# University of Otago BMLSc programme year 4 student abstracts Semester 2, 2021

## Validating the method for inhibited specimens for the analysis of faecal pathogens

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**Objectives:** The Molecular Pathology Department at SCL, Dunedin is planning to implement extraction free faecal PCR from AusDiagnostics Faecal Buffer specimens. In order to have a backup method in the event of sample inhibition, we aimed to validate the performance of faecal PCR assay with extraction of Faecal Buffer by AusDiagnostics MT-Prep, a nucleic acid extraction instrument.
**Methods:** Faecal samples (44) were inoculated in Faecal Buffer tubes and tested in this experiment. At least 2 samples positive for each faecal pathogen target in the AusDiagnostics Faecal Pathogens assay were included. For each specimen, PCR was performed on both extraction-free supernatant and nucleic acid extract from MT-Prep, by AusDiagnostics High-Plex instrument. The take-off values for all target pathogens and corresponding spikes were measured. Eleven of the 44 included faecal samples were also inoculated in STAR buffer and had PCR performed after MT-Prep extraction.
**Results:** All the faecal samples inoculated into the faecal buffer and then extracted on the MT prep had later target and spike take off values than those from extraction-free faecal buffer samples. There were also 4 extracted samples with spike inhibition. The outcome of MT-Prep extracted specimens has shown poor agreement with extraction-free ones. Regarding the extracted STAR buffer samples, none were inhibited, and their target and spike take-off values were closer to results from their corresponding extraction-free specimens.
**Conclusion:** This study suggested that it is unsuitable to apply MT-Prep extraction to Faecal Buffer specimens as the back-up method for extraction-free faecal PCR. Limited testing of extracted STAR buffer samples showed better performance. Therefore, we may consider specimens in STAR buffer as the alternative plan after future studies.

## EvaGreen-based digital droplet PCR for copy number variation analysis: A systematic review of the literature

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**Objectives:** Digital droplet polymerase chain reaction (ddPCR) is purported to be a robust platform for quantitating copy number (CN). This literature review aimed to assess the accuracy and assay optimization of EvaGreen-based ddPCR for CNV analysis. Findings from this review will help guide an in-house validation project for adopting EvaGreenbased ddPCR to orthogonally confirm CNVs detected by a targeted next-generation sequencing (NGS) panel at LabPLUS, Auckland.
**Methods:** PubMed and Embase (Ovid) databases were searched for relevant studies. Studies were included which reported adopting EvaGreen-based ddPCR for CNV analysis using any sources of DNA template and/or assay optimization of EvaGreen-based ddPCR. All studies were written and published in English. Relevant information on assay protocols, accuracy for quantitating CN, assay optimization, and multiplexing strategies of EvaGreen-based ddPCR was extracted.
**Results:** Seven studies were eligible for data extraction. The assay protocols reported by all studies emphasized the significance of primer design, DNA preparation, droplet generation, and thermal cycling conditions on the final assay performance. The accuracy for quantitating CN was high, with an average calculated percentage error of 4.86% across five studies. Assay annealing temperature, primer concentration, and the amount of input DNA added per reaction were highlighted as parameters critical in assay optimisation. Multiplexing could be achieved through manipulation of amplicon length or the final primer concentration.
**Conclusion:** EvaGreen-based ddPCR is an accurate platform for CNV analysis, which is relatively easy to optimize and multiplex. Although EvaGreen-based ddPCR could be potentially employed as an orthogonal method for confirmation of CNVs detected via NGS, further validation and studies are required to fully evaluate its diagnostic performance.

## Investigation of an alternative method for managing patients with EDTA mediated platelet clumping

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**Objectives**: In the diagnostic haematology laboratory, platelet clumping is a cause of pseudothrombocytopenia, which can lead to potential interpretation errors. Platelet clumping is typically due to platelet activation during venepuncture or the action of an EDTA mediated antibody. It is critically important to obtain accurate platelet counts, as treatment is often transfusion dependent, which is a dangerous procedure if the platelet count is incorrect. The efficacy of ThromboExact and gentamicin in preventing platelet clumping is currently unknown and poorly characterised in the literature. This study aimed to assess the validity of ThromboExact and gentamicin for overcoming platelet clumping and to compare their effectiveness in rectifying EDTA-mediated platelet clumping.
**Methods:** A full blood count was performed on thirty normal controls and three known platelet clumping patients. Normal controls had one EDTA and one ThromboExact bled, whilst the clumping population had two EDTA, one ThromboExact and one sodium citrate bled. The normal control samples were tested upon lab arrival, and after five hours, while the known platelet clumping samples were tested hourly over six hours. This was to assess the tube stability in reducing platelet clumping. Analysis for both groups was carried out on the same Sysmex XN-20 Automated Cell Counter.
**Results:** Preliminary results indicated EDTA was more stable in normal controls than in patients with a known history platelet clumping. Gentamicin and ThromboExact were deemed the preferential method in known platelet clumping patients, due to the smaller decrease in platelet number over six hours.
**Conclusion:** This study suggests the use of gentamicin as the preferred method for rectifying EDTA mediated platelet clumping, due to statistical and cost analysis and overall performance.

