8 years of age. Most bacteria were not bound by IgA or IgE, although the proportion of bacteria in feces bound by these antibodies increased between 1 week and 6 months of age.

Conclusion: Low numbers of bacteria in feces during infancy may be related to allergies in later childhood. However, the number of bacteria in feces does not vary between farm and control infants, and so does not explain why farm children have lower allergies.

A validation study of microdialysis of glycerol with a Cuprophane catheter in abdominal adipose tissue of lean, obese and individuals with type two diabetes.

Stricter protocols may be needed too decrease the random error

By Hedda Ahlsén

Batchelor thesis in biomedical laboratory science preformed at Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg 2021

Supervisor Per-Anders Jansson MD, PHD

Background
With Microdialysis (MD) the concentration of substances in specific tissues can be measured as opposed to whole body concentration like through a blood sample. This paper will focus on its usage within metabolite monitoring of glycerol in adipose tissue to further research in obesity and diabetes.
Purpose
The purpose of this paper is to establish the coefficients of variance (CV), standard deviation (SD) and, additional validation of the reliability of microdialysis of glycerol with a custom made Cuprophane catheter in abdominal adipose tissue. To further validate microdialysis as a method and to provide valuable information on how future studies shod be designed.
Method
This paper includes 15 participants divided in three groups 7 individuals with type two diabetes, 6 obese individuals without diabetes and 2 lean individuals. Microdialysis with custom made Cuprophane catheters in the adipose tissue of the abdomen was performed during an oral glucose tolerance on two occasions with one week separation test. CV and interindividual standard deviation (IISD) was calculated with windows excel. Intraclass correlation (ICC) and Wilcoxon signed rank test was performed with SPSS.
Results
The overall CV was calculated to 27%. The CV per group was also computed per group. For the T2D group the CV was 19%, for the obese group 22% and for the lean group it was 3.6%. The overall IISD was calculated to 37 µmol/l. Each group had IISD values as follows: T2D 26 µmol/l, obese 30 µmol/l and, lean 5.5 µmol/l. Wilcoxon signed rank test did not indicate any statistically significant systematic deferens between glycerol concentrations in samples collected visit 2 and 3. The SPSS analysis of ICC gave a value of 0.676. As the ICC is between 0.5-0.75 the method of the study can be considered moderately reliable.
Discussion
The CV, IISD and the ICC all indicate a acceptable reliability for this method though less than desired. This random error could be due to the method or the variability of glycerol intra individually.
Conclusion
Research with the log of the mean, larger groups (especially lean individuals) and stricter protocol shou be conducted. Before definitive conclusions about the reliability of microdialysis of glycerol with a costume Cuprophane catheter in adipose tissue can be drawn.

Degree project in biomedical laboratory science, 15 higher education credits Basic level.

Preparation of alkaline phosphatase from Escherichia Coli C90 using osmotic shock and ion exchange chromatography in mini format

By Ibrahim Aleissa

Department where the project is carried out: Institution of Biomedicine, Sahlgrenska Academy, University of Gothenburg.2021

Supervisor: Martin Lidell

Alkaline phosphatase (ALP) is an enzyme found in various tissues and is linked to skeletal and liver function. The enzyme helps to cleave phosphate groups from different molecules in the body. The bone isoenzyme may be involved in mammalian bone calcification and the intestinal enzyme is thought to play an important role in the transport of phosphate into the intestinal epithelial cells. Cytochemical studies of Escherichia coli have shown that ALP is present in the preplasmic space and on the cell surface. ALP was detected with several substrates (ethanolamine phosphate, glycerophosphate, p-nitrophenyl phosphate and glucose-6-phosphate) over a wide pH range in a bacterial strain (C-90) known to be constitutive of this enzyme. The aim of this project is to further develop a previous laboratory, where ALP is purified from periplasmic lysate from E. coli with ion exchange chromatography, so that it can be performed with smaller packed columns and a faster elution process so that a greater focus can be placed on evaluating the contents of the fractions collected during the separation procedure. A periplasmic lysate from E. coli C90 produced by osmotic shock will be prepared and will be the starting material for the purification of ALP by ion exchange chromatography on 1 ml columns. Different elution gradients and types of anion exchangers (HiTrap DEAE FF, Q FF, ANX FF (HS) and Q XL) will be tested. The separation procedure is evaluated by studying the obtained chromatograms, by analyzing ALP activity in fractions and evaluating the purity of ALP by SDSPAGE with Coomassie staining and western blot. Finally, ALP will be purified from three different periplasmic lysates. The initial tests of protein separation on HiTrap DEAE FF with three different elution gradients showed that essentially three peaks appeared in the chromatograms. Enzyme activity measurements and analyzes by SDS-PAGE and western blot showed that the middle peak contained ALP with good purity centrally in the peak. The separation between the three peaks increased with a less steep elution gradient, but the gradient where Buffer B was increased by 0.5% / ml mobile phase was judged to have a sufficiently good separation of the three peaks with good peak height. During protein separation with this elution gradient on other anion exchangers, the three peaks were also seen, but the separation between the peaks was not significantly better than on HiTrap DEAE FF. The final purifications of ALP from three different periplasmic lysates showed that the three peaks were visible in all samples but that the central ALP peak varied slightly in peak height in relation to the other peaks. The results obtained show that it is possible to purify ALP in a fast way with the methodology developed here and that greater focus can be placed on evaluation of fractions' ALP content and ALP purity in a future laboratory on the BMA program.

Anti-neutrophil cytoplasmic antibody test identified Autoantibodies against eosinophils - Signs of Liver Disease?

By Noah Al Jabban

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Immunology – Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Pontus Thulin, M.D., MSc.

Routine testing for anti-neutrophil cytoplasmic antibodies by indirect immunofluorescence (IIF) at the Department of Clinical Immunology at Sahlgrenska University hospital revealed that approximately 1 % of all anti-neutrophil cytoplasmic antibody (ANCA) IIF ordered tests were positive for antibodies that reacted with eosinophils. Background, eosinophils have been observed in autoimmune diseases of the liver such as primary biliary cirrhosis, and antibodies specific for the eosinophil specific protein eosinophil peroxidase have been detected in several liver diseases such as primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) but also in infectious hepatitis. However, in the only study of patients with autoantibodies to eosinophils that have been published they identified eight patients using the ANCA IIF method, and none of these patients were found to have liver diseases. Due to the frequent finding of these autoantibodies, about 25 cases yearly only in our department, and the lack of scientific literature about these antibodies a study was initiated. The aim of this project is to investigate the possibility that these patients may have developing autoimmune liver disease. Methods in this study, patients (n=36) were included on the basis of a positive eosinophil staining in routine ANCA IIF analysis, and their medical records were evaluated, new samples were analysed for a wide spectrum of autoimmune diseases and they are currently on follow up to investigate any developing disease. Routine methods at the department of Immunology includes ANCA IIF to ensure that all patients have antibodies reactive with eosinophils, measurements of 11 different autoantibodies that are associated with liver diseases, we measured serum immunoglobulins (IgG, IgA and IgM) levels since increased levels of IgG is commonly seen in AIH, and increased levels of serum IgM is commonly seen in PBC. As a result, two dyeing patterns could be discerned, a pattern with negative neutrophils and positive eosinophils (group-1), and a pattern where both stained (group-2). In total 29 patient's serum were analysed in immunoblots, of those samples four hade at least one positive result and it was noted that group-1 had six additional patients with one to three limit values. and we conclude that the included patients need further follow-up, and that we have reasons to initiate studies to evaluate the presence of anti-eosinophil antibodies in patients with different liver diseases, especially autoimmune liver diseases.

Evaluation of various diagnostic biomarkers in histological material from patients with primary and metastatic cutaneous malignant melanoma

By Mohamad Alslamah

Basic level in Biomedical Laboratory Science performed at the pathology and cytology laboratory, Halland hospital, Halmstad city, sweden 2021
Supervisor: Tomas Seidal, Consultant, Assistant Professor

Abstract

Background: Malignant melanoma **(**MM) is a type of skin cancer that arise from melanocytes (melanin-producing cells). The disease has been steadily increased dramatically worldwide within the last decades (almost 4000 incidence/year in Sweden). It causes the death twice more than traffic accidents caused. The major risks for melanoma development are the fair skin that does not tan, solar UV-radiation, the number and type of pigmented nevei and a family history of melanoma.Tumors can be primary (confined in situ) or metastasis(cancer cells migrate to another organ and builds a new colony). Early and correct diagnosis, and radically surgical excision is still the best way for survival rates.. Immunohistochemistry is one of the methods that are used to detect and distinguish MM, because of the high sensitivity and specificity between some antibodies and the target antigen in the inspected tissue. In this study five antibodies (HMB45, SOX10, S100, Melan A, MITF) were used on primary and metastasis samples with high content of tumor cells, to see which of the antigens that gave the highest expression in primary and metastasis MM.

Material and methods: A total of 40 samples were used in this study. All the samples were picked up from the archive between Jan 2020 and Sep 2021, with the help of the data program "CGM, Analytix" at the pathology and cytology department in Halland hospital in Halmstad, Sweden. Haematoxylin and Eosin stained preparates were tested in the microscope to choose the samples that contained most tumor cells**.** 20 histologic samples with primary MM were used to check the expression of HMB45, SOX10, S100, MITF and Melan A. 20 samples with metastasis MM were used to check the expression of SOX10, S100, Melan A and MITF

Results and interpretation: The study showed that the median age of the patients when diagnosed with MM was about 71 years old. The primary incidence for the men was less than it was for the women (40% for men, 60% for women). On another side was the metastasis incidence higher for men (65% for men and 35% for women). That could be because the women expose themselves more to the UV-radiation and at the sam time they are more careful than the men and visit the dermatologist earlier. All the antigens were expressed at different levels, HMB45 was positive in 17/20 (85%) of primary MM samples. SOX10, S100 were expressed in 40/40 (100%) of primary and metastasis MM samples. Both Melan A and MITF were expressed in 20/20 (100%) of primary MM samples and in 19/20 (95%) of metastasis MM samples. Both S100 and SOX10 showed high sensitivity but HMB45, Melan A and MITF showed a higher specific

Conclusion: The study revealed that SOX10 and S100 were the most reliable markers and they were expressed in all samples, therefor they can be used with a great confidence in the laboratory diagnose of MM.

Identification of a plasma-borne signal that stimulates the release of sphingosine-1-phosphate from red blood cells

By: Nisrein Al-Suttari

Bachelor thesis in Biomedical Laboratory Science performed at the department of Biology and Environmental Science, University of Gothenburg, 2021.

Supervisor: Anders K. Nilsson, PhD. Researcher

Sphingosin-1-phosphate (S1P) is a bioactive sphingolipid metabolite that is important in many physiological and cellular processes such as inflammation, atherosclerosis, immunity and cell proliferation. S1P is excreted from red blood cells and platelets in the circulation. The aim of this work is to set up a method and verify the previous results that plasma carries a signal that stimulates the release of S1P from red blood cells (RBCs), Further; the work involves the isolation and characterization of a plasma-borne signal that can stimulate S1P from RBCs.

Blood was collected from one individual and after separating RBCs and cell-free plasma, RBCs were incubated in various concentrations of plasma (0- 25% - 50% - 75% - 100%) and then the plasma/media were analyzed for S1P. Different incubation times were also examined (0-4 hours). In addition, the amount of RBCs released S1P was analyzed after incubation with different types of plasma (EDTA, sodium citrate and lithium heparin) or serum. Furthermore, isolation of a plasma fraction that could stimulate S1P release from RBCs was sought for by ultra-filtration, protein precipitation and albumin/IgG depletion. S1P was quantified by liquid chromatography coupled to mass spectrometry.

The results show that S1P concentration is higher in plasma incubated with RBCs. Also, the plasma S1P concentrations were increased with longer incubation time. The protein precipitation studies suggested that the S1P-inducing factor is a protein. The ultra filtration method results showed that concentrates retained the activity signal of S1P and that the S1P-inducing factor has a molecular mass over 100 kDa. This factor disappears after albumin/IgG depletion of serum.

In conclusion plasma-induced release of S1P from red blood cells is a process that takes place over several hours and release of S1P is depending on a factor, most likely a large protein or a protein complex, found in plasma.

Left ventricle diameter versus left ventricle volume – a strong association between the two variables and their importance for assessment of left ventricular size

By: Rebecka Ardevik

Bachelor thesis in Biomedical Laboratory Science performed at the department of physiology at Norra Älvsborgs Länssjukhus, Trollhättan, 2021

Supervisor: Sofie Ahlin, MD PhD

Introduction: Dilatation of the left ventricle can be a sign of several pathological conditions in the heart. The measurement of left ventricular dimension is therefore an important parameter included in the standard echocardiographic examination of the heart. Today, the two mainly used methods to estimate left ventricular size are measurements of end diastolic diameter in parasternal long axis view and volume estimation using Simpson's biplane method in apical two- and four-chamber views. The aim of this study was to investigate the association between left ventricular size estimated with end diastolic left ventricle diameter and left ventricular volume measured with Simpson biplane method and investigate which variable of these variables that was primarily used for final assessment of left ventricular size.

Method: Data from 653 echocardiographic examinations, was collected at the department of a clinical physiology at Norra Älvsborg Länssjukhus in Trollhättan. Left ventricular diameter measured in parasternal long axis view and left ventricular volume calculated by Simpsons biplane method were assessed. Left ventricular size based on the two measures were then estimated according to the department's reference values and was then compared with the final assessment in the echocardiographic report. To compare distribution of categorical data, Fisher's exact test and Chi-Square test were used and for comparison of continuous data, student's T-test was used. Correlation analysis of ordinal data was tested with Chi-square test and the correlation between end diastolic left ventricle diameter and left ventricle volume was tested with Pearson's correlation test.

Result: For the left ventricle volume estimated with the Simpson biplane method, 87,2% (n=260) of the estimation of left ventricle size based on the reference values matched the final assessment. For the left ventricle diameter measured in parasternal along axis view, 84,3 % (n=413) of the estimation of left ventricle size based on reference value was consistent with the final assessment. In 16 exams, the final assessment of ventricle size did not match any of the left ventricle size assessment achieved with left ventricle diameter or left ventricle volume based on reference values. Furthermore, the left ventricle diameter was present in a significantly higher proportion of the examinations than left ventricle volume. However, in examinations where both left ventricle diameter dimensions and left ventricle volume were specified but did not match, the final assessment of ventricle size tended to be based on left ventricle volume (13,3 %, n=39) instead of left ventricle diameter (7,5 %, n = 22).

Conclusion: There was a strong association between left ventricle size based on the reference assessments of left ventricle volume and left ventricle diameter and the final left ventricle assessment. In cases where both left ventricle diameter and left ventricle volume were available but did not match there was a tendency to rely more on the of the left ventricle volume estimation.

Validation of B-cell isolation and memory B-cells for children with inflammatory bowel disease.

