Otago BMLSc student research project abstracts Semester 1, 2022

Group B *Streptococcus* detection from recto-vaginal swabs of pregnant women: a comparison between Alethia loop-mediated isothermal amplification and culture-based methods

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Objectives: Group B *Streptococcus* has the ability to cause early onset disease in neonates which is the most common cause of severe neonatal infection within the first 24 hours of life in most developing countries. This is preventable if the colonised mother is identified and treated with intrapartum antibiotic prophylaxis during labour. My objective is to compare the current culture-based method for detection of group B *Streptococcus* with the Alethia benchtop loop-mediated isothermal amplification (LAMP) method.
Methods: Recto-vaginal swabs were taken from pregnant women, streaked on blood agar and incubated in Lim broth for 18-24 hours. One loop of Lim broth was tested using chromogenic group B Streptococcus agar and 50 µL of the same Lim broth was tested using the Alethia LAMP method.
Results: Recto-vaginal swabs were tested using both methods. Of the 66 swabs tested, there was a 79% agreement between methods. The total turnaround time for the Alethia was much shorter than the total turnaround time for the culture-based method, however the cost of the Alethia was 10 times greater.
Conclusion: The Alethia method was simple to perform, with a much shorter turnaround time than the current culture-based method but, due to outside factors, the sensitivity and specificity in this comparison was unsatisfactory. The outside factors have not been defined but may include technical error and lag time between subculturing the LIM broths and testing on the Alethia. Therefore, I would suggest that this method requires further investigation before it is implemented in the diagnostic laboratory.

Comparison of glycated haemoglobin with oral glucose tolerance tests for diagnosis and monitoring of type II diabetes mellitus

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Objectives: Glycated haemoglobin testing has emerged as the preferred test for diagnosing and monitoring patients with type II diabetes mellitus and was recommended as the assay of choice in 2011 by the New Zealand Society for Study of Diabetes. Previously, the oral glucose tolerance test was widely used, however this test is no longer favourable but still routinely used in diagnostic laboratories. The purpose of this study was to compare and validate the use of HbA1c assay over the oral glucose tolerance assay.
Methods: Glycated haemoglobin patient tests performed by staff at Pathlab Waikato were compared with oral glucose tolerance tests performed by staff at Pathlab Tauranga. An analysis of the two different test methods was completed, taking into account the clinical value of the different tests, test costs, patient suitability, biological variability and the ability to predict chronic complications.
Results: The analysis identified several advantages of the glycated haemoglobin test method compared with the oral glucose tolerance test method. This included lower cost of testing due to reduced staff time, less consumables and ability to share collection tubes between other tests. It also showed lower patient discomfort as it does not require fasting, more accurate determination of future diabetes related cardiovascular and microvascular pathologies, and the ability to also screen for other haemoglobinopathies (such as thalassaemia).
Conclusion: Glycated haemoglobin testing proved to be a more reliant testing method for the diagnosis and monitoring of type II diabetes mellitus. The use of oral glucose tolerance testing should not be completely disregarded as it still has some clinical value, particularly when diagnosing diabetes in patients with indeterminate glycated haemoglobin results.

Method comparison analysis: Abbott c series Gamma - Glutamyl Transferase (GGT) and Gamma - Glutamyl Transferase2 (GGT2)

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Objectives: Gamma-glutamyl transferase (GGT) is a peptidase enzyme that facilitates gamma-glutamyl group transfer to an acceptor. This enzyme is present in multiple organs. However, serum elevations can originate from the liver. Serum GGT measurements are relevant for conditions that mostly affect the hepatobiliary system such as alcoholism, hepatitis, and hepatobiliary obstruction. A method comparison analysis was carried out to compare the two Abbott Alinity c series assays, GGT and GGT2, for measuring serum gamma-glutamyl transferase enzyme. The aim of the analysis was to determine the assay performance of the new g-glutamyl transferase assay (GGT2) and if it is able to replace the original g-glutamyl transferase (GGT) assay without inducing a change in measurement of the enzyme concentration.
Methods: Thirty refrigerated serum samples with known previous GGT enzyme measurements were collated and tested using the reference assay (GGT) and the test assay (GGT2). The results were compared to determine if there was a change in enzyme measurement between the current assay and the new assay.
Results: The measured GGT enzyme concentration using the new gamma-glutamyl transferase assay (GGT2) showed a negative bias across the entire range of measurement. Intra-batch and inter-batch precision indicated high precision of the GGT2 assay as the notable variations were still within acceptable limits.
Conclusion: Despite the constant negative systematic bias, it