## Comparison between BD BACTEC™ blood culturing and standard culturing for suspected prosthetic joint infections

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**Objectives:** The New Zealand Health Quality and Safety Commission's (HQSC) prosthetic joint infection (PJI) sampling and culture guide states that any broth culturing performed must be in addition to solid media culturing. Multiple studies have shown improved detection of PJI pathogens with the use of blood culturing broth systems. This study was performed to determine whether a testing protocol using the BD BACTEC™ FX40 blood culture instrument could replace the Canterbury

Southern Community Laboratories' (CSCL) current solid media and thioglycollate broth method.

**Methods:** Tissue samples (44) from 16 patients were homogenised and cultured using the current CSCL and study blood culture methods. MedCalc Software was used to analyse data.

**Results:** The 16 true positive samples were all *Cutibacterium acnes* shoulder infections. The current method identified 16 true positives (100% sensitivity; 82.14% specificity; 76.19% positive predictive value; 100% negative predictive value) with an average true positive identification time of 8.06 days. The study method identified 14 true positives (87.5% sensitivity; 85.71% specificity; 77.78% positive predictive value; 92.31% negative predictive value) with an average of 10.38 days.

**Conclusion:** This study suggests that the blood culturing method cannot replace the current CSCL PJI method. The current standard method detected all true positives and, on average, did so faster by two days. Only *Cutibacterium acnes* was isolated in true PJI patients in this study. In another study, this microorganism was shown to be poorly recovered specifically in PJI blood culturing compared to standard culturing. Further investigation could assess, especially if other PJI organisms are frequently isolated, if blood culturing (without negative vial terminal subculturing as per the HQSC PJI guidelines) may replace thioglycollate broth culturing.

**Acknowledgement:** The author thanks staff at Canterbury Southern Community Laboratories, Christchurch for supervision of this project.

## Verification study of the Abbott® i-STAT total βhCG Point-of-Care test for rural hospital use

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**Objectives**: A need for a point-of-care (POC) testing method to detect βhCG was identified for the Dunstan Hospital, to assist with the differential diagnosis of abdominal pain in women of childbearing age presenting to the hospital if miscarriage or ectopic pregnancy were suspected. This study was designed to assess both the accuracy and precision of the i-STAT βHCG quantification POC test, to verify this method for use in clinical decision making in a rural setting.

**Methods**: Measurements (40) were made, on two available i-STAT devices, for samples with a HCG quantification test request or known second trimester pregnancy. These were measured against the reference method (Roche® 2010/ E170 assay) for correlation analyses. Individual samples (38) were analysed. Samples at each end of the measurement range were used for precision calculations: co-efficient of variation (CV) and uncertainty of measurement (UoM). Quality control (QC) was also performed on all days the patient samples were measured to calculate CV and UoM.

**Results**: The I-stat method showed high correlation with the reference method, adjusted $R^2$=1.0. Bland-Altman analysis showed a mean difference of 1.88% between the methods. CV values obtained from patient precision were 5.6 and 7.2, which was consistent with CV values calculated from daily precision runs which had CV values for each level of 6.6, 3.7, and 6.0. Sensitivity and specificity were 100% for the I-Stat method.

**Conclusion**: The i-STAT β-hCG POC test provided hCG values that were consistent with the reference method and sufficiently precise for the purpose of emergency clinical decision making where pregnancy may be suspected.

## Verification of the Hologic Panther Fusion system for diagnostic testing of parainfluenza, adenovirus, human metapneumovirus and rhinovirus

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**Objectives:** Respiratory viruses are seen globally, producing similar symptoms while requiring different treatments and clinical management. This makes testing and diagnosis important. Wellington Southern Community Laboratories use the (verified) AusDiagnostic Respiratory 24 assay for such testing. This method is time-consuming and laborious unlike the Hologic Panther Fusion, already used to test some respiratory viruses in the laboratory.

The aim of this study was to verify the Hologic Panther Fusion for diagnostic testing of parainfluenza 1/2/3/4, adenovirus, human metapneumovirus and rhinovirus in comparison to the AusDiagnostic.

**Methods:** The Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance (SHIVERS) study-five criteria was used to determine the sample population. All samples were run on the Hologic Panther Fusion for these respiratory targets and their cycle thresholds recorded. Five positive samples (covering upper and lower limits of detection) were selected for each target. These samples were then tested on the AusDiagnostic, which produces a take-off value. These values are two different measurements making direct comparison difficult.