By Fanny Asp

Bachelor thesis in biomedical Laboratory Science performed at the Department of Infectious diseases, Sahlgrenska Academy, University of Gothenburg, Gothenburg, 2021.

Supervisor: Hardis Rabe, PhD

Background: Inflammatory bowel disease (IBD) is a chronical inflammatory conditions in the gastrointestinal tract that can be divide in to the two subgroups, Crohns disease and Ulcerative colitis. Children with IBD are more often affected by severe inflammation compare to adults with IBD.

Aim: The aim of this study is to validate and optimize the isolation of B cells with the use of the commercial kit MagnisortTM Human B cell Enrichment KIT (Invitrogen). Another aim was to examine how treatment of Crohn's disease and ulcerative colitis affected the levels of these children's CD27 + memory B cells in the blood.

Method: Blood from 10 healthy adults were used to optimize the B-cell isolation kit MagnisortTM Human B-cell Enrichment KIT. In IBD study, the proportion of $CD27^+$ memory B cells were examined by flow cytometry in the blood of children diagnosed but not treated for ulcerative colitis (n = 12) and Crohn's disease (n = 8) or children with an ongoing treatment, for ulcerative colitis (n = 3) and Crohn's disease (n = 11), as well as a control group (n = 9) that included children whit no inflammation in the gastrointestinal tract.

Result: The percentage of purified B cells when optimizing the commercial kit were at its lowest 3% and at most 30% isolated B cells, compared to the promised 83% in the kit protocol. Furthermore, we found that children with newly diagnosed untreated Crohn's disease had lower levels of $CD27^+$ memory B cells (10%) compared to control children (26%). While treatment of the Crohn's disease increased the proportion of circulating $CD27^+$ memory B cells to 16%. There was no significant difference in the percentage of CD27 + memory B cells in children with ulcerative colitis and control children.

Conclusion: The best results of B cells isolation were obtained when the amount of antibodies were doubled and the incubation time for both antibodies and magnetic beads were increased. We confirmed that children with Crohn's disease have lower proportions of memory B cells in the blood compared to control children. Treatment with anti-inflammatory drugs increases the proportion of memory B cells in the blood of children with Crohn's disease.

NO IMPACT ON SEROTYPE EXPRESSION WHEN COMBINING MUTANT AND WILD TYPE *wbeT* GENES IN O1 VIBRIO CHOLERAE

By Julie Ayala Edman

Bachelor thesis in Biomedical laboratory science performed at the department of immunology and microbiology, Institute of Biomedicine, Gothenburg University.
Sahlgrenska Academy, BMA062, VT2021
Supervisor: Dr. Michael Lebens, scientist

Abstract

Background and aim: Cholera is a severe diarrheal disease caused by infection with the bacterium *Vibrio cholerae*, primarily of the O1 serogroup. The O1 serogroup is further divided into two serotypes named Inaba and Ogawa that are both known to cause disease. Oral cholera vaccines (OCV) are an important aspect of cholera control, but the currently licensed OCVs have a complex and expensive manufacturing process due to the use of several different *V. cholerae* strains to achieve sufficient antigen representation. In previous studies, O1 *V. cholerae* strains expressing both Inaba and Ogawa antigens have been produced by manipulation of the *wbeT* gene, in efforts to simplify the composition and reduce production costs of OCV. In the current study we explore an alternative method of regulating serotype expression by combining a mutant wbeT gene with an endogenous wild type gene of a O1 *V. cholerae* of the Ogawa serotype. **Methods:** Plasmid vectors with the wbeT mutants were constructed using PCR, restriction analysis and ligation by DNA ligase. The vectors were transformed using electroporation into electrocompetent cells for cloning and were then introduced to the Ogawa O1 *V. cholerae* strain by conjugation. Serotype was tested using agglutination with Ogawa- and Inaba-specific antibodies. **Results:** Two different mutants of the wbeT gene (S158F and S158P) were conjugated into recipient strains of *V. cholerae*. Agglutination tests of the two produced strains showed agglutination with anti-Ogawa antibodies and no agglutination with anti-Inaba for both strains. **Conclusion:** Combination of mutants used in this study with a wild type *wbeT* gene does not affect serotype expression. The gene product of the wild type *wbeT* gene seems to be dominant when combined with a mutant *wbeT* gene product.

The role of telomere derived noncoding RNA in maintaining the alternative lengthening of telomeres phenotype

By Frida Bengtsson

Bachelor thesis in Biomedical Laboratory Science performed at Department of Laboratory Science at Sahlgrenska University Hospital, Gothenburg 2021
Supervisors: Tanmoy Mondal, Associate Professor and Roshan Vaid, Post-Doctoral Researcher

Neuroblastoma is a childhood cancer and origin from an embryonic neoplasm arising from primitive neuronal crest cells in the sympathetic ganglia or adrenal medulla. The cancer can be divided into four stages; very low, low, intermediate and high-risk neuroblastoma. The three stages first mentioned usually have a good prognosis compared to high-risk neuroblastoma which has a high mortality rate and the patients do not respond well to the drugs given today. Some neuroblastomas are positive for alternative lengthening of telomere through homologous recombination. In this project the role of telomere derived non-coding RNA (TERRA) will be studied which may contribute to the alternative lengthening of telomere. Further, better understanding could lead to the development of new better drugs. In this study U2SO cells are treated with DNA damaging agent to induce telomere damage and upregulate TERRA expression from telomere. After the cells are fixed with formaldehyde to stabilize the RNA-protein complex followed by nuclear lysis by sonication. Beads coated with streptavidin are added to pull down TERRA which is captured by biotin oligo. TERRA is later isolated and converted to cDNA, expression of TERRA is measured by qPCR. The TERRA interacting proteins will be captured with an antisense probe tagged with biotin and analyzed by using western blot or mass spectrometry. After three different trials the study has come up with a good protocol to target TERRA. Unfortunately, no TERRA interacting proteins could be detected by using western blot method. Proteins have also been sent for mass spectrometric analyzes, but so far, no results have been attained. In conclusion this study has come up with a good protocol to capture TERRA but RNA bound proteins still need to be characterized. The result from mass spectrometry will determine how to proceed. If new proteins are detected this will allow further studies of how RNA bound proteins help in telomere maintenance in alternative lengthening positive cells. If no proteins are detected the procedure has to be troubleshooted and improved.

Cancer-related splice variant of phosphoinositide-3-kinase increases phosphorylation of signaling proteins and impacts cell metabolism – despite lacking a catalytic domain

By Therese Bidesjö

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Chemistry laboratory, Sahlgrenska Academy, University of Gothenburg, 2021.

Supervisor: Victoria Rotter Sopasakis, PhD, Associate Professor.

Background: Phosphoinositol-3-kinase (PI3K) is a protein highly involved in cell metabolism and proliferation since it is a central node in the PI3K-pathway. The protein consists of two subunits: a regulatory subunit, p85, and a catalytic subunit, p110. A novel splice variant of the p110α, named p13α, has recently been discovered by the research group in which this study takes place. Though this protein lacks a catalytic domain it has been found in human tumors and shows increased proliferative effects and activation of Akt when overexpressed in cell culture.

Aim: This study aimed to elucidate the impact of p13α on cell metabolism and circulating nutrients.

Materials and methods: Human embryonal kidney cells (HEK293) were used for Western Blot-analysis to study the effects of p13α on intracellular proteins involved in cell metabolism. In addition, hemolymph of *Drosophila melanogaster* flies and larvae were used for enzymatic measurements of circulating glucose, trehalose and diglycerides. Both the HEK293-cells and *D. melanogaster* were previously genetically modified to resolve the effects of p13α in comparison to the reference p110α and controls.

Results: The results demonstrated that p13α increased the cellular levels of p-Akt and p-GSK3β which indicates impact on the PI3K-pathway. Moreover, p13α increased the cellular levels of p-AMPKα, PPARα and PGC1α and hence impact the fat metabolism by upregulating beta oxidation of fatty acids thereby reducing the concentrations of circulating diglycerides. The results also suggest that p13α enhance gluconeogenesis thus elevating glucose concentrations in the circulation.

Conclusion: The impacts of p13α are beneficial for cancer cells since this protein might provide such cells with large amounts of energy from beta oxidation and gluconeogenesis. It is also believed to induce a diabetes-type-2 like state and thereby create a vicious circle by increasing tumorigenesis. Further research should be done to elucidate the full mechanisms of p13α and its impact on cancer and diabetes-type-2.

THE LEVEL OF COMPLEASOME COMPLEX (ANTISECRETORY FACTOR - COMPLEMENT FACTOR C3c) IS HIGHEST IN CEREBROSPINAL FLUID IN THE ACUTE PHASE OF PATIENTS WITH TICK BORNE ENCEPHALITIS (TBE).

By Emil Blomgren

Bachelor thesis in Biomedical Laboratory Science performed at Clinical microbiology, Sahlgrenska Academy, University of Gothenburg, 2021 Supervisor: Ewa Johansson, Phd

Background: Tick borne encephalitis (TBE), is one of the most common causes of viral encephalitis in Sweden. The complement system is activated in different ways in the central nervous system in TBE and in herpes simplex encephalitis (HSE). Antisecretory factor (AF) is a protein that reduces inflammation and normalizes secretion processes. The proteasome subunit AF forms a complex with complement factors, called compleasome. With antibodies directed against AF or against another subunit of the proteasome together with antibodies against complement factor C3c, the basal compleasome level or level of specific AF compleasome is determined in a sandwich ELISA. High levels of compleasom have been measured in cerebrospinal fluid (CSF) collected from patients suffering from HSE especially in the acute phase of the disease.

Aim: Was partly to produce a stable reference sample and to use it as a relative reference in sandwich ELISA analyzes. Then analyze the compleasom level in CSF (n=42) and in plasma (n=33) collected from patients (n=27) in the acute and later phase of TBE and examine the relationship between AF complex level in the acute phase and degree of recovery over time. Also compare the levels of AF complex at TBE with previously determined levels at HSE.

Method: Plasma was activated in vitro so that a reference sample (p-sample) was prepared with 10x elevated levels of AF compleasome shown in sandwich ELISA and by Western blot analysis. With two variants of sandwich ELISA, AF compleasome and basal compleasome levels were analyzed in CSF and plasma samples from TBE patients compared with samples taken from healthy control subjects (n=17).

Results: AF compleasom and compleasom levels in CSF were highest in the acute phase of the disease and decreased over time and were significantly higher than the levels in healthy control individuals (p = 6.50E-07 and p = 2.04E-05, respectively). The overtime pattern of AF / compleasom is similar to that previously seen in CSF in HSE patients. Western blot analysis confirmed the formation of C3c in acute phase samples. The levels of AF compleasom and compleasom in plasma samples from TBE patients were significantly higher than the levels in healthy control subjects (p = <0.05), but unlike CSF levels did not decrease over time. There is a tendency for there to be a correlation between high levels of compleasom in the acute phase and full recovery, determined by the Glasgow outcome scale (GOS).

Conclusion: After the introduction of relative unit units based on the produced reference protein, future analyzes can become comparable. The high levels of AF / compleasom in the acute phase may indicate that this complex has an important part in the immune response in TBE infection and may be a marker for which phase the disease is in.

Confirmation of transfection containing dCas9 to Saccharomyces cerevisiae strains before detection of switches between DNA polymerases at physical obstacles on the DNA strand

By Erika Bystedt

Bachelor Thesis in Biomedical Laboratory Science performed at the Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Anders Ranegaard Clausen, PhD

DNA polymerases can lose their processivity if they encounter DNA-binding proteins during synthesis. Dead Cas9 (dCas9) is a DNA binding protein that can bind to specific sites in the genome. The aim of the project is to find out if dCas9 is overexpressed after transformation into strains of Saccharomyces Cerevisiae that carry various mutations of DNA polymerases. A plasmid containing guideRNA carrying dCas9 was used for the transformation. This dCas9 is designed to simulate a DNA binding protein and bind to specific sites in the genome and this in turn can result in more switches between different DNA polymerases. If dCas9 is overexpressed, it means that the transformation has been successful. It was measured using qPCR and compared with the reference gene actin.

This will then be used to study with HydEn-Seq which is a whole genome sequencing and thus see if there are switches between different polymerases as dCas9 binds to the DNA strand and stops the replication fork. If a change occurs, it could be to a polymerase with lower processivity and this in itself could result in more mutations which need to be studied further to develop the method of dCas9 as a roadblock of replication and study this process.

Children who have undergone liver transplantation has higher levels of cytokines and activation of T-cells.

By Linnea Sofie Ellburg

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, 2021

Supervisor: Hardis Rabe, PhD

Background: The prevalence of liver transplanted children who develop post-transplant disorders has increased rapidly over the last few years. Autoantibodies, IBD and autoimmune hepatitis are some of the common conditions. However, the frequency of developing food allergies is greatest. The condition is known as LFTA (liver transplanted associated food allergy) and affects roughly 40 % of the children. The mechanism causing the development of food allergy after paediatric liver transplantation is unknown, however it is believed it is initiated by an immunological dysfunction caused by the transplantation itself, liver failure or the consumption of immunosuppression at an early stage of life.

Aim: The aim of this study was to investigate the cytokine profile and the activation of T-cells in the adaptive immune system in children who have undergone liver transplantation and compare the activation mechanism with healthy controls and food allergic children with no history of liver failure. We wanted to increase the knowledge surrounding the immunological dysfunction causing LFTA.

Material and methods: A cross-sectional study including serum from liver transplanted children (n=43), healthy controls (n=26) and food allergic children (n=8) was performed. The levels of the cytokine's TNF-α, IL-13, IL-4, IL-10, IL-6, IL-2, TNF-β, IFN-γ, IL-17A, IL-12p70, APRIL, BAFF and sCD40L were measured utilizing flow cytometry. The levels of activated $CD4^+$ and $CD8^+$ T-cells who express HLA-DR and CD38 were also measured using flow cytometry.

Results: Children who have undergone liver transplantation had higher levels of cytokines compared to the healthy and food allergic children. Children who developed LFTA even had higher levels of IL-10, IL-17A and IFN-γ compared to food allergic children with no history of liver failure. Liver transplanted children also had a higher percentage of $CD4^+$ and $CD8^+$ T-cells which express $HLA\text{-}DR^+CD38^+$ compared to the healthy and food allergic children.

Conclusion: Children who have undergone liver transplantation regardless development of LFTA has a more activated immune system compared to healthy and food allergic children.