**Results:** Each sample's position within both measurements' limits of detection were approximately the same, indicating the Hologic Panther Fusion detected results similar to the AusDiagnostic. A linear regression graph indicated the two measurements had a strong concordance as discordant results were only found in the lower limits of detection, which have increased chance of sampling bias due to low viral loads and less clinical significance. There were no positive parainfluenza 1/2 samples in the study population so these could not be verified.

**Conclusion:** This report indicated the Hologic Panther Fusion is verified for diagnostic testing of parainfluenza 3/4, adenovirus, human metapneumovirus and rhinovirus at Wellington Southern Community Laboratories.

## Verification of total prolactin and post-PEG monomeric prolactin reference intervals validation of phosphate-buffered saline polyethylene glycol method

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**Objectives:** Macroprolactin complexes positively interfere with the Roche prolactin assay. Precipitation of macroprolactin by 25% polyethylene glycol provides an estimate of monomeric prolactin. Wellington SCL is reviewing its methodology by comparing the performance of phosphate-buffered saline polyethylene glycol (PBS-PEG) to de-ionised water polyethylene glycol (DI-PEG) solution, as monomeric prolactin recovery may be more consistent with PBS-PEG.

**Methods:** Forty samples with normal prolactin concentrations, 25 with elevated concentrations, and 20 normal-health male samples had serum frozen for testing. After warming to room temperature and removing particulate matter, 200μL was combined with 200μL DI-PEG or 200μL PBS-PEG and vortexed, incubated, then centrifuged and tested on the Cobas e602. Monomeric prolactin recovery rates and percentage difference between the methods were calculated. Normal serum (4mL) and elevated-prolactin serum (4mL) were repeatedly tested to determine CV%.

**Results:** Monomeric prolactin recovery was 2.3% less using PBS-PEG for the normal group, and 5.6% less in the elevated group. Method correlation was strong, $R^2$ of 0.95 for the normal group and 0.99 for the elevated group. Recovery reduction was more pronounced with higher total prolactin concentrations. The 20 normal-health male total prolactin concentrations ranged from 98.5mU/L to 380.6mU/L. CV% using DI-PEG was 3.8% and 2.0% for normal and elevated groups, whereas PBS-PEG was 2.1% and 1.9% respectively. Most method differences fell within the RCPA prolactin analytical performance specification.
**Conclusion:** Wellington SCL's current total prolactin and post-PEG monomeric prolactin reference intervals were verified for the testing population, as well as the 40% cut-off for macroprolactinaemia. PBS-PEG had better reproducibility, supporting a change from DI-PEG.

## Phoenix 100 identification and antimicrobial sensitivity validation

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**Objectives:** The Nelson hospital laboratory microbiology department has a BD Phoenix 100 bacterial identification and antimicrobial susceptibility analyser. The primary goal of this project is to validate the Phoenix 100 for diagnostic use by verifying that the Phoenix 100 produces the same results as would be produced by the laboratory with their current methods. The current methods include MALDI-TOF MS as the gold standard for identification and phenotypic bacterial profiles as the gold standard for antimicrobial susceptibility and as another identification method.
**Method**: A comparison of 24-gram positive organisms and 21-gram negative organisms was constructed. Their identification was compared on genus and species levels and antimicrobial susceptibility results were compared with the reference methods for any variation.
**Results**: All 45 organism identifications correlated with the reference methods on the genus level and 38 on the species level. There were 21-gram positives and 17-gram negatives with the same species identifications as the reference methods. Not all organisms had antimicrobial sensitivity panels performed but of the 36 organisms that did, there was only one discrepancy which was the determination of the source of an AmpC. The percentage of corroborating identifications were comparatively low considering results from other studies, but this was due to the way the project was executed and organisms were selected. Organisms which would routinely have confirmatory testing with the Phoenix 100 were appropriately identified.
**Conclusion**: The Phoenix 100 analyser should be appropriate to include in routine confirmatory testing as it produces the same results as methods already in use. This can help improve patient outcomes by reducing turnaround times and providing confirmatory diagnostic information.

## Platelet function analysis on PFA-200 and the introduction of preanalytical variation by pneumatic tube transportation

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**Objectives:** Determine the preanalytical variation and corresponding clinical significance of introduced to samples undergoing pneumatic tube system transportation prior to Platelet Function Analysis testing.
**Methods:** Paired samples were collected from participating donors through venepuncture with a 21-gauge needle. As per the standard operating procedure of Waikato District Health Board, one sample was walked to the laboratory, the paired samples were allocated a randomly assigned Lamson station located throughout Waikato Hospital for pneumatic tube transportation to the laboratory. Upon reception, paired samples were tested in parallel on the Siemens INNOVANCE® PFA-200 System measuring closure time of specimens in PFA Collagen/ Epinephrine Test Cartridges.
**Results:** Removal of outliers following a Shapiro-Wilk Test, normally distributed data plotted on a Bland-Altman plot expressed agreement between the two methodologies. The obtained bias of -2.9 seconds, p-value of 0.5, R-squared value of 0.89 and difference of interquartile ranges of 0.5 seconds identified a statistically insignificance between the difference in closure times of walked and Lamson transported samples. The null hypothesis stating no difference exists between the two methodologies failed to be rejected (p-value >0.05).
**Conclusion:** A statistically insignificant difference was identified between the closure times of walked and pneumatic tube transported samples for Platelet Function Analysis. Further continuation of this study is recommended to finalise an overall conclusion and to establish the appropriate clinical and statistical significance of the preanalytical variation introduced by Waikato District Health Board Lamson system.

## Evaluating the performance of visual observation to detect haemolysed, icteric and lipaemic samples: Are our eyes good enough?

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**Objectives:** Common preanalytical interferences such as haemolysis, icterus and lipaemia can significantly affect test results and compromise patient safety. The current method for haemolysis, icterus and lipaemia detection in routine coagulation samples is visual observation. The aim of this study was to evaluate the accuracy and agreement between visual observation and automated serum indices methods.
**Methods:** This study was performed on 40 routine coagulation samples. Visual observation was performed by five trained laboratory staff members, according to standard operating procedures. Automated haemolysis, icterus and lipaemia detection was completed using the Cobas c501 serum indices Generation 2 assay. The accuracy and agreement between visual observation and automated methods was determined.
**Results**: Accuracy of 100% was achieved for the correct handling of samples with haemolysis values over 100mg/dL. This agreement was reflected in the k value of 1. Observer error occurred most frequently for samples with haemolysis around 50mg/dL. No clinically relevant levels of lipaemia or icterus were present in the sample population, which likely contributed to the high level of accuracy and agreement achieved.
**Conclusion:** Very low frequencies of clinically relevant levels of haemolysis, icterus and lipaemia were detected in the sample population. This is representative of the quality of samples received in the Oamaru, Southern Community Laboratory. Routine coagulation specimens with observed interference should have automated serum indices results obtained, particularly when observed haemolysis is around 50mg/dL. This is to safeguard the accuracy of routine coagulation results. A more extensive study to assess the performance of visual observation for varying degrees of haemolysis, icterus and lipaemia would be useful to reveal further errors of sample categorisation.

## Evaluation of an established urine total porphyrin screening protocol for implementation in Southern Community Laboratories, Dunedin.

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**Objectives:** Porphyria is a group of disorders affecting haem metabolism, resulting in the accumulation of toxic haem precursors, porphyrins. Porphobilinogen is a porphyrin and can be detected in a screening test for acute intermittent porphyria. A positive porphobilinogen result indicates the requirement for more specific testing, such as total urine porphyrin testing. Currently, total urine porphyrin testing is conducted by Canterbury Health Laboratories. Our laboratory intends to bring this test in-house by evaluating an established protocol utilised by Southern Community Laboratories, Wellington.
**Methods:** Twelve Royal College of Pathologists Australasian (RCPA) samples of known median concentration and two ClinChek quality control samples were utilised to evaluate the respective accuracy and precision of this protocol. The samples were acidified using hydrochloric acid to dissociate porphyrin chelation and scanned between 300nm and 600nm on the Thermo Fisher Evolution 220 spectrophotometer. The peak absorbance was determined, quantified, and reported in umol/L for comparison to the expected value.
**Results:** A strong correlation was observed between the median value of RCPA samples and the calculated results. A calculated bias of 4.5% from the median was acceptable and within the RCPA analytical performance specification of 10%. A Student t-test was performed (p=0.296), demonstrating statistically insignificant differences. Additionally, this protocol displayed high precision with a CV of 0-1.16% when repeatedly analysing the quality control samples.
**Conclusion:** This study showed agreement between the expected and calculated results. The differences between these results were not statistically or clinically significant. This evaluation has validated the reliability of this protocol to determine the concentration of total porphyrins in fresh urine and should be adopted by Southern Community Laboratories Dunedin.

## A comparison between GMS (manual) and PASF (automated and manual) for the diagnosis of fungal infection

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**Objectives:** The effective management of fungal infection heavily depends on the early diagnosis and timely initiation of treatment. Periodic Acid Schiff (PAS) and Gomori methenamine silver (GMS) are the two most common stains for fungal diagnosis. The aim of this research project was to compare the quality and sensitivity of both stains using automated and manual methods.
**Methods:** PAS and GMS were performed on 20 specimens that were previously positive for fungal infection. Sixty slides were evaluated based on a 5-grading scale. Statistical tests were also performed to compare the different staining methods.
**Results:** There were 15 out of the 20 specimens that were positive for one or both stains for fungi. The mean score of the stain quality for both manual PAS and GMS was evaluated using a paired sample t-test (p=0.21) which is statistically insignificant. The automated PAS and manual PAS also failed to show a significant difference with a p-value of 0.58. Furthermore, a chi-squared test was performed to assess the difference in sensitivity of both stains (p-value<0.05) which was statistically significant and both stains are strongly associated.
**Conclusion:** PAS and GMS stains were slightly different in quality and positivity rate. However, both stains are still considered optimal for fungal detection as they failed to show significant difference and they were both strongly associated. Overall, PAS was easier to perform whereas GMS was easier to read.