THE IMPACT OF SEX AND EXPRESSION OF THE ALFA 7 NICOTINIC ACETYLCHOLINE RECEPTOR IN ATHEROSCLEROSIS

Alice Enoh Jansson

Essay: 15 hp

Program: Biomedicinska analytikerprogrammet

Level: First Cycle

Semester/Year: St 2021

Supervisor: Maria Johansson, PhD, Associate Professor

Background

Cardiovascular disease is the main cause of death globally, where atherosclerosis is the most common underlying cause. Women who have not yet entered menopause are at lower risk of developing cardiovascular diseases than men. In atherosclerosis there is an accumulation of fat and immune cells in the arterial wall creating plaques that occlude the bloodstream. This can eventually cause heart attack and stroke. Atherosclerosis is characterized by several inflammatory processes, but exactly what types of cells and receptors that contribute to this chronic inflammation is yet to be determined. One receptor that is believed to have immunoregulating properties is the alpha 7 nicotinic acetylcholine receptor (α7nAChR).

Purpose

The aim of this study was to investigate whether lack of α7nAChR affects the progression and immune cell composition of atherosclerosis in mice and to explore potential differences between the sexes.

Methods

In this study we performed immunohistochemistry targeted towards the two cell markers VCAM-1 and CD68 on the aortic root retrieved from both male and female mice. A portion of the mice were “knockout mice” lacking α7nAChR and were matched with sibling controls with an intact receptor expression (wild type).

Results

The results showed no significant difference in the plaque composition between the knockout mice and the wild type mice within the same sex. However, there were significant differences between the sexes in total plaque area, area of positive staining and perimeter, where female mice showed higher degree of atherosclerosis, increased amount of CD68 and VCAM-1 expressing cells, as well as increased vascular remodeling compared with male mice.

Conclusion

The study shows that sex has an influence in progression of atherosclerosis in mice. Since human plaques are thought to have similar composition and progression as plaques in mice, this conclusion may be applicable to atherosclerosis in humans as well.

Typing of norovirus GII.4 with the Ion Torrent technique

Preparation and sequencing of patient samples

By Isabella Erlandsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, 2021

Supervisor: Maria Andersson, Biomedical Scientist, PhD

Background: The norovirus is highly infectious and causes acute gastroenteritis with vomiting and diarrhoea as main symptoms. The spread of noroviruses in hospital environments lead to a great strain on the care system, with staff on sick leave and fragile patients being at risk of dying from the infection. The most common virus genotype to cause big outbreaks is GII.4. NGS has previously been proven useful to keep track of which genotypes are in circulation. Ion Torrent is a type of next-generation sequencing where the release of protons during the addition of correct nucleotides to a complementary DNA strand is measured, and the template strand is simultaneously sequenced.

Aim: The aim of this part of the study was to prepare samples for Ion Torrent and to sequence those samples. Later on in the study, after this project, the main goal is to investigate whether Ion Torrent could be helpful in finding deficiencies in the care system that lead to a higher spread of infection, and by that limit future norovirus outbreaks in hospital environments.

Method: Stool samples were gathered from hospitalized patients with an ongoing norovirus infection. The samples were prepared for sequencing through RNA extraction, identification of GII.4 through real-time PCR, production of cDNA and addition of fusion primers and barcodes to the amplicons. The sequences' size and quantity were then examined through gel electrophoresis before the libraries were prepared for, and later sequenced with, the Ion Torrent technique. A phylogenetic tree was created to interpret the results.

Results: Out of the 130 original norovirus samples, in the end 94 samples were identified as GII.4 and successfully sequenced. The phylogenetic tree showed two main groups of closely related viruses.

Conclusion: We found that the method for preparation gave satisfactory sequencing results. The completion of the larger study is needed before any definitive conclusions can be drawn, but the results suggest that the Ion Torrent method could be a good candidate for making contact tracing more effective in hospitals in the future.

Whole genome sequencing of SARS-CoV-2

” A comparison between different sequencing technologies”

By Leyli Faqiripour

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Josefin Olausson, Hedvig Engström Jakobsson

It is becoming increasingly common today for alternative developed molecular biological methods to replace the classical methods in microbiological diagnostics. Next Generation Sequencing (NGS) allows, unlike previous methods, massive parallel sequencing so that millions of sequences can be analyzed bioinformatically. Illumina and Ion Torrent are dominant well-functioning NGS platforms for sequencing, but the Nanopore technology can provide several benefits such as the ability to read longer fragments and a time and cost efficiency. To maximize the sequencing efficiency and quality, size selection is performed on the library, this by using methods based on gel electrophoresis or magnetic beads. The purpose of this study is to investigate whether Nanopore technology is equivalent in specificity and performance compared to Ion Torrent and illumina and whether AMPure XP Beads can be used for size selection and give equivalent results as Pippin Prep.

To obtain whole-genome-sequences directly from clinical samples, nanopore sequencing was performed using a modified ARTIC protocol in a flow cell. The results were compared with the same whole genome sequences obtained from Ion Torrent and Illumina. Brain biopsies and cerebrospinal fluid previously purified with Pippin Prep were purified using AMPure XP Beads following a protocol for size selection of adapter-ligated DNA from BioLabs.

The results from the sequencing showed that Nanopore provides the same virus type as Ion Torrent and Illumina and that all platforms used in this study have their advantages and disadvantages. Size selection with AMPure XP Beads is an effective method for cleaning libraries, however, Pippin Prep is recommended to be used for more accurate results, especially when you have low input such as RNA libraries.

Abstract

A comparison between rapid testing devices and Liquid Chromatography-High Resolution Mass Spectrometry for detection of drugs of abuse in oral fluid

By: Catrin Fredriksson

Bachelor thesis in Biomedical Laboratory Science performed at Clinical chemistry, Diagnostic Mass Spectrometry & Chromatography, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Moa Andresen Bergström, PhD

Clinical analysis for drugs of abuse are usually carried out in urine. During the last 10 years oral fluid has become a more commonly used sampling matrix since the collection is easier to observe and analytical techniques have improved. Following this trend, several commercially available rapid drug testing devices for oral fluid have emerged. Rapid testing devices are based on immunochromatography for the detection of drugs of abuse and produce test results within 5-10 minutes, as they do not require the sample to be sent for laboratory analysis. Three rapid testing devices Multi-drug cup, Surestep™ and abc2Multi16-88 were evaluated using authentic patient samples and compared to drug analysis using qualitative and quantitative analysis by liquid chromatography-high resolution mass spectrometry analysis (LC-HRMS). Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated for each device.

Patient samples were tested and pooled to generate a test set of 20 samples which covered 15 dugs/drug classes. For the rapid testing devices, results showed a good total sensitivity (>80%) for Surestep and Abc2multi16-88 tests. Specificity was satisfactory (>80%) for all tests. None of the tests gave a positive tetrahydrocannabinol result and gave several false positives for methadone and fentanyl. Detection of amphetamine and opiates gave the highest true positive results out of the targeted substances. A major concern regarding the rapid testing devices is the subjectivity in interpreting the results. Rapid testing devices offer a preliminary analysis and positive samples must undergo a confirmatory analysis. LC-HRMS can provide full scans of samples but requires several steps for sample extraction and processing of result. This process is time consuming and a disadvantage for the LC-HRMS. Nevertheless, LC-HRMS continues to be an excellent tool for detection and identification of drugs of abuse.

Evaluation of a CHROMagar MRSA for more efficient detection of Methicillin resistant *Staphylococcus aureus* (MRSA)

By Tiffany Giang

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Microbiology laboratory at Kalmar County Hospital, 2021
Supervisors: Jorge Hernandez, PhD and Annika Wistedt, PhD

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) occurs worldwide and is a common cause of nosocomial infections. Treatment of infections caused by MRSA is problematic because these stains are resistant to all beta-lactam antibiotics. MRSA infections are often associated with longer hospital stays, longer antibiotic treatment, and higher costs. The ideal laboratory method to detect MRSA should have high sensitivity and specificity and give results within the shortest time possible.

Aim: The aim of this study is to evaluate a selective and differentiation cefoxitin-containing chromogenic agar for MRSA detection and to determine the minimum concentration of MRSA-strains required to grow on the agar plate compared to currently used agar media (SAIDE) for clinical diagnostics.

Method: Three control stains were diluted 1:10 in seven steps. Each dilution step was inoculated on horse blood agar and CHROMagar MRSA. Susceptibility testing was also performed on a positive control strain inoculated on a Mueller Hinton agar with various antibiotics, including Cefoxitin. Known MRSA strains were used and diluted from 1:10 in five steps. The last three dilution steps were inoculated on horse blood agar, SAIDE agar and CHROMagar MRSA.

Results: The number of colonies did not vary between the different agar with the same dilution. Some samples only containing 15 CFU/ml were able to grow on CHROMagar MRSA but samples containing 150/ml all grew on the agar plate. Most MRSA strains were detected on CHROMagar MRSA after 24 hours of incubation meanwhile some slow-growing MRSA stains were detected only after 48 hours.

Conclusion: The growth of MRSA strains does not vary between different agars and CHROMagar MRSA can detect samples containing only 150 CFU/ml MRSA stains. MRSA strains can be detected on CHROMagar MRSA after 24 hours, but some slow-growing strains can take up to 48 hours. To compare CHROMagar MRSA with the enrichment method, more tests must be performed.

Production and purification of S1-domains from different coronaviruses, for future use as serological antigens

By Maria Güven

Bachelor thesis in Biomedical Laboratory Science performed at the Mammalian Protein Expression core facility, Sahlgrenska Academy, University of Gothenburg, 2021.

Supervisors: Malin Bäckström, associate professor, Tomas Bergström, professor & Mikael Andersson, M Sci

Background: One issue that has taken centre stage in the fight against the global pandemic is to be able to determine immunity after the coronavirus infection and after Covid-19 vaccination. The lack of IgG-tests with high sensitivity and specificity is large for the following coronaviruses that infect humans: HCoV-HKU1, HCoV-229E, HCoV-OC43 and HCoV-NL63. The S1-domain on the Spike protein in the virus envelope is probably the domain that works best as a virus-specific antigen, as it is involved in receptor binding to the host cell and enable infection and is therefore a good target for antibodies.

The aim of this project was to use recombinant technology to express, produce and purify the S1-domain of the Spike protein from SARS-CoV2, HKU1, 229E, OC43 and NL63 for later use as antigen to ELISA in order to see if previous infections with HKU1, 229E, OC43 and NL63 that usually cause colds lead to increased resistance to SARS-Cov2.

Method: Expression vectors containing the gene segment of the viral envelope component S1 from SARS-CoV2, HKU1, 229E, OC43 and NL63 had been constructed since earlier. At this point, test expressions of the proteins in HEK293F cells and CHO-S cells were performed by transient transfection. Analysis of the protein expression in the cells was performed with Western blot. A major transient transfection was performed in HEK293F cells for the production of the 229E-S1 and NL63-S1 proteins. The 229E-S1 protein was purified with affinity chromatography and purity was analysed with both SDS-page and Western blot.

Result: In the test expression of the proteins, all proteins were expressed better in the HEK293F cells than in the CHO-S cells, where 229E-S1 and NL63-S1 were the proteins that gave the highest expression. The result of the 229E-S1 purification from the larger production (800 mL) provided sufficient purity for the use of the protein as an antigen to ELISA with a protein amount of approximately 8 mg.

Conclusion: All five S1-domains could be produced in HEK293F cells and one of them, 229E-S1, was produced and purified on a larger scale. How well this protein and the others work in serological tests remains to be investigated. No conclusion has yet been reached on how well the protein acts as an antigen in ELISA, as serology analysis is yet to be performed.

ThinPrep - PreservCyt® Solution is an optimal preparation method for semen samples when analyzing Human Papillomavirus

By Emelie Hansson

Bachelor thesis in Biomedical Laboratory Science performed at the Reproductive medicine laboratory, Sahlgrenska University Hospital, Gothenburg, 2021

Supervisor: Kersti Lundin, Associate Professor

The Annual Report 2020 of the National Quality Register for Assisted Reproduction (Q-IVF) reports that 10-15% of all heterosexual couples in Sweden have fertility problems. The man's infertility is mainly due to impaired sperm quality; low sperm count, affected morphology and/or motility. Human papillomavirus (HPV) is the most common sexually transmitted virus. Previous studies have shown that HPV can be a risk factor for infertility in men and also affect assisted reproduction, embryo development and pregnancy.

The purpose of this pilot study was primarily to find the optimal preparation method for the detection of HPV in semen samples and to investigate whether HPV in seminal plasma and/or on sperm could be analyzed using TaqMan. The intention was also to study the prevalence of HPV in the men who participated.

The samples were prepared with ThinPrep – PreservCyt ® or directly frozen with cryoprotectant in liquid nitrogen. The samples were divided into two fractions; in one fraction, HPV virus cloned in plasmid vectors were added. The second faction remained native. The samples were then analyzed with TaqMan for HPV 6,11,16 and 18.

In the study, 8 men were included, all were found to be negative for HPV 6,11,16 and 18. The preparation methods give equivalent results and did not create any disturbances during analysis.

The conclusion is that both ThinPrep® and cryoprotectant can be used as a preparation method in combination with qPCR and TaqMan analysis. ThinPrep® is less costly and quicker and may be used as first choice. The prevalence could not be assessed. The pilot study provides a basis for further research that aims to look at possible links between HPV infection in men and results (fertilization, embryo development and pregnancy) after IVF treatment.

Validation of 7-color leukocyte panel for diagnosis of paroxysmal nocturnal hemoglobinuria with flow cytometry

By Paola Hjelm

Bachelor thesis in Biomedical Laboratory Science performed at the Flow cytometry laboratory, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor:
Linda Fogelstrand, Associate professor, Senior consultant, Department of Clinical Chemistry, Sahlgrenska University Hospital and Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg.
Anastasia Soboli, Practical supervisor, Biomedical Scientist, section Flow Cytometry, Department of Clinical Chemistry, Sahlgrenska University Hospital.

Paroxysmal nocturnal hemoglobinuria (PNH) is a hematological condition caused by a mutation in the *PIGA* gene, which is involved in the production of glycosylphosphatidylinositol anchor proteins (GPI-APs). Lack of GPI-APs can result in blood cells being attacked by the complement mediated immune system. Detection and quantification of GPI-AP-deficient cell populations is essential for PNH diagnosis and treatment follow up, as well as in other hematological disorders such as aplastic anemia or myelodysplastic syndrome, and requires a method with high sensitivity.
Due to updated guidelines suggesting a switch from a 5-color to 7-color PNH panel, and the acquisition of a newer flow cytometer at the flow cytometry laboratory at Sahlgrenska University Hospital, a method validation is required. The aim of this project was to validate a 7-color panel for leukocytes (monocytes and neutrophils) on BD FACSLyric cytometer for the assessment of PNH with flow cytometry following ICCS / ESCCA PNH guidelines. The markers used in this panel were CD45 (expressed on all leukocytes), cell specific markers CD15 (neutrophils), CD64 (monocytes), and specific the GPI-APs markers CD24 (neutrophils), CD14 (monocytes), CD157 and FLAER (both neutrophils and monocytes).
Titration of antibodies, comparison of two different erythrocyte lysis methods (bulk and Euroflow) were conducted and a gating strategy to access PNH populations in neutrophils and monocytes was designed. Assay sensitivity was defined by establishing limit of detection (LOD) and lower limit of quantification (LLOQ) using whole blood as material. For LLOQ a dilution series was created with concentrations of 0.01, 0.1, 0.2, 0.5 and 1% artificial PNH cells. Precision was determined by analyzing four patient samples of which two contained PNH populations. The same samples were used for comparing the 7-color panel with the currently used 5-color panel. The results showed a LOD of 0.004%, LLOQ of 0,1%, and as for precision a CV of 1.4% for neutrophils and 2.3% for monocytes. The comparison of the two panels showed overall agreement, and the small variations seen could be explained by the use of different markers in 5-color and 7-color panels. In summary, the here validated 7-color panel showed good sensitivity and precision. Due to the rarity of PNH positive samples, analyzing more samples for precision and panel comparison is needed in order to complete the validation at the flow cytometry laboratory.