## Evaluation of the Roche Ca15-3 assay on the Cobas 8000

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**Objectives**: Ca15-3 is a tumour marker used for monitoring breast cancer treatment or progression. Southern Community Laboratories (SCL), Dunedin, currently sends serum samples requiring Ca15-3 analysis to Canterbury Health Laboratories (CHL) but will soon bring the assay in-house. The aim of this evaluation was to compare the Ca15-3 results obtained by CHL using the Beckman-Coulter DXL800 analyser to the results obtained on the Roche Cobas 8000.
**Methods:** Both analysers employ a chemiluminescent microparticle immunoassay method but differ in their monoclonal antibody specificity. Breast cancer patient samples (n=17) were analysed in parallel and their results compared using statistical analysis. Two levels of Roche PreciControl Tumour Marker (TM) were analysed daily on the Cobas 8000 over a two-week period to assess assay precision.
**Results:** A mean difference of 53.02 U/mL was identified between the two assays. The Bland-Altman plot had limits of agreement (95%) from -102.676 to 208.724 U/mL and the Passing-Bablok generated a slope with the equation y= 0.6033 + 1.56x. The t-test derived a p-value of 0.0142. The CV% for PreciControl TM1 (n=16) and PreciControl TM2 (n=17) was 3.54% and 3.64%, respectively.
**Conclusion:** The results obtained by the Cobas 8000 have an upwards bias of 56% compared to the Beckman-Coulter DXL800. This is statistically significant as deemed by the p-value. It is clinically significant as it is higher than the analytical performance specification for Ca15-3 set by the Royal College of Pathologists of Australasia. This indicates that while the Cobas 8000 has good precision, the two assays are not interchangeable. During the period of assay changeover at SCL, patient results should not be directly compared to their older results obtained from CHL.

## A comparison of the growth of *Streptococcus pneumoniae* in aerobic and anaerobic conditions

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**Objectives:** *Streptococcus pneumoniae* is a Gram-positive diplococcus capable of causing infections such as pneumonia. Scientists at Canterbury Health Laboratories (CHL) currently culture respiratory samples using aerobic incubation in 5% carbon dioxide ($CO_2$) to identify *S. pneumoniae* colonies. The aim of this study was to compare the growth of *S. pneumoniae* in aerobic and anaerobic conditions in order to determine if the permanent addition of anaerobic incubation for all respiratory samples would facilitate more efficient *S. pneumoniae* identification.
**Methods:** Twenty-two *S. pneumoniae* clinical isolates were each inoculated onto three 5% blood agar plates. Each plate was incubated aerobically in 5% $CO_2$, anaerobically or held for 3 hours in a flow jar in 5% $CO_2$ at room temperature prior to anaerobic incubation. Colony size and appearance was recorded after 24 hours and 48 hours of incubation. Microsoft Excel was used for data analysis.

**Results:** Average colony size after 48 hours of incubation aerobically in 5% $CO_2$, anaerobically and in the flow jar was 0.91mm, 2.32mm and 2.12mm, respectively. ANOVA and post hoc tests indicated a significant difference between aerobic incubation and both anaerobic incubation ($P < 0.001$) and the flow jar ($P < 0.001$). Colony appearance after aerobic incubation resembled draughtsman and were grey and moist. Anaerobic and flow jar colonies were mucoid, white, and convex.
**Conclusion:** Colony size was found to be consistently larger after both anaerobic and flow jar incubation along with more efficient identification of colonies due to a greater ease of recognition. Despite confirmation of anaerobic incubation increasing the efficacy of *S. pneumoniae* detection, the low numbers of samples positive for *S. pneumoniae* received by CHL and the increase in costs associated with a change in incubation methods has encouraged CHL to maintain their current protocols.

## Assessment of a new serological kit for detecting SARS-CoV-2 neutralising antibodies

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**Objectives:** To assess a new serological kit for detecting SARS -CoV-2 neutralising antibodies (NAbs) and compare results to other serological and molecular assays.
**Methods:** Sixty-six frozen serum or plasma samples were selected to cover a wide range of previously performed tests' results. Two serological assays were previously performed, enzyme-linked immunosorbent assay (ELISA) for anti-SARS-CoV-2 Spike Protein IgG antibodies (S-IgG), and ELISA for anti -SARS-CoV-2 Nucleocapsid Protein antibodies (NCP-IgG). Additionally, nasopharyngeal swabs from all 66 patients were previously tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to detect SARS-CoV-2 RNA. The new kit is a surrogate virus neutralisation test (sVNT), which does not require live virus, hence does not need biosafety level 3 equipment. sVNT was standardised under CHL conditions, then performed on the selected samples. Results were compared to those obtained from assays previously performed.
**Results:** All positive and negative controls were within the manufacturer's defined range, showing validity of results under CHL conditions. Comparing sVNT results with previous tests, showed agreement of 65.2%, 86.4%, and 74.3% with qRT-PCR, S-IgG, and NCP-IgG assays, respectively. The discrepancies between sVNT and the other assays can be explained by one or more of several reasons: qRT-PCR only detects viral RNA in current infections, while sVNT detects NAbs from current or cleared infections; not all infected individuals seroconvert; some samples were from vaccinated individuals; antibodies are not produced at early infections; and the experiment's small sample size due to the limited kit supply.
**Conclusion:** sVNT was performed successfully at CHL. Commercial kit may need to be introduced in the future for the detection of NAbs. Standardisation and full comparison are required with the commercial kit with a bigger sample size.

## CD56 immunohistochemistry optimisation

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**Objectives**: An RCPA evaluation of the CD56 immunohistochemistry protocol used in the Department of Histology, Taranaki Pathology Services indicated weak staining with high background pigmentation. The purpose of this study was to review the current protocol and determine if alterations to the methodology could produce higher quality staining.
**Methods**: Three tissue specimens were selected based on previous positive staining under the current CD56 protocol, and as such were known to have CD56 positive tissue regions for analysis. Each tissue was stained using multiple different protocols with heightened or lowered antigen retrieval or antibody incubation times as well as a repeat of the current protocol.
**Results**: The staining of these tissues demonstrated that the current protocol produced intense, selective staining in all three specimens, and did not display the background or weak staining observed in the RCPA tissue. The alterations to the antigen retrieval stage of the protocol were observed not to affect staining significantly unless the time was severely reduced, which resulted in pale, sub-optimal staining. Increasing the antibody incubation time was observed to increase the staining intensity and background staining of the section, while decreasing the antibody incubation was observed to result in paler staining. While the reduced antibody incubation still resulted in acceptable staining of strongly CD56 positive tissue, weakly positive tissue was unacceptably pale.
**Conclusions**: It was determined that the current protocol is capable of consistently producing acceptable staining of CD56 positive tissue, and that other factors besides the antigen retrieval time and antibody incubation times must have interfered with the staining of the RCPA assessment tissue, such as tissue fixation or processing.

## The comparison of three commercial rodent tissue indirect immunofluorescence assays for detection of anti-liver kidney microsomal type 1 autoantibodies and other tissue autoantibodies

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**Objectives:** Rodent tissue indirect immunofluorescence assays (IIFAs) are used for detection of tissue autoantibodies to aid in the diagnosis of autoimmune diseases. The aim of this study was to compare the MeDiCa mouse stomach/kidney IIFA, currently used at Southern Community Laboratories (SCL) Dunedin, with the NOVA Lite and ImmuGloTM rodent stomach/ kidney/liver IIFAs, for the detection of anti-liver kidney microsomal type 1 (anti-LKM 1) autoantibodies. The secondary aim was to compare the overall performance of the three IIFAs for detection of other tissue autoantibodies routinely screened for.
**Methods:** Fifteen tissue autoantibody positive serum samples were tested according to manufactures instructions, with the MeDiCa, NOVA lite, and ImmuGloTM IIFAs. No anti-LKM1 positive samples were able to be obtained, a commercial anti-LKM 1 human serum positive control was used as substitute. The slides were viewed under fluorescence microscope and blind read by five of the immunology staff at SCL Dunedin.
**Results:** The anti-LKM1 positive control showed distinct staining and was easily identified by the readers with all three IIFAs. The detection of anti-mitochondrial and anti-gastric parietal cell autoantibodies was consistent across all three IIFAs and with the original reported result. Two discrepancies were seen with the MeDiCa IIFA and four were seen with the NOVA lite IIFA in low titre (1:40) anti-smooth muscle antibody positive samples.
**Conclusion:** All three IIFAs showed good detection of anti-LKM1 autoantibodies, and it was concluded that the MeDiCa IIFA currently used is adequate to identify these autoantibodies. Overall, the MeDiCa IIFA produced comparable results to the NOVA lite, and ImmuGloTM IIFAs, and should continue to be used routinely.