Analysis of Lynch Syndrome with Tissue Microarray Immunohistochemical expression analysis of Mismatch Repair proteins in patients with endometriosis associerated ovarian cancer

By Max Holmgren

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Pathology at Sahlgrenska University Hospital, University of Gothenburg, 2021.

Introduction: Clearcell cancer and Endometroid Ovary cancer are two of the most prevalent Ovary cancers in today's society. At the moment there is a lack of effective screeningmethods for the cancers which leads to many of the cancers not being spotted until later stages. Lynch Syndrome have previously shown signs to be able to be associated with both Clear cell and Endometroid ovary cancer. If this sthudy shows a correlation between Lynch syndrome and the cancers it will mean that a more effective screening method may be applied.

Aim: The purpose of this study is to examine for Lynch syndrome in Clear cell and Endometroid Ovary cancer. A supplementary purpose is to further analyse the characteristics for Clear cell cancer and Endometroid ovary cancer for the cancer patients in the study.

Method: To perform the study 64 Endometroid ovary cancer and 38 clear cell cancer biopsies from archived paraffin wax blocks were collected and turned to Tissue Microarray Blocks. These were screened with immunohistological stain for the genes MLH1, MSH2, MSH6 and PMS2. During the study the medical history of the patients were used to collect data of the characteristics for the cancers.

Results: Lynch Syndrome was assumed not optimal for screening by immunohistochemistry for any of the two cancers according to the results of the study. A correlation between Lynch Syndrome and Synchronus corpus cancer was however discovered. This will however need a larger study to confirm.

Collected data for the cancers included occurence of endometriosis, age when diagnosed, presence of adenomyosis, grade of differentiation and other cancer found in the patients.

NO DIFFERENCE IN RIGHT ATRIUM STRAIN IN ELDERLY HYPERTENSION PATIENTS WITHOUT AND WITH HEART FAILURE WITH PRESERVED EJECTION FRACTION

BY Fadumo Ibrahim

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska University Hospital Ostra, University of Gothenburg 2021. Supervisors: Magnus Johansson MD, PhD, Bente Grüner Sveälv PhD, Pari Allahyari leg. BMA.

Background: Heart failure is a condition that usually presents with symptoms during exertion. Heart failure with preserved ejection fraction (HfpEF) is a common form of heart failure in elderly patients with hypertension (HT). HT is likely an underlying risk factor for HfpEF. In diastolic heart failure with preserved systolic function (HfpEF), the dysfunction occurs mainly in the left ventricle, leading to a worsening relaxation pattern and eventually to increased filling pressure. The increased pressure in the left atrium leads to higher pulmonary arterial pressure and increased stress on the right ventricle. Thus, the right atrium may also be affected.

Aim: The aim of the study was to evaluate right atrium strain in elderly patients with HT without heart failure and a group with HfpEF.

Methods: A total of 33 patients participated in this study, of which 17 patients had HfpEF. The patients with HfpEF were recruited from a study at Sahlgrenska University Hospital Ostra. The patients without heart failure were selected from a randomly selected population from the area of Gothenburg. All participants were elderly patients with hypertension and were divided into a group with heart failure and a group without heart failure for comparison of the strain method. Both the HfpEF group and the control group were matched for gender and age. Statistical methods were used to evaluate the study. Mann-Whitney test was used to assess the significance difference in the different variables between the two independent groups. Coefficient of variation (CV) was calculated to assess the interindividual variability between measurements from two observers and interindividual variability between measurements from the same observer at different time points.

Results: No differences were found between the control group and the HfpEF group: reservoir strain: 25.32±4.48 vs 26.05±8.82, p=0.861, contraction strain: 13.78±3.75 vs 14.12±4.81, p=0.965, strain rate: 1.79±0.38 vs 1.80±0.50, p=0.861. Coefficient of variation (CV%) between measurements of two observers showed: reservoir strain 25.4%, contraction strain 30.4% and strain rate 18.4%. CV between measurements of the same observer was: reservoir strain 15.7%, contraction strain 18.7% and strain rate 9.5%.

Conclusion: RA strain could be performed in 69% of patients and showed similar values in both groups with no statistically significant difference. This suggests that RA function was not affected by HfpEF in this group of elderly patients with HT.

Antibiotic resistance determination of clinical Helicobacter pylori isolates

By Farah Issa

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Microbiology laboratory, Sahlgrenska Academy, University of Gothenburg, 2021
Supervisor: Kaisa Thorell, PhD Microbiologist

Background
In 2017, the World Health Organization (WHO) classified clarithromycin-resistant Helicobacter. Pylori (H. pylori) as a high-priority pathogen, and the development of new antibiotics for this pathogen is a great need, especially since there is no vaccine against the disease or any effective preventive measures. Early diagnosis of H. pylori infection can help reduce the risk of developing gastric cancer. About 30% of the population in Sweden is infected with H. pylori, in some areas even more. Recent studies show that in the elderly 20-40% are infected and among the younger 5-20%

Purpose and method
This study aimed to identify AMR-associated variants of H. pylori. The strains grown on Fastidious Anaerobic Agar (FAA) plates and the biomass of the growing ones used for DNA extraction, and then the quantity and quality of DNA were determined. The goal was to study at least 50 strains. We also studied statistics on antibiotic resistance in H. pylori isolates from Clinical Microbiology, Sahlgrenska University Hospital between the years 2011-2021.

Results
H. pylori strains were subjected to antimicrobial susceptibility testing by E-test method against 3-7 antibiotics. MIC results of the H. pylori isolates showed the highest resistance to Metronidazole (MTZ) 33% and the lowest Erythromycin (EM-E) and Levofloxacin (LEV) 4%, resistance to Amoxicillin (AML), Ampicillin (AMP), Clarithromycin (CLR) and Doxycycline (CD-DO) were 26%, 10%, 15% and 8%. Subsequently, the results obtained were divided according to seven antimicrobial agents into mono- and multi-resistance, where 19% of H. pylori isolates had resistance to a single drug, while 65% had resistance to two antimicrobial agents and 12% had resistance to 3 antimicrobial agents.

Conclusion
It was a challenge to grow from freshly frozen strains and the strains that have been grown and extracted will be able to be an important piece of the puzzle in studies of antibiotic resistance in H. pylori. The determination of resistance is made primarily to find out if bacteria grown from a patient can be treated with topical antibiotics. When bacteria are classified as resistance bacteria then it can not be expected to respond to treatment, this poses a threat to public health. This requires knowing how bacteria have acquired this ability and finding solutions to counteract this resistance.

Larger differences in interobserver variability for stroke volume measured with Simpson biplane than for stroke volume measured with LVOT VTI/ LVOT diameter

By Elin Jernberg

Bachelor thesis in Biomedical Laboratory Science performed at NU Hospital Group, Trollhättan, Sahlgrenska Academy, University of Gothenburg, 2021.

Supervisor: Sofie Ahlin, MD PhD

Introduction: The difference in measured values that does occur between different observers are called interobserver variability since the observers own personal knowledge and experience plays a vital role when it comes to measure and assess the echocardiograms. This study aims to investigate differences in interobserver variation in the staff working for NU Hospital Group in Trollhättan, Sweden

Material and method: Six echocardiographic exams with varying image quality were chosen whereafter the staff one by one and without knowledge about any previous results measured Left Ventricular Outflow Tract, LVOT, and Velocity Time Integral, VTI to estimate stroke volume. They also performed measurement for end diastolic volume and end systolic volume in a four-chamber view and a two-chamber view to estimate stroke volume with the Simpson biplane method.

Results: Stroke volume estimated with LVOT VTI had lower interobserver variability (8.6-20.2) than the interobserver variability for stroke volume estimated by Simpson biplane (19.6-29.7), (p-value 0.000471). Neither image quality or sepal hypertrophy inflicted on the interobserver variability between the different echocardiograms. Significant difference were found in one of the parameters (time measured from the beginning of the picture loop to the measuring of the variable end systolic volume in four chamber view) for comparing job role and experience, where the group with 0-5 years of experience had lower coefficient of variation than those with more experience (p-value 0.008) and doctors had lower coefficient of variation than BMA (p-value 0.030).

Conclusion: The variables measured for LVOT and LVOT VTI had lower interobserver variability than the variables measured for Simpson biplane regardless of job role, experience, image quality and septal hypertrophy. The lower interobserver variation found in LVOT and LVOT VTI makes it a more reliable method for estimation of stroke volume.

Programmed cell death-ligand 1 (PD-L1) expression in non-small cell lung cancer: comparison between paired biopsies and cell blocks

By Felicia Johansson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Pathology and Cytology, Halland hospital in Halmstad, spring 2021
Supervisors: Mohammed S. I. Mansour, MSc, PhD student and Tomas Seidal, MD, Assistant professor

Background: Lung cancer is one of the most common types of cancer and the main form of cancer that causes the most deaths, because this tumor is often aggressive and spreads quickly. Programmed cell death-ligand 1 (PD-L1) is expressed on tumor cells while its receptor programmed cell death-1 (PD-1) is expressed on activated T-cells. The PD-1/PD-L1 pathway plays a significant role in cancer therapy and today there are immune checkpoint inhibitors that are used as treatment for lung cancer which prevent PD-1/PD-L1 binding. Currently immunohistochemical detection of PD-L1 is only standardized in histological specimens.

Aim: The study aimed to compare the expression of PD-L1 positivity in paired histological and cytological specimens from patients with non-small cell lung cancer, and whether cytologically obtained material in cellblocks can replace biopsies and be used for the detection of PD-L1.

Methods. A total of 45 cases with paired histological biopsies and cytological specimens (17 bronchial brushes, 9 endobronchial ultrasound (EBUS) or 19 pleural fluids) were stained with the PD-L1 antibody, clone 28-8 from Dako. Only cases containing more than 100 malignant cells were assessed and only membranous staining in these cells was considered positive. The number of positive tumor cells was scored at ≥1%, >5%, >10% or ≥50% cutoff levels.

Results: Positivity was observed in 26 of 45 (57.8%) of histological cases and 19 of 45 (42.2%) of cytological cases at cutoff ≥1%. Cohen's Kappa (κ) shows moderate agreement between all paired cases at all cutoff levels, at ≥1% κ =0,44 and at ≥50% κ=0,49. For overall percentage agreement (OPA) a concordance of 71% was observed at ≥1% and 82% at ≥50%. When comparing different cytological samples, bronchial brush specimens have been shown to have the best concordance, followed by EBUS.

Conclusion: The study has shown that cytological and histological samples are corresponding to each other but varies depending on the type of cytological specimen. However, many different preanalytical and biological factors must be taken into account prior to further research, such as preparation technique of cytological material, type of biopsy, cellularity, sampling location and tumor heterogeneity.

Verification of an LC-MS/MS method for quantitation of pregabalin in urine.

By Sofia Johansson

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Chemistry laboratory, Northern Älvsborg County Hospital, Trollhättan, 2021
Supervisors: Elmira Luks, hospital chemist
Jenny Brunnegård, hospital chemist PhD

Introduction: Pregabalin is a drug commonly used to treat neuropathic pain, generalised anxiety disorder and partial epileptical seizures. In recent years there has been an increased awareness for the potential misuse of pregabalin and due to this there is a need to detect and quantify the substance in various specimens. At the clinical chemistry laboratory at Northern Älvsborg County hospital, detection and quantification of pregabalin in urine samples is performed by routine. The method used for this purpose is liquid chromatography tandem mass spectrometry on instruments Agilent 6470 and Waters Xevo TQ.

Aim: The aim of this study was to verify a method on an additional Agilent 6470-instrument for the purpose of having two equal instruments available for the analysis of pregabalin with high precision in accordance to accreditation goals. Furthermore, the goal was to have a high agreement and good correlation between the two different Agilent 6470 instruments.

Method: A method comparison was made between the two Agilent 6470-instruments using 49 urine samples from daily routine analysis. An evaluation of in series and inter series variation was made to determine the new method's reproducibility and repeatability. The lowest limit of quantification was determined and potential matrix effects were examined by an ion suppression test.

Results: The method comparison showed a high agreement (98 %) and a good correlation (R2=0,9966) between the two instruments. The method also showed acceptable variation coefficients regarding reproducibility and repeatability, which confirms high precision. The lowest limit of quantification was determined to be 60,48 µg/L. No matrix effects of significance were observed in the ion suppression test.

Conclusion: The method comparison showed a good correlation and the new method meets the goals of the verification regarding precision. The method however requires fine tuning before it can be applicated into daily routine analysis of pregabalin.

Low reliability for microdialysis of lactate in adipose tissue

- A quantitative study of microdialysis

By Mathilda Johansson Lorentsson

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Per-Anders Jansson, MD, Ph.D, Wallenberg laboratory

Background: Microdialysis is a method used to examine organs and various tissues that are difficult to access with other methods. Using a catheter with a thin centered semipermeable membrane, it is possible to extract fluid using a perfusion fluid. In this study, the focus is on lactate in adipose tissue. Lactate is part of the metabolism and has an important role in the immune system, which makes it interesting to study.

Aim: The aim of this study is to analyze the reliability of microdialysis in adipose tissue with lactate as the major metabolite.

Method: The study involved 7 women (47%) and 8 men (53%) with either type 2 diabetes, healthy but obese (BMI between 25.0 - 40.0 kg / m2) or healthy lean (BMI between 18.0 - 24.9 kg / m2). The participants attended three visits, of which the two later visits consisted of microdialysis combined with an oral glucose tolerance test (OGTT). The statistical methods used were Wilcoxon's signed ranked test, correlation diagram, intraclass correlation coefficient (ICC), and individual diagrams to compare how the values for the different visits matched.

Results: Wilcoxon's became 0.964 which makes it non-significant. A significant value indicates systematic errors between the visits. The ICC was 0.518, which shows that the reliability of the method is on the verge of low as it falls within the limit value between 0.5 - 0.75 with indicates moderate reliability. The study resulted in an overall CV of 24% which indicates a low reliability for the method and that the method requires a stricter protocol. An overall CV <10% indicates a method with high reliability.

Discussion: The conclusion of the study is difficult to determine as the sample only included 15 subjects. The ICC value indicates that the reliability is relatively low. Wilcoxon's test shows that there is no significant difference, which indicates no systematic difference between the values.

The largest source of error in the study was that the method protocol was changed halfway through. This makes the values unreliable, and it is therefore difficult to conclude how valid the method is.

Decreased work capacity is a risk factor for myocardial infarction with an ST elevation.

By Filip Jurkovic

Bachelor thesis performed at Sahlgrenska university hospital, Sahlgrenska academy, university of Gothenburg 2021

Supervisor: Araz Rawshani, Leg medical doctor.

Background

It is known that physical exercise provides an improved cardiovascular risk factor profile and improved vascular function. However, it is unknown whether the risk of total coronary occlusions correlates with work ability. We studied the risk of ST elevation myocardial infarction (STEMI) among healthy participants in the United Kingdom Biobank (UKB) in relation to work ability measured on an ergometer cycle.

Method and statistical analysis

We included all participants in UKB who completed a work test (work ECG) provided that they did not suffer from a STEMI before inclusion in the study. Participants were grouped into 5 groups (groups 1 to 5) based on work ability (<60, 60–79, 80–99, 100–119 and 120–150 watts). The risk of STEMI in relation to work ability was analyzed with Cox regression and adjustment was made for age, sex, smoking, systolic blood pressure, diastolic blood pressure, resting heart rate, body mass index, Forced expiratory volume for 1 second (FEV1), C-reactive protein (CRP), Cystatin C, Hemoglobin A1c (HbA1c), Low density lipoprotein (LDL), High density lipoprotein (HDL), total cholesterol, lipoprotein A, hematocrit and hemoglobin.

Result

Compared with <60 W load, groups 2, 3 and 4 had lower point estimates for the hazard ratios (HR, hazard ratio) but the confidence intervals included 1.0 and were therefore not significant. No trend was noted regarding the point estimate, which was around 0.75 for groups 2, 3 and 4. Compared to group 1, however, group 5 (120–150 W) had a hazard ratio of 0.23 (95% CI 0.06–0.85). HR for men compared to women was 4.05 (95% CI 2.46–6.66). Each year of smoking, the risk of STEMI increased by 1%. Each unit increase in systolic blood pressure (mmHg) increased the risk of STEMI by 1%. Diastolic blood pressure, resting heart rate, body mass index (BMI), maximal heart rate achieved during workout, FEV1, total cholesterol, hematocrit, hemoglobin, CRP, LDL, and lipoprotein A were not associated with the risk of STEMI. For each unit increase in HbA1c, the risk of STEMI increased by 2%. For each unit increase in cystatin C, the risk of STEMI increased by 132% (HR 2.32, 95% CI 1.72– 3.14).

Conclusion

Compared to performing <60 W, the risk of STEMI is 77% lower if you succeed in achieving a load of 120–150 W.

Comparison between small VOI and whole kidney segmentation method to calculate kidney dosimetry of patients treated ^{177}Lu-DOTATATE.

By Nahrin Kioarkis

Bachelor thesis in biomedical Laboratory Science performed at the section for clinical physiology and Radiophysics, Sahlgrenska academy, University of Gothenburg, 2021

Supervisor: Jehangir Khan

Purpose: The kidneys and bone marrow are risk organs in peptides receptors radionuclide therapy (PRRT) with ^{177}Lu-DOTATATE. Therefore, it is important to have an accurate individual kidney dose measurement for neuroendocrine tumour patients, to reduce the toxicity on these organs and to increase the efficacy of ^{177}Lu-DOTATATE treatments on the tumour. Manually segmentation approach using patients CT images is commonly used for accurate kidney dosimetry. However, this approach is time-consuming, might be inter and intra-observer dependent. The purpose of this study was to establish a fast and reliable method for the calculation of the kidney absorbed doses of patients treated with ^{177}Lu-DOTATATE. Hence, to evaluate the kidney absorbed doses measured using a small spherical volume of interest (VOI) of volume 2 ml or 4 ml were compared to whole kidney segmentation technique.

Methods: Total 18 patients with disseminated neuroendocrine tumours underwent targeted radionuclide therapy. A SPECT/CT scans over the abdomen area were acquired at 0, 1, 2, and 7 days after ^{177}Lu-DOTATATE infusion. The activity concentrations (counts per ml) were measured in serial of SPECT images using a small VOIs (2 ml or 4 ml) drawn on the kidney cortex and in manually segmented right and left kidneys. The kidney absorbed doses were calculated based on the activity concentrations obtained using the small VOIs on SPECT images and were compared with absorbed doses obtained with whole manually segmented right and left kidneys.

Results: The absorbed doses in the testing cohort (n=18) between the whole kidney segmentation and small VOI method (2ml – 4ml) were strongly correlated for both kidneys ($r= 0.80$, $p=0.000$). Moreover, the Bland-Altman indicates good agreement for kidney absorbed dose estimate between the small VOI of 2ml and whole segmented kidney method, where the mean difference was equal to (Bias= -0.3, SD= 0.9) for the right kidney and for the left kidney (-0.7, 1.2), respectively. Likewise, for the small VOI of size 4 ml and whole segmented kidney, the bias was -1.0 (1.1) for the right kidney, and for left kidney -1.3 (1.5), respectively.

Conclusion: This work showed that the small VOI method has the potential to calculate accurate kidney dosimetry. In addition, the small VOI approach is a fast method to calculate kidney absorbed doses and complied with the whole kidney segmentation method.

Keywords: ^{177}-Lu DOTATATE, Kidney Dosimetry, Neuroendocrine tumour, VOI segmentation.

Validation of SealSafe with the use of formaldehyde as a method for fixation of histopathological samples

Part of quality and method development at Department of Clinical Pathology, Sahlgrenska University Hospital

By Louise Koponen Edvardsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Pathology, Sahlgrenska University Hospital, 2021
Supervisor: Katarina Junevik, PhD and Sofia Tedelind, PhD

The uterus is part of the female reproductive system and is responsible for retaining, protecting as well as nourishing the fertilized ovum, up until the fetus is ready to be born. Leiomyoma is the most common benign tumor in the uterus and cervical cancer is the most common malignant tumor in the uterus. To set a diagnosis, the surgically removed uterus is sent to the Department of Clinical Pathology at Sahlgrenska University Hospital to get processed and analysed under a microscope. The first and most important step in the process is the fixation of the tissue to stabilize and inhibit autolysis. Currently 4% formaldehyde (formalin), which is a cancerogenic reagent, is used as the fixative. The reagent is used in great amounts as well as in an open system. SealSafe is an instrument that automatically adds formalin to the tissue in a closed system with vacuum sealing and a reduced ratio between formalin and tissue. This study was performed to validate the use of SealSafe as transportation as well as fixation tool at the Department of Clinical Pathology, Sahlgrenska University Hospital. A control group consisting of 8 benign uteri was handled according to the current routines of the clinic and the fixation was judged based on grade of fixation, consistency as well as bloodiness. Due to the Covid-19 pandemic, surgeries were cancelled which caused a lack of tissue that was supposed to be managed by the SealSafe instrument and compared to the control group. The results of this study is therefor unfortunately incomplete. Based on the information handed out by the supplier of the SealSafe instrument, and from results of previous studies, the standardisation that comes with SealSafe is expected to have a great advantage in terms of elevating the quality of the current method. The reduced volume of formalin and vacuum sealing is not expected to impair the morphology of the tissue.

Effects of estrogen on immune cells and osteoarthritis progression

By Clara Kristoferson

Bachelor thesis in Biomedical Laboratory Science performed at the Krefting Research Center, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Ulrika Islander, Carmen Corciulo

Osteoarthritis (OA) is a rheumatic disease with an inflammatory component. It affects all joints and causes chronic pain, cartilage degeneration and leads to a reduced quality of life for those affected. Estrogen levels have been found to affect the development of OA through the estrogen receptors ERα and ERβ. This study was aimed to examine how different concentrations of estradiol affects adenosine receptor A_2A and A_2B expression and cell proliferation in macrophage and lymphocyte cell lines. We also investigated the effect of estradiol on articular cartilage of an OA mouse model.

Two different types of cell lines were used in the study: Raw 264.7 cells which are macrophage-like cells and Jurkat cells which are lymphocytes. The cells were kept in media consisting of DMEM medium with 1% P/S and 10% FBS and in atmosphere 37C, 5% CO2 and 95% humidity. Western blot was used to detect the adenosine receptors from Raw and Jurkat cells. MTT assay was used to observe cell proliferation in Raw cells. Bone samples from a previous animal experiment with mice was used for the histochemistry analysis.

Results showed that the adenosine receptors expression in Raw 264.7 cells indicated no major difference between the E2 concentrations as they did in the Jurkat cells. MTT showed insufficient proliferation at lower E2 concentrations and a beneficial proliferation at higher concentrations. The histochemistry analysis showed that mice treated with estradiol had a reduced cartilage degeneration while treatment with vehicle showed an increased effect on degeneration.

Estrogen effects the cells in a positive way through giving protection against cartilage degeneration and the development of OA. The estrogen also stimulates the proliferation of cells leading to an immune response.

CHARACTERIZING DNA POLYMERASE δ VARIANT R808H FOUND IN COLORECTAL CANCERS SUGGESTS IMPACT OF MUTATION ON CELL PHYSIOLOGY AND REPLICATION ENZYMOLOGY

By Anja Lidén Österberg

Bachelor's thesis (15 hp) in biomedical laboratory science at the Clausen laboratory, University of Gothenburg, 2021. Supervisor: Anders R. Clausen, Research Assistant.

Genomic instability is a major feature of colorectal cancers, of which 16% are hypermutators. Accurate DNA replication is a primary defence against accumulation of mutations and the replicative polymerases Polδ and Polε have been found to contain numerous mutations in many hypermutated cancers. Because replicative DNA polymerases are direct sources of introducing mutations into the genome, understanding how mutations in these polymerases change the synthesis of DNA and contribute to genome instability is of great importance. Yet, a systematic evaluation of Polδ variants in CRC is missing to date. This study aimed to characterize Polδ variant R808H found in colorectal cancers by comparing *S. cerevisiae* strains with and without the homologous point mutation. Growth analysis and spot assays were performed for physiological characterization and HydEn-seq was used for mapping Polδ replication enzymology. Strain differences were observed in cell growth as well as replication enzymology. These findings suggest a functional impact of the mutation on replication enzymology and cell physiology, making it interesting for further investigation.

OPTIMIZING NANOPORE SEQUENCING OF THE SARS-COV-2 GENOME BY AFFINITY PURIFICATION WITH MAGNETIC INSTANT CAPTURE BEADS

By Ina Liljestrand-Landin

Bachelor Thesis in Biomedical Laboratory Science performed at the Department of Clinical Microbiology and Immunology, institute of biomedicine, Sahlgrenska Academy, University of Gothenburg, 2021
Supervisors: Maria Andersson, PhD, Johan Ringlander MD, PhD

ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) is the betacoronavirus responsible for the ongoing pandemic by causing the disease Covid-19 and has since the outbreak in Wuhan, China 2019 caused over 2,5 million deaths worldwide. The virus is a part of the *Coronaviridae* subtype in the *Nidovirales*-family and share a lot of its genetic features and caused symptoms with its predecessor SARS-CoV-1. The virus is a positive sensed RNA-virus with a genome of ~30kb.

Nanopore sequencing is a relatively new sequencing method is also known as "Third generation sequencing" and has many benefits such as adjusting the nanoscale-pores to the project depending on what nucleic acid that is to be identified. The aim of this project was to optimize two different protocols to follow from Oxford Nanopore Technologies; The "Direct RNA sequencing" and "PCR cDNA barcoding" protocols, to find the most suitable to successfully map the SARS-CoV-2 genome and transcriptome, and also look at the risk of integration of the virus into the human genome. The sequencing was conducted on a MinION-unit using the EPI2ME program for mapping the reads produced. The samples were collected from Sahlgrenska University Hospital, the Department of Microbiology, Virology and had been tested positive by the routine qPCR-analysis on site. One sample of in-house Vero cell culture infected with SARS-CoV-2 was also used.

Before the sequencing an affinity purification protocol using Magnetic Instant Capture Beads "MagIC Beads" was performed to witness if the results from the sequencing improved, which is indicated when results are compared with previous not yet published experiment results.

The results from sequencing showed that when performing the Direct RNA sequencing the fragment sequences were longer (~1000 bases) than the fragments from the PCR-cDNA protocol (~220 bases). The fragments did although show signs of being error-prone since <1% of the reads got classified as betacoronavirus-reads compared to the 11% when conducting the PCR-cDNA protocol on the same samples.

The Direct RNA-protocol is ideal for this extensive and complex genome but needs to be optimized to enhance the quality of reads.

The results of the bioinformatical analysis on the reads mapped from the sequencing experiments shows no signs of viral integration into the human genome. Our results therefore also suggest that integration of SARS-CoV-2 into the human genome does not occur in the human respiratory epithelia.

Single Nucleotide Polymorphism in Children with Gastroenteritis

By Freja Lindstedt

Bachelor thesis in Biomedical Laboratory Science performed at the Molecular Microbiology Unit at Sahlgrenska University Hospital, University of Gothenburg, 2021
Supervisor: Maria Andersson, PhD

Background: Single nucleotide polymorphisms have been shown to affect virus-host-interactions for a number of viruses. Examples of these polymorphisms are rs368234815 and rs12979860 in interferon lambda 4, linked to upper respiratory tract infection, and rs601338 in FUT2, known to affect norovirus and rotavirus infection.

Aims: The aims of this study were to determine the effectiveness of using rectal swabs as opposed to blood samples for genotyping assays, as well as to study the relationship of the aforementioned mutations to pathogen persistence and clearance in children with gastrointestinal infection.

Methods: DNA extraction was performed on 30 randomly chosen blood samples and 127 rectal swab samples from children with known gastrointestinal infection. Beta globin was used as extraction control. For the genotyping, allelic discrimination qPCR assays were used. In order to compare genotyping on rectal swabs and blood samples, the blood samples were analysed for beta globin and genotyped for the rs12979860 locus. The rectal swabs were analysed for beta globin and then genotyped for rs368234815, rs12979860 and rs601338 using qPCR. The rectal swab sample genotypes were statistically compared to known patient data of pathogen persistence and clearance using Pearson's chi-squared test.

Results: All blood samples were positive for beta globin and successfully genotyped. Out of the 127 rectal swab samples, 121 were positive for beta globin. All these samples were successfully genotyped. Of the 6 samples negative or weak for beta globin, only one sample, weak but not fully negative for beta globin, was able to be genotyped. In comparing the genotypes to pathogen persistence and clearance, some significant differences were shown, but were ultimately inconclusive.