## A comparison of the precision and stability of pancreatic amylase and lipase

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**Objectives**: Serum enzymes specific to the pancreas are an important metric in the diagnosis of acute pancreatitis. Traditionally, amylase has been the analyte of choice due to the inexpensive and rapid nature of its test. However, with the increased availability of lipase testing kits, both enzymes are frequently requested to diagnose pancreatitis. The aim of this study was to compare the stability and precision of amylase to that of lipase to determine the redundancy of amylase.
**Methods:** Twenty-eight samples were selected at random. For stability testing, 15 samples were aliquoted into two sets and kept at different temperatures. Lipase and amylase levels were measured in samples from both sets every two days. Precision was measured by repeating each test three times on 13 samples. Statistical values were calculated, and data was graphed and compared.
**Results:** Both enzymes remained within the acceptable limit of the original value, despite some minor drift. The largest drift was observed in the serum amylase in the samples kept at room temperature, and the least in the serum lipase in the samples from the same set. All repeats fell within the allowable limits of the precision test. Amylase was found to be slightly more precise than lipase, and the increase in lipase concentration did not correlate to increased imprecision.
**Conclusion:** The stability of serum pancreatic amylase and lipase were comparable, with any drift being clinically insignificant. The precision of lipase and amylase assays were comparable. Therefore, the decision to use the serum pancreatic amylase or the serum lipase assay should not depend on these factors. Further considerations investigators should take into account are sensitivity, specificity, price and availability.

## Validation of the Roche lipase assay across the Wellington SCL Laboratories

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**Objectives:** To review the current literature on serum pancreatic amylase vs serum lipase in the diagnosis of acute pancreatitis. Validate the Roche Lipase assay across the WSCL laboratories to replace the pancreatic amylase assay currently reported.
**Methods:** The validation of the Roche lipase assay was undertaken across the four WSCL laboratories on three different Roche Cobas platforms: Wellington (c8000) Hutt and Masterton (c501) and Kenepuru (c311). Patient samples within a range of 5.1 – 3281 IU/L were collated at the Wellington Site and distributed to the other three sites for testing on the same day. Each laboratory performed lipase calibrations and quality control before patient samples were tested. Inter-laboratory precision was reviewed using the RCPAQAP analytical performance specifications.
**Results:** Results from each site were statistically analysed using Analyse-it® for site specific reporting on: Co-efficient of Variation%, Passing – Bablock regression and Bland Altman. Inter laboratory precision was reviewed using the RCPAQAP analytical performance specifications.
**Conclusion:** The literature review showed the specificity of lipase is slightly superior to pancreatic amylase in patients with acute pancreatitis. This in combination with the fact that serum amylase is elevated earlier and persists longer in acute pancreatitis clinically justifies the replacement of pancreatic amylase with lipase testing. Validation of the Roche lipase assay across the WSCL laboratories showed good correlation of performance between testing platforms. Assay performance was acceptable and within RCPA specifications for lipase. The lipase assay was implemented across the WSCL Laboratories and replaced pancreatic amylase in the diagnosis of acute pancreatitis.

## Evaluation of a real-time rt-PCR method on the BD Max™ platform for detecting Mumps orthorubulavirus (MuV) in clinical samples

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**Objectives:** This project aims to evaluate the performance of a real-time reverse-transcriptase Polymerase Chain Reaction (PCR) method for *Mumps orthorubulavirus* (MuV) detection in clinical specimens using the BD MAX™ automated RT-PCR platform, with comparisons to a method for the ABI 7500 RT-PCR platform.
**Methods:** A ten-fold dilution series of PRIORIX MMR vaccine containing vaccine-strain MuV was tested via a newly developed in-house "BD MAX™ method", and Canterbury Health Laboratories' current "ABI method". Twenty positive and 22 negative patient samples tested previously with the ABI method for MuV were tested in parallel via the BD MAX™ method; any samples that gave conflicting results were retested via the ABI method in duplicate.
**Results:** The BD MAX™ method consistently detected MuV in dilutions down to $10^{-5}$ times the original concentration, whereas the ABI method could only detect down to the $10^{-4}$ dilution step. The BD MAX™ method appears to have greater analytical sensitivity. Specificity of the BD MAX™ method appears similar to the ABI, as none of the previously negative patient samples tested positive for MuV on the BD MAX™. Unexpectedly, one of the previously positive samples tested negative for MuV on the BD MAX™; this sample later tested positive for MuV twice on the ABI.
**Conclusion:** This in-house method for the BD MAX™ to detect MuV in samples is at least as specific as the method on the ABI 7500 and has the potential to be more sensitive. Considering the analyser's automated nature and the method's room for future optimisation, the BD MAX™ is well suited for MuV testing.