Conclusion: While rectal swabs are not quite as dependable as blood samples for genotyping, they can be used. Further optimisation is required. Beta globin is a reliable extraction control. Further study is required to properly establish the relationship between the rs12979860, rs368234815 and rs601338 genotypes and gastrointestinal infection persistence and clearance.

Comparison of kidney dosimetry of patients treated with ^{177}Lu-SPECT images based on 30 projections versus 120.

By Noushik Manuel

Bachelor's thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology at University Hospital Sahlgrenska /Östra, University of Gothenburg 2021.
Supervisors: Jehangir Khan, Hospital Physician
CO-Supervisor: Peter Bernhardt, Professor/ Doctor/ Hospital Physician in Radio Physics.

Background: Peptide receptor radionuclide therapy (PRRT) with ^{177}Lu-DOTATATE is an effective treatment option for the treatment of positive neuroendocrine tumours. However, kidney and bone marrow are found to be critical organs. Therefore, individualised kidney dosimetry is important in PRRT with ^{177}Lu-DOTATATE treatment. The main purpose of this study was to compare the image-based kidney dosimetry based on SPECT/CT reconstructed images consisted of 120, 30 and 120 artificial intelligences (AI)projections of NET patients treated with ^{177} Lu-DOTATATE.

Method: Eighteen (n=18) patients underwent ^{177}Lu-DOTATATE treatment. SPECT acquired data were consisted of total of 120 projections. Furthermore, 3 of 4 projections were removed from 120 projection data set to obtain 30 projections. In addition, SPECT acquisition data with 120, 30 and 120 artificial intelligence (AI) projections obtained with synthetic intermediate projections (SIP) were reconstructed. Furthermore, kidney activity concentrations were determined in manually segmented kidneys based on CT images, by applying a volume of interest (VOI) over the right and left kidneys on SPECT images. Thereafter, activity concentrations in SPECT images were measured for the estimation of the kidney dosimetry.

Results: Pearson's correlation coefficient analysis demonstrated a strong positive correlation ($r=0,99$, $p<0,01$), of calculated kidney absorbed doses based on reconstructed SPECT images; 120 reference (REF) versus 30, 30 versus 120 artificial intelligence (AI), and 120REF versus 120AI projections, respectively. According to Bland-Altman, the mean difference (mean bias) was found of (0.2) with a standard deviation (SD) of (0.3).

Conclusion: Our results demonstrated that kidney dosimetry based on SPECT images with 30 projections complied with 120 projections data. Moreover, image reconstruction based on 30 projections is fast, and equally accurate as 120 projections.

Keywords: Neuroendocrine tumour, ^{177}Lu-DOTATATE, Kidney dosimetry, SPECT reconstruction.

Optimization for detection of Epstein Barr-Virus miRNA with Two-tailed RT-qPCR

By Linda Mellert

Bachelor thesis in Biomedical Laboratory Science performed at the department for Clinical Microbiology, Sahlgrenska academy, University of Gothenburg, 2021
Supervisor: Ka-Wei Tang, MD, PhD

Introduction: Micro-RNAs (miRNAs) are short, non-coding RNA molecules whose function is to regulate gene expression. Due to their properties, they can act as potential biomarkers for a number of diseases. Connections between Epstein Barr-virus miRNAs have been seen with a number of diseases. Due to the immunosuppressive medication that is given after transplantation the virus, which is latent in B lymphocytes, reactivates and proliferate. This can develop an aggressive and potentially life-threatening malignancy such as post-transplant lymphoproliferative disorder (PTLD). There are methods for detecting miRNAs and the most common method is RT-qPCR. However, it is not optimal and therefore a new method, two-tailed RT-qPCR has been developed.

Aim: The aim was to optimize the protocol for detecting miRNAs by reducing background noise, testing controls for easier interpretation of results and setting a minimum detection limit. The correlation between EBV DNA and EBV miRNA was to be investigated.

Method: The samples was synthetic EBV-miRNA and 27 patient samples (plasma and serum) previously analyzed for EBV-DNA. To reduce background noise, two dilution steps for cDNA, 1: 5 and 1:40, were added to the previously developed standard protocol. The lowest detection limit was determined by making a dilution series from 10^7 to 10^0 of synthetic EBV-miRNA. Two different RT controls and an extraction spike-in control were tested. The method used for all sub-steps, for synthetic EBV miRNA and patient samples, was two step two-tailed RT-qPCR using SYBR Green. The correlation between EBV-DNA and EBV-miRNA was examined in plasma patient samples along with two synthetic spike-in controls.

Results and conclusion: The results are not complete and need further studies. Dilution series in which cDNA from synthetic EBV-miRNA was diluted 1:5 and 1:40 were compared and showed reduced background noise in the 1:40 dilution. For patient samples however, this dilution can reduce the concentration so that they fall below the lowest detection limit that from the dilution series was set to 1000 copies/ml. Only one RT control, cell-miR-76, gave results and no correlation between EBV-DNA and EBV-miRNA was observed. The conclusion is that further experiments needs to be performed but can be based on the results obtained from this work.

Lower degree of TRAP-induced platelet aggregation in platelet concentrate units with much visible aggregates as compared to little visible aggregates, without any obvious connection to P2Y12 receptor haplotype

By Mirna Mirza

Bachelor thesis in Biomedical Laboratory Science performed at laboratories of Sahlgrenska Academic hospital, University of Gothenburg, 2021
Supervisor Camilla Hesse (senior lecturer), Ali- Reza Moslemi (senior lecturer).

Platelets are small cell fragments found in the blood along with white and red blood cells. Their role is to detect damages in the blood vessels and stop bleeding. Platelets are created in the bone marrow, and they have no cell nucleus but contain granules with substances important for their function. They live in the blood circulation for eight to ten days before they are destroyed in the spleen. Platelets heal wounds in the blood vessels by moving to the damaged area, sticking together, and forming a plug.

Platelet transfusion is essential for patients with thrombocytopenia that could be caused by surgery, chemotherapy, or disease. Platelet concentrates for transfusion can be processed from whole blood donations or by apheresis techniques.

The P2Y12 receptor (ADP-receptor) is important for platelet aggregation, and it has been reported to occur in different variants (haplotype 1 and 2). It has been reported that platelets from individuals with haplotype 2 has a higher aggregability.

The purpose of this study is to investigate "mini-platelets" that contain aggregates and how the aggregates affect the aggregation ability of platelets and to study what form of the P2Y12 receptor they have.

This study was conducted on 18 blood donors in the laboratories of the university of Gothenburg and Sahlgrenska academy hospital, and the presence of haplotype 1 and 2 was investigated among donors. Also, the platelet aggregability was investigated using the Multiplate® instrument and three different platelet agonists (ADP, ASPI, TRAP). Since aggregation occurs during the processing of whole blood, the platelets were inspected and the aggregation graded as no aggregates, little or much aggregates.

Among the 18 blood donors, only one individual was identified with the haplotype 2 and therefore it was not possible to evaluate any differences in aggregability related to different haplotypes. For this purpose, more samples must be analyzed.

When the aggregability response to the three different agonists were evaluated in relation to the aggregate-level in the platelet units, a significant difference was found for TRAP, with a lower response in the group with much aggregates as compared to the group with little aggregates. There was a trend for a lower response also for ADP and ASPI, however it was not significant.

Since aggregates in the platelet units is not expected, and is an unwanted side effect of the processing, this preliminary data encourage more studies.

Validation of a LC-MS/MS method for detection and quantification of tramadol, oxycodone and methadone in urine on Agilent 6470

Method comparison between two Agilent 6470 instruments

By Golshan Moniri

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical chemistry laboratory, Northern Älvsborg County Hospital, 2021

Supervisors: Elmira Luks Hospital Chemist, Jenny Brunnegård Hospital Chemist PhD

Opioids, synthetic substances are widely used in clinical treatment of acute and long-existing pain. Because of their highly addictive nature and possible misuse the need for analysis is high. The purpose of this study was to validate an existing method for detection and quantification of tramadol, oxycodone and methadone in urine samples on a new LC-MS/MS instrument; Agilent 6470 instrument 199. Anonymized frozen urine samples with concentrations within the range 200 µg/L-5000 µg/L were analysed. Each substance was analysed in 10 different urine samples each day for a total of five consecutive days.

Analytes in urine samples were separated by size and polarity by liquid chromatography (LC) and ionized by electrospray ionization. Precursor ions were selected and fragmentized into product ions by tandem mass spectrometry before passing through to a detector. The results were compiled in chromatograms.

Inter and intra assays carried out on low (200 µg/L) and high control (1000 µg/L) for each substance in order to investigate the reproducibility and repeatability of the method on instrument 199 and the obtained results were within the set limits. The obtained concentration results from the urine samples on instrument 199 and the operating instrument 198 were compared to determine correlation and bias between the instruments. Absolute (µg/L) and relative (%) bias plots were obtained which showed low difference between the two instruments. Correlation between the two instruments for all three substances was over 0,95. Furthermore, oxycodone showed low signal response and showed higher bias in comparison with tramadol and methadone. The method on instrument 198 shows none of this which means the issues are related to instrument 199. The conclusion of this study is that the method will need further validation and adjustments for oxycodone on instrument 199.

An Assessment of the Effect of the Concentration of Mannitol, Salt and Nitrogen Source on the Ability to produce Polybeta-hydroxybutyrate as a Bioplastic Production Material by Streptomyces Hygrocopicus Bacteria

Boshra Moradi

Department: Azad University Science and Research Branch, Iran

Handledare: Mojtaba Taran

Abstract

Among biodegradable plastics, polyhydroxyalkanoates and their copolymers have received more attention than other biodegradable polymers due to their complete degradability, flexibility, water resistance and simplicity of production process. Despite its many advantages over petrochemical polymers, its use has been limited its production is not cost-effective. One of the most common ways to solve this problem is to use a cheap substrate. The present study used hygroscopic bacteria isolated from soil contaminated with oil. Using the Taguchi method, the three important parameters of mannitol, soy flour and salt were taken into account for their effect on the production of polymer, polyhydroxybutyrate. The share of factors and their amount in PHB production with maximum yield by hygroscopic streptomyces has proven that PHB yield can be significantly increased by optimizing effective factors. The optimum amount of mannitol factor was 20 mg/ml units, soy flour 8 mg/ml and salt 6 mg/ml. The predicted value for PHB production was 1.56% under these conditions.

Key words: Hygroscopic streptomyces, bioplatic, polyhydroxybutyrate, Taguchi method.

Expression of the platelet receptor PAR1 and its importance in the formation of aggregates in platelet apheresis

By Nermin Muhammad

Stem cell and Component laboratory, Clinical Immunology and Transfusion medicine, Sahlgrenska University Hospital, Gothenburg

Supervisors: Helena Barreto Henriksson, associate professor, Camilla Hesse, senior lecturer

Nowadays, platelet transfusion is extremely important for medical care and other research measures. Platelets have an important function in the body to stop bleeding. If a blood vessel or an organ tissue is damaged, which in turn, it sends out signals to the platelets that accumulate in the damaged area. The platelets form a plug (clot) to repair the damage. Platelets express several specific receptors on the surface and secrete various agonists that play an important role in primary hemostasis. When platelets are collected for transfusion, it is important to avoid platelet activation because the platelets should be able to aggregate after the transfusion to a patient. Transfusion medicine at Sahlgrenska Hospital has occasionally detected deviation with aggregates in platelets for transfusion. In case aggregate occurs, the bags should be discarded, which causes a great lack of platelet for transfusion, wasting time, effort, and resources. The purpose of this study was to study whether there is a significant difference between non-aggregated (normal) platelets and aggregated by measuring the platelet aggregation ability and expression of platelet activation markers CD-62 and PAR1. 10 aggregated and 10 normal platelet samples were studied. The ability to aggregate was studied on days 1 and 2 after donation via impedance aggregometry and tested with three different agonists such as arachidonic acid (ASPI), thrombin receptor activating peptide (TRAP) and adenosine diphosphate (ADP). Furthermore, expression of platelet activation markers on surface CD-62 and PAR1 was measured by flow cytometry. The found results illustrate how aggregated platelets had higher activation rate and poorer ability to aggregate with TRAP and it also shows that the activation marker CD62 was expressed more in aggregated platelets. Further studies covering a wider range of measures and boasting a larger number of samples are needed to further evaluate the results.

TOWARDS UNDERSTANDING THE PHOSPHOR-CODE GOVERNING PROTEOLYTIC CLEARANCE OF ALZHEIMER'S DISEASE-RELATED TAU PROTEIN BY CALPAIN-1 AND -2

By Magda Niewolik

Bachelor Thesis in Biomedical Laboratory Science
Department of Chemistry & Molecular Biology, Faculty of Science, University of Gothenburg
Supervisors: Björn Burmann, PhD, Associate professor, and Irena Matečko Burmann, PhD

Background: Tau, a microtubule-associated protein, is physiologically expressed in neurons, where it plays a significant role in microtubule assembly and stability. Its function is modulated by a series of post-translational modifications (PTMs), whose dysregulation can lead to the deposition of abnormal Tau isoform conformations. This deposition is characteristic for a group of neurological disorders known as tauopathies, with the most common tauopathy being Alzheimer's disease (AD). One of the histopathological hallmarks of AD is the deposition of intraneuronal aggregates of Tau (neurofibrillary tangles), mostly comprised of a hyper-phosphorylated form of the protein. Among the kinases regulating Tau phosphorylation is glycogen synthase kinase 3β (GSK-3β). This kinase has the ability to phosphorylate Tau at 28 different phosphorylation sites, many of which are notedly AD-related. Calpain-1 and -2 are calcium-activated intracellular proteases mediating the cleavage of Tau, and their activity has been linked to AD.

Aim: This work aims to explore and review the interplay between site-specific phosphorylation and associated calpain proteolytic activity by delineating the effect of Tau phosphorylation on the efficacy of Tau proteolytic clearance by calpain-1 and calpain-2.

Method: The two most abundant Tau isoforms, Tau39 and Tau40, were cloned into a pET28-vector with a His6-SUMO-Tag on the N-terminus and subsequently overexpressed in a heterologous system using *E. coli* BL21 (lDE3) Star™ cells. After successful protein purification, using affinity and subsequent ion-exchange chromatography, the proteins were phosphorylated *in vitro* by GSK-3β. Nuclear Magnetic Resonance (NMR) was used to determine the phosphorylation sites. The proteolytical activity of calpain-1 and calpain-2, depending on the Ca^{2+} concentration, was analysed by a combination of NMR, electrophoresis in SDS-polyacrylamide gel and Western blot.

Results: After successful purification and phosphorylation, the methods used showed a notable difference between the proteolytic activity of calpain-1 and -2 on the two Tau isoforms. Cleavage of phosphorylated Tau39 was more rapid than the non-phosphorylated protein, while for Tau40, both counterparts were cleaved at a similar rate. Calpain-1 cleaved Tau39 at a greater proteolytic rate in 0.5mM Ca^{2+}, compared to 3mM Ca^{2+}. Lastly, proteolytic activity assay of calpain-2 showed an enhanced aggregation propensity of both isoforms, especially in the case of non-phosphorylated Tau.

Conclusion: These findings provide new insight into the phosphor-code governing proteolytic clearance of AD-related Tau protein. Further evaluation of this data should be performed to understand better the connections between Tau, its phosphorylation, and proteolytic clearance by calpain-1 and -2. Prospective studies should include mass spectrometry analysis and Western blot using fragment specific antibodies.

No difference in incidence of cardiovascular events between individuals with right- and left-sided carotid plaque

By: Isabella Paulsson

Bachelor thesis in Biomedical Laboratory Science performed at the
Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg, 2021.
Supervisor: Caroline Schmidt, leg BMA, Docent

Introduction
Cardiovascular disease is the leading cause of death worldwide and many risk factors have been identified, such as age, smoking, hypertension, and hyperlipidemia. Cardiovascular disease is often caused by atherosclerosis, which frequently presents in the carotid arteries. The thickness of the carotid wall and the presence of plaque has been shown to be a good predictor for future illness. However, few studies have explored the relation between plaque location and its effect on the development of cardiovascular disease.

Aim
The aim of this study was to assess whether unilateral plaque in the left or right carotid artery is associated with a difference in risk regarding the development of cardiovascular disease.

Method
The study included a total of 1032 participants living in the Gothenburg area. Participants were either 58-year old men (n=386) or 64-year old women (n=646), with no prior history of cardiovascular disease. Subjects were examined with ultrasonography to identify carotid plaque, and serum samples were collected to assess for known risk factors for the development of cardiovascular disease. Cardiovascular events were recorded during a mean follow-up period of 9 years.

Results
No significant differences were found between the groups with right- and left-sided carotid plaque regarding cardiovascular events during the follow-up period. However, multiple significant differences were found between the groups regarding risk factors for later development of cardiovascular disease. Individuals with unilateral left-sided carotid plaque were found to be significantly younger, and had an elevated risk regarding lipid-related risk factors. The group with right-sided plaque were older, and had higher blood pressure as well as glucose levels.

Conclusion
No association was found regarding plaque location and cardiovascular events during the follow-up period. Significant differences were however observed regarding risk factors, which suggests a possible difference in the development of unilateral plaque depending on side.

Abstract

"Sex-dependent differences in collagen turnover during subcutaneous adipose tissue expansion"

By: Gabriela Pereira

Bachelor thesis in Biomedical Laboratory Science performed at the department of Physiology/Metabolic Physiology; Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Milica Vujičić, Phd

Background: Obesity has become prevalent globally in the last decades. With it follows the health conditions that occur as a consequence of the obese state, putting an increased burden on the health system worldwide. Although obesity is quite significantly present in both male and female population, it has been shown to affect the sexes unevenly males have a greater tendency to suffer the metabolic consequences of obesity more harshly. Due to the location of fat accumulation as well as hormonal factors, these differences affect the sexes inflammatory response to growth in the adipose tissue differently.

Purpose: This study aims to determine sex-differences in collagen turnover during subcutaneous adipose tissue expansion, as well as differences in adiposity, inflammation and metabolic health between the sexes in a healthy and obese state.

Methods: Male and female mice were fed either chow or high-fat diet (HFD). The animals were weighted and glucose levels measured before they were sacrificed. Inguinal white adipose tissue (IWAT) and liver were extracted from the mice. The IWAT samples were stained with picrosirius red to determine total collagen in subjects. Protein levels of collagen III (COL3) were measured by Western blot. mRNA expression of inflammatory-, collagen-, adipocyte function-, macrophage-, and extracellular matrix-related markers were examined using qPCR. Livers were stained with Hematoxilin-Eosin to determine the lipid composition of the liver by observing lipid droplets

Results: Both body and IWAT weight were increased by HFD in males and females. Blood-glucose levels and lipid composition of the liver were significantly increased in males on HFD but not females. A higher total collagen amount was observed in females compared to males in chow but not in HFD. A tendency towards higher amount of COL3 was also observed in females compared to males on both diets on a protein level. A sex-difference in regulation of collagen genes on HDF was found. The regulation of adipocyte function and macrophage genes also differed between the sexes upon HFD.

Conclusion: In conclusion, this study shows there is a significant difference in collagen composition between males and females. In addition males are more harshly affected by a HFD when it comes to tissue inflammation, liver fat content and blood-glucose levels compared to females at the same degree of obesity.

Low-grade chronical inflammation after radioterapy in pelvic-organs

Optimisation protocol for labeling and quantification of neutrophil granulocytes in colon tissue in mice

By Ann Quach

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Onkology, Department of Clinical Science, Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Sravani Devarakonda M.S in molecular biology

Radiotherapy has its pros and cons in cancer treatment. The benefits are obvious; radiotherapy is a local, non-invasive treatment that can cure cancer. Its disadvantages include often severe side-effects that make day-to-day life more difficult for the pelvic cancer survivors. Damage to normal tissue may lead to chronic disorders, where, in the case of the intestine within the radiation field, one is forced to live with gas discharge, diarrhea and fecal leakage. Diet rich in fiber has anti-inflammatory properties and has been studied on its protective nature towards maintaining the mucous membrane. There has been an increased incidence of neutrophil granulocytes up to 20 yeras after radiation. Neutrophils are recruited in bacterial infections, so this may indicate chronic inflammatin of the intestine. The main goal of the study was to use the immunohistochemistry technique and optimise a staining protocel for the marker MPO and quantify the number of MPO-positive cells in different groups that received a fiber-free or fiber-rich diet and to investigate whether the diet could affect the number of neutrophils after radiotherapy. C57BL/6 male mice were divided into four groups accordingly; fiber-rich diet + no irradition (control group), fiber-rich diet + irradition, fiber-free diet + no irradition (control group) and fiber-free diet + irradition. Quantification was preformed at week 1 (acute phase) and week 18 after radiation (late phase). The tissue were stained according to the optimised protocol and counting was preformed using light microscope. We found that 1 week after radiation, neither diet or radiation had an effect on numer of neutrophils. 18 weeks after radiantion, the irradiated mice had more neutrophiles than the control group and the increase was slightly greater in the mice that received a high-fiber diet. The results indicate that radiation cause an infiltration of neutrophils and a high fiber diet stimulates this infiltration. This can cause the fiber diet to stimulate the immune response to infiltarting bacteria. This hypothesis should be futher tested as it may be important for prevention against radiation-induced low-grade chronic inflammation of the colon.

Establishment of the Neoferontest, an immunological method for detection of latent *Neoehrlichia mikurensis* infection

By Michael Rapp

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Infectious Diseases, Institute of Biomedicine

Sahlgrenska Academy, University of Gothenburg, 2021

Supervisor: Anna Grankvist, Överbiolog (Main supervisor Linda Wass, Special biomedical scientist)

Neoehrlichia mikurensis is a gram negative obligate intracellular bacterium that infects humans through tick-bites. The primary vector is *Ixodes ricinus* that is spread out over large parts of Europe. The bacteria give cause to the infection neoehrlichios which in sever form causes vascular and thromboembolic complications. The only diagnostic method that is available for now is PCR-based. The purpose of this project is to try and establish a new method for detecting latent infections. The method is based on the Interferon-γ release assay. Peripheral blood mononuclear cells were isolated from whole blood taken from healthy and *N. mikurensis* infected individuals. The cells were cultivated in a 96-well plate, stimulated with a *N. mikurensis*-specific protein and endothelial cell homogenate positive for *N. mikurensis.* The cells were then cultivated for 5 days after which the spent culture media was retrieved, and the cells were discarded. The spent culture media was then used to perform a sandwich ELISA for both Interferon-γ and Interferon gamma-induced protein 10. The method was performed on 5 individuals, two positives for infection and three negative controls. One control showed positive against the *N. mikurensis*-specific protein when Interferon-γ was tested. Interferon gamma-induced protein 10 showed that one control and one patient was positive for the *N. mikurensis*-positive endothelial homogenate. A different control showed positive for the *N. mikurensis* specific protein. The purpose of the study was not achieved since a reliable method could not be established. The method must either be looked over and changed or alternative methods needs to be used.

Microbroth dilution more reliable than E-test when determining MIC-value for Viridans Streptococci

By Lisa Rask

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology,
Sahlgrenska Academy, University of Gothenburg, 2021
Supervisor: Erika Lindberg PhD

For some serious infections it is important to determine the antimicrobial resistance of the bacteria. The European Committee on Antimicrobial Susceptibility Testing recommends the microbroth dilution method to determine the antimicrobial resistance. Clinical Microbiology, Sahlgrenska University Hospital in Gothenburg, strive to follow the recommendations and implement the microbroth dilution method for the determination of resistance in Viridans Streptococci. The purpose of this study is therefor to validate the microbroth dilution method for Viridans Streptococci.

Ten control strains and ten clinical isolates of Viridans Streptococci were used for validation of a microbroth dilution plate (SEMST7) for nine different antibiotics. A bacterial suspension of 0.5 McFarland was added to Mueller Hinton Fastidious broth and the microbroth plate was inoculated with 100 µl/well and incubated for 18-24 hours at 34-36°C. The minimal inhibitory concentration was read and categorized as sensitive, sensitive to increased exposure or resistant. With the values obtained, statistical data were calculated to produce essential agreement, categorical agreement, minor error, major error, and very major error to determine the accuracy and precision of the method.

The control strains obtained a total essential agreement of 99.7 % and a categorical agreement of 97.8 % with 1.54 % minor error, 0 % major error and 0.62 % very major error.

Using the clinical isolates, the microbroth dilution method was compared with the current gradient method. The total essential agreement was 91.1 % and total categorical agreement was 98.9 %, with one minor error (1 %) and no deviations in forms of major error or very major error.

The result of the study shows that the microbroth dilution method provides more accurate values of minimal inhibitory concentrations than the gradient method and will therefore be introduced as the determination method for Viridans Streptococci at Sahlgrenska University.

Elevated concentrations of cystatin C and associations between increased intima-media thickness, calcium score and plaque in a population with different types of glucose metabolism

By Filip Rolin

Bachelor thesis in Biomedical Laboratory Science performed at Wallenberg laboratory, Sahlgrenska Universitetssjukhuset, Göteborg.

Supervisor: Caroline Schmidt, Associate Professor

Introduction: Diabetes is a common risk factor to atherosclerosis, which is the most common reason for myocardial infarct. IMT (intima-media thickness), plaque and CACS (coronary artery calcium score) are all different types of diagnostic measurements for thickness of arterial walls and assessment of plaque in the process of atherosclerosis. Previous studies have shown that cystatin C, a biomarker usually used for kidney function, plays a role in the process of atherosclerosis.

Aim: The aim for this study is to investigate the relationship between cystatin C and the different types of measurements for atherosclerosis and compare it to individuals with different types of glucose metabolism.

Method: 1965 volunteers in the ages 50 - 64 were examined using ultrasonography of carotid arteries, computed tomography, blood work and questionnaires. Data was divided into groups and categorized in different types of glucose metabolism. Atherosclerotic measurements and risk factors were compared to a control group with normal glucose metabolism. Cystatin C concentrations were compared with the atherosclerotic measurements CACS, IMT and prevalence of plaque.

Result: The results show a significant association between plaque and cystatin C ($p<0.001$). IMT and CACS shows significant associations to cystatin C. Elevated concentrations in cystatin C (mg/l) increases IMT with 0.094 – 0.195 mm dependent to artery segments ($p\leq0.006$). Also, a negative correlation was observed between HDL (high density lipoprotein) and cystatin C ($p<0.001$).

Conclusion: There are significant associations between cystatin C and the atherosclerotic measurements CACS, IMT and prevalence of plaque in individuals with different types of glucose metabolism.

Establishing an Immunohistochemistrial Triple Stain using p63, CK34BE12 and AMACR as a tool in the Diagnosis of Prostate Adenocarcinoma

Essay/Thesis: 15hp
Program and/or course: Biomedical Laboratory Science, BMA062 Bachelor Thesis in Biomedical Laboratory Science
Level: Bachelor
Semester Year: ST2021
Supervisor: Martin Johansson, Doctor and Professor in Pathology
Examiner: Martin Lindell
Report No:
Keywords: Prostate Adenocarcinoma, Diagnosis, p63, CK34BE12, AMACR, p504S, Immunohistochemistry, Triple Stain

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Purpose: Establishing an immunohistrochemistrial triple stain using antibodies against p63, high molecular weight cytokeratin 34 Beta E12 and AMACR, with two protocols – one where the antibodies were added one by one within separate incubation periods, and second where p63 and 34BE12 were mixed into a cocktail and added together before AMACR (alike double stain). Successful procedure was to be added to the laboratory and used when ordered.

Theory: Prostate cancer is the second most common cancer around the world. Benign neoplasia develops from basal cells that forms a membrane around acini in the stroma. On top of the cells lays secretory cells, which produces enzymes and can become malignant when they penetrate the basal membrane and form acini in stroma. These cells produce an enzyme, AMACR, which can be used in diagnosis, when H&E stain is not enough. By introducing antibodies with a chromogen against the protein, malignant cells can be stained. Same goes for benignant cells when antibodies against p63 and 34BE12 are used. By combining all three antibodies, it is possible to detect malignant and benignant acini, but also cells that are precursors to malignant cells.

Method: 24 formalin fixed and paraffine embedded blocks were picked, which before had been diagnosed with some sort of prostatic cancer. The tissues were sliced and stained with H&E, single antibodies, and triple stains (separate, cocktail 1:100 and 1:50) using visualisation methods from Dako. AMACR was visualised with magenta red-chromogen, and p63/34BE12 with DAB.

Result: All the slides were successfully stained with single antibodies, and triple stains. Triple stains made it simpler to analyse the slides and detect neoplastic changes in tissues. Triple stain where a cocktail diluted 1:100 was used showed the best results, therefore can be a candidate for usage in the laboratory.

VERIFICATION OF A NOVEL METHOD FOR IGG DIAGNOSIS OF HERPES SIMPLEX VIRUS AND VARICELLA-ZOSTER VIRUS

By Niki Rostamzadeh

Bachelor Thesis in Biomedical Laboratory Science
Department of Clinical Microbiology, Sahlgrenska University Hospital
Supervisors: Ka-Wei Tang, M.D., Ph.D. and Ine Lurquin, Biomedical Laboratory Scientist

Background: Neurological infections caused by herpes simplex virus (HSV) and varicella-zoster virus (VZV) are potentially fatal conditions if left untreated. Their acute phase diagnosis is based on PCR analysis of serum and cerebrospinal fluid (CSF) samples. At later stages of disease, assays of intrathecal IgG antibody synthesis serve as powerful diagnostic tools. The determination of pathogen-specific antibody indices (AI) of serum and CSF by using Reiber's formula is frequently recommended for accurate assessment of intrathecal antibody synthesis.

Aim: In this study the diagnostic value and precision of a fully automated enzyme-linked immunoassay (ELISA) for the detection of anti-HSV IgG and anti-VZV IgG in serum and CSF using the system Euroimmun Analyzer I have been evaluated. Automated assays have been compared to current, manual in-house assays, in terms of their sensitivity and specificity for the assessment of pathogen-specific intrathecal antibody production.

Method: Paired serum and CSF from 43 patients that had previously been analyzed for detection of HSV- and VZV-specific IgG antibodies with the manual ELISA were analyzed with the automated assay. Total IgG and albumin concentrations was then measured for samples with quantifiable levels of specific antibodies. This allowed calculation of AI for the paired samples, which made assessment of intrathecal antibody production possible. The distribution of positive and negative results between methods were then compared and statistically analyzed using McNemar's test with binominal distribution. Furthermore, the intraassay and interassay variations of automated ELISAs were calculated and compared to those of the current manual ELISAs.

Results: When comparing the distribution of results from manual and automated methods significant differences ($p = 0.035$) were noted. Several cases of discrepancy were discovered when comparing the results from the different methods, indicative of falsely positive and negative findings from the manual ELISAs. The intra- and interassay coefficients of variation were between 4-17 % for all parameters tested, with values showing a maximum fluctuation of +/- 3.3 standard deviations from the mean.

Conclusion: The automated assays and their evaluation algorithm were deemed more suitable for sensitive and specific assessment of intrathecal antibody synthesis when compared to the current manual methods. Due to substandard precision testing, further evaluation of intra- and interassay variation for the automated assay is recommended.

Assessment of Galectin-3 as a marker for oral cancer using western blot and ELISA

By Marcus Rådberg

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Odontology, University of Gothenburg, 2021.
Supervisor: Karin Christenson, PhD.

Background: Immunological factors play an important role in the development of oral cancer. Galectin-3 is a carbohydrate binding protein that is strongly suggested to contribute to the progression of oral cancer through interactions with immune cells. The purpose of this study was to analyse the concentration of Galectin-3 in saliva collected from patients diagnosed with oral cancer before and 3 – 6 months after receiving treatment and compare it to healthy controls to assess the possibility of using Galectin-3 as a biomarker for the progression of oral cancer.

Method: Analysis was performed on saliva samples collected from 7 patients diagnosed with oral cancer both at the time and diagnosis and either 3- or 6-months post-treatment as well as from 7 healthy controls. Qualitative analysis was performed using western blot on centrifugated saliva. Both pellet and supernatant were analysed with antibodies against the CRD unit and full-length Galectin-3. Quantitative analysis was performed using a sandwich ELISA.

Result: Galectin was found in saliva from both cancer patients and healthy controls. The mean concentration for patients was 163 ng/ml before and 232 ng/ml after receiving treatment. The mean concentration for healthy controls was 171 ng/ml. No significant difference between the groups could be detected.

Conclusion: It was not possible to confirm that Galectin-3 can function as a biomarker for oral cancer in this study.

Elevated Concentrations of High-Sensitive Cardiac Troponin T in Inividuals with Diabetes Mellitus Is Associated with Coronary Artery Calcium Score, Intima Media Thickness and The Presence of Plaque in the Carotids

By: Simon Sjölund

Bachelor thesis in Biomedical Laboratory Science performed at Wallenberg Laboratory, Sahlgrenska Akademin

Supervisor: Caroline Schmidt, Associate Professor

Background: cTnT is a cardiac specific biomarker, commonly found in straited cardiac tissue. In recent years, novel ways of implementing high sensitive (hs) immunoassays have been exposed to tests in order, not to only to be used in clinical practice as a tool to predict myocardial infarction (MI), but also to prognosticate individuals with incipient atherosclerosis. This has been confirmed by the literature. Moreover, hs-cTnT is a better tool when specifying cut-off-values and predicting lower concentrations of cTnT with a higher degree of accuracy.

Aim: The present study aims to investigate the potential associations between cardiac troponin T (cTnT) and factors typically synonymous with atherosclerosis in a patient sample with varying degrees of glucose metabolism.

Method: The present study uses data from the data set of *The gut microbiota, future type 2 diabetes and cardiovascular disease*. A randomized study population including the same proportion of both sexes with ages varying between 55 – 64 was compiled. A control group with healthy subjects was enrolled. The study population responded to a questionaire with queries concerning general health and the participants were later subjected to radiological examinations. Coronary Artery Calcium Score (CACS) was obtained by ultrafast computed tomography (UFCT) with a dual source Stellar Detector. Ultrasound images, depicting the intima media thickness (IMT) and precence of plaque in the carotids, were collected by Siemens Acuson S2000 ultrasound with a 9L4 linear transducer.

Results: Linear regression analysis performed in IBM SPSS (version 27) showed significant associations between cTnT CACS ($p < 0{,}001$), IMT ($p < 0{,}001$) and the presence of atherosclerotic plaque in the carotids ($p < 0{,}001$).

Conclusion: The results from the present study indicate that there are significant associations between elevated concentrations of the biomarker cTnT and diabetes. Furthermore, associations between individuals with varying degrees of impaired glucose metabolism and the factors typically synonymous with atherosclerosis: CACS, IMT and the presence of plaque in the carotids, are presented in this study.

HEPATITIS B VIRAL DNA INTEGRATIONS AND THEIR EXPRESSION IN LATE-STAGE LIVER DISEASE USING LONG-READ NANOPORE SEQUENCING

By Joakim Stenbäck

Bachelor's thesis in Biomedical Laboratory Science performed at the Virology Research Laboratory, Sahlgrenska University Hospital, 2021.

Supervisors: Maria Andersson, PhD, Johan Ringlander M.D and Doctoral Student

Hepatitis B is one of the most common chronic infections and infects the liver. Transmitted through sexual contact and blood, despite the presence of an effective vaccine the disease continues to be a major health concern around the globe. When infected at birth or during childhood, the disease often becomes chronic and progress into liver cirrhosis and hepatocellular carcinoma. Hepatitis B virus can integrate parts of its genome into the chromosomes of human hepatocytes, which may affect tumor suppressing genes and oncogenes, giving rise to a larger risk of cancer among chronically infected patients. The lack of a cure for chronic hepatitis B has led to more studies of these integrations to establish their role in disease progression and clinical implications.

The aim of this study is to use long-read sequencing to capture longer reads or entire transcripts to further study their integrational patterns into the host. A supplementary aim is also to establish which sample processing method provide optimal results for genetic analysis of hepatitis B virus.

Two separate liver extracts from two separate patients were analyzed using the methods nanopore sequencing, Capture Probe enrichment, Semi-specific PCR enrichment, as well as no enrichment to compare the method efficiency. A total of 6 samples were prepared using the three different enrichment methods. The results were then passed through an in-house bioinformatic pipeline to study the integrations of the virus.

The results of this study show that the optimal method seems to be using capture probe enrichment. Several transcripts were captured in their full length, most often with a starting 5' end in the HBV S2 region, had a HBV/Human Breakpoint at HBV positions 1750-1830 and ending at human genomic sites followed by a polyA tail.

The sample quality is vital to successful enrichment of hepatitis B viral transcripts and differences in RNA fragmentation was observed between two samples used. This thesis used only two samples due to the time limit and cost of sequencing. The study is planned to continue and include more patient samples to investigate differences in HBV integrations between patients.

No Observed Association Between Vitamin D and Intima-Media Thickness nor Plaque in Carotid, but Seen Between Vitamin D and Coronary Artery Calcium Score: A Retrospective Study Performed on Individuals with Varying Glucose Metabolism.

By: Felicia Linn Sörqvist

Bachelor thesis Biomedical Laboratory Science performed at Wallenberg Laboratory, Sahlgrenska Academy.

Supervisor: Caroline Schmidt, Associate Professor

Background. Diabetes and prediabetes is a common disease which is associated with an increased risk for cardiovascular disease. 66 % of the death among diabetics are caused by cardiovascular disease. Atherosclerosis is a complication of diabetes. Coronary Artery Calcium Score (CACS), plaque and Intima-Media Thickness (IMT) in the carotids are some of the atherosclerotic measurements. The amount of plaque in the coronary arteries and the amount of calcium are proportional to each other, therefore a CACS can be estimated from the amount of plaque by measuring the amount of calcium. There is an association between an increased IMT in the carotids and cardiovascular disease. IMT is generally elevated among diabetics and therefore it has a prognostic value in diabetic populations. Studies have shown that vitamin D has a certain type of influence in the progression of diabetes, the risk factors for diabetes and for CACS. The exact mechanisms behind them is not fully understood.

Aim. The aim of this study is to analyze the association between vitamin D and the atherosclerotic measurements.

Method. 1965 men and women between the ages 50 and 64, born in Sweden, were examined to investigate the incidence of newly discovered diabetes, prediabetes and an increased risk for diabetes. The prevalence of atherosclerosis in the carotids and coronary arteries was examined with ultrasonography and computed tomography, respectively.

Result. The results show that diabetics have an increased IMT in common carotid arteries, higher prevalence of plaque and higher CACS. CACS decreases when vitamin D increases, also individuals with a lower vitamin D have a higher CACS.

Conclusion. There are associations between vitamin D and CACS and potentially between vitamin D and IMT in the common carotids.

Trial, evaluation and optimisation of tissue processing in clinical pathology

By Henrik Torstensson
Bachelor thesis in Biomedical Laboratory Science preformed at Södra Älvsborgs Hospital
Sahglrenska Academy, University of Gothenburg, 2021
Supervisor: Pia Gabrielsson, Biomedical analyst. Roman Krenz, Med.dr.

A critical part of the method within clinical pathology is tissue processing. This must be well optimised and meet certain standards. To meet the higher demands in clinical practice the methods such as dehydration and clearing must provide a good result. Therefore, in this thesis project several new dehydration programs on the LOGOS (Milestone, Bergamo, Italy) tissue dehydrator were trialled, optimised, and validated for clinical practice. The method used in this study is the golden standard for most clinical pathology laboratories: Fixation, dehydration, sectioning, staining, and mounting. This method ensures that the processed and sectioned samples show representative morphological results in microscopical evaluation. Experienced pathologists at the laboratory evaluated the product from all tests to uphold quality ensuring practices. The results from this project indicated that the tissue dehydration timetable used in the evaluation of the method provides us with reliable results in staining methods such as Hematoxylin and Eosin, Van Gieson and Alcian Blue PAS in routine staining. Immunohistochemistry was evaluated and the final tissue processing program showed promising results in retention of antigen epitopes after tissue processing. Antigens tested in this project were: ER, PR, HER2, KI-67, VIM, CEA and CD10. The thesis concludes that the examined tissue processing program may be utilized in clinical practice in the laboratory for routine dehydration.

Investigation of the interferences hemolysis, icterus and lipemia for different concentrations of cortisol and testosterone on Alinity i

By Ebba Varildengen

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Chemistry, Södra Älvsborgs Hospital, Sahlgrenska Academy, University of Gothenburg, 2021.
Supervisor: Karin Lundberg, MSc

In most analysis in a laboratory, HIL-index is measured, which means that the degree of hemolysis, icterus and lipemia is measured. This is so that the result will be reliable and evaluated correctly. Each analysis has a limit on how high the interference can be before the result is affected, which means that samples can sometimes not be answered or need to be retaken, which is resource demanding for the healthcare. Therefore, a high HIL-index limit is desirable, but the limit must not be so high that the interference causes a clinically significant difference. In general, the limits are based on the reagent suppliers recommendation, which usually looks at the analytical quality instead of clinical relevance. Therefore, it is important that laboratories perform their own inference investigations. The aim of this study is to investigate the effect of hemolysis, icterus and lipemia for the analytes cortisol and testosterone on the instrument Alinity ci.

Patient samples with different levels of each analyte were collected and interfering substances in different concentrations were added to 4-9 different levels of cortisol and testosterone. To a blank, dilute solution was added. The change in percentage that occurred was compared with each starting value.

For cortisol and testosterone when investigating hemolysis, no major change in percentage were seen, at most 13% at hemolysis level 782 mg/dL for cortisol and 10% at hemolysis level 775 mg/dL for testosterone. When investigating lipemia, a decrease in percentage for both cortisol and testosterone was seen for most of the lipemia levels, with -33% and -24% at most. For both cortisol and testosterone when investigating icterus, no significant changes were seen except at a low analyte concentration, with -23% and +41% at most.

The results obtained from the investigation of hemolysis indicate that the current limit can be raised for both analysis. For lipemia, further investigations need to be made before a decision to raise the limit can be made. For icterus, the results indicate that the limits may not be changed, as low analyte concentrations seem to be greatly affected by a high level of icterus.

CAPILLARY PT ANALYSED ON MICROTAIONER EDTA-PLASMA

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Bachelor thesis in Biomedical Laboratory Science Performed at the Clinical Chemistry laboratory at Östra hospital, Sahlgrenska Academy, University of Gothenburg, 2021.
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Background: The prothrombin time is used to analyze the extrinsic coagulation pathway and blood samples are taken from patients who use the medicine Waran®, among others. Prothrombin time analyzes clot formation time which shows how fast or slow the blood coagulates. Patients using Waran® need regular follow-up as dose monitoring is important, a too high dose of the drug increases the risk of bleeding and a too low dose increases the risk of clot formation. ***The aim*** of the study is to investigate whether prothrombin time can be analyzed on EDTA plasma and to investigate the durability of EDTA tubes after sampling blood. ***Material and method:*** The study uses samples from 30 different individuals, 10 healthy individuals who do not undergo any coagulation treatment and 20 Waran® treated patients. The patients were at the hospital for follow-up blood tests and gave consent to participate in the study orally before the blood samples were drawn. The analysis was performed on Sysmex cs-5100 from Simens. The test tubes used were capillary EDTA mictrotainer tubes, venous citrate vaccutainer tubes and a fale bottom tube with 400µL Owren buffer. The study was divided into 3 different sektions for analysis: durability of the EDTA tubes, capillary EDTA vs. capillary Owren's buffer and the capillary samples vs. the venous. For statistical calculations, Excel was used and a pre-made template was used for the regression and difference plots. ***Results:*** The EDTA tubes showed a durability of up to 5 hours. Comparison of capillary EDTA and capillary Owren's buffer indicates a connection between the assays, also comparison of venous citrate tubes and capillary Owren's buffer indicates a clear connection. However, when comparing capillary EDTA and venous citrate as well as capillary EDTA and capillary Owren's buffer, the result does not indicate any connection. ***Conclusion:*** Samples taken in EDTA tubes have a durability of 5 hours and should be analyzed before then. Further investigation is required when comparing Capillary EDTA samples and venous citrate samples before the method can be applied in daily practice.