## Evaluation of the clot waveform analysis of the non-anticoagulated population in Christchurch

*Noelle Watanabe[1] and Rebecca Lu[2]*
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**Objectives:** The clot waveform is a reaction profile that can be generated from the prothrombin time (PT) and activated partial thromboplastin time (APTT) measured by the Sysmex CS-2500 automated coagulation analyser. The clot waveform analysis (CWA) allows for the evaluation of both qualitative and quantitative data derived from the clot waveform, which is known to show different patterns in certain clinical conditions compared with normal. This project was carried out to assess the PT and APTT CWA data of non-anticoagulated patients in Christchurch.
**Methods:** CWA of 52 non-anticoagulated patients were generated on the Sysmex CS-2500 automated coagulation analyser using Dade Innovin and Dade Actin FS reagents for PT and APTT, respectively. Normal distribution of data was checked by constructing a QQ plot for each parameter.
**Results:** As the QQ plots supported normal distribution, hypothetical reference ranges (mean ± 2SD) were calculated for each quantitative parameter (with one exception). 12 parameters (PT:6, APTT:6) were generated for every sample tested, each representing a different aspect of the clot waveform; maximum velocity, time taken to reach maximum

velocity, maximum acceleration, time taken to reach maximum acceleration, maximum deceleration, and time taken to reach maximum deceleration. A range for the time taken for maximum acceleration of PT could not be calculated as all results were identical. Due to lack of time, the initial plan of comparing the non-anticoagulated CWA results to the results of patients undergoing warfarin therapy, could not be executed.
**Conclusion:** By using normal CWA results as a baseline, various conditions and anticoagulant usage may be detected at a higher sensitivity and detail than just clotting time.

## Monocyte distribution width: extension of sample age up to 7 hours

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**Objectives:** Monocyte distribution width was previously demonstrated to be stable for a period of 4 hours. The aim of this study was to evaluate whether monocyte distribution width remained stable in samples aged between 4 and 7 hours.
**Methods:** Rotorua Hospital Emergency Department and inpatient samples collected in ethylene diamine tetra acetic acid vacutainer tubes were selected between the hours of 4 am and 10 am. Monocyte distribution width was tested at 0, 4, 5, and 6 hours in 28 samples based on the collection time or received time. For 26 samples, monocyte distribution width was tested at 7 hours. Testing was performed on board the UniCel DxH 900 analyser (Beckman Coulter, Incorporated, Brea, California).
**Results:**
The average coefficient of variation at 7 hours was 5.54%. The average mean increased from 18.04 U at 0 hours post collection, to 19.31 U at 7 hours post collection. By separating the data into 3 groups; monocyte distribution width below 20 U, monocyte distribution width which rose above 20 U after the initial 0 hour test, and monocyte distribution greater than 20 U at 0 hours, it was identified that the two latter groups had a similar trend, while the former had a dissimilar trend between 4 and 7 hours. Monocyte distribution width did not decrease below 20 U in the group which had monocyte distribution width greater than 20 U at 0 hours.
**Conclusions:**
The results of this study found monocyte distribution width to be stable up to 7 hours, with the greatest amount of deterioration occurring during the first 4 hours. Further studies with larger sample sizes are required to confirm results.

## Comparison of Hemocue haemoglobin and HGB-O in XN-20 for lipaemic samples

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**Objectives:** Haemoglobin is one of the basic parameters tested in the haematology department. The method used in LabPLUS, called SLS-Hemoglobin, produces falsely high results for lipaemic samples. LabPLUS is using a new parameter HGB-O in XN-20 to substitute the haemoglobin value. Hemocue, a point -of-care analyser is also known to be unaffected by lipaemia. The main aim of this research was to evaluate the comparability between Hemocue haemoglobin and HGB-O.
**Methods:** Forty-five patient samples were picked for this research according to the lipaemic level, L value (L=50-149: 31, L=150-350: 10, L>350: 4). All the samples were tested by both XN-20 and Hemocue analyser. In XN-20, SLS-Hemoglobin and HGB-O were done. QCs for XN-20 were performed as routine and Hemocue analyser did its QC automatically.
**Results**: The paired t-test (mean difference 0.42 g/L; 95% confidence interval -0.87-1.71 g/L; p=0.51) and Bland-Altman plot (mean difference 0.42 g/L; Lower LOA -8.00; Upper LOA 8.85) indicated a statistically insignificant difference between the Hemocue haemoglobin and HGB-O results. Passing-Bablok fit for all samples (y = 1.0494x - 5.9965, R2 0.97) showed a positive bias (approximately 5%) for HGB-O compared to Hemocue haemoglobin, which is clinically insignificant according to RCPA Haematology Participant Handbook 2014. However, for samples with L values greater than 150, the Passing-Bablok fit (y = 1.1856x - 23.005, $R^2$ = 0.9737) showed proportional bias of 18.5%, which is not acceptable.
**Conclusion:** HGB-O and Hemocue haemoglobin both statistically and clinically agreed with each other according to the statistical tests for all 45 samples. However, Passing-Bablok fit showed they might not be comparable to each other in samples with greater L values. Further investigations with more highly lipaemic samples are necessary.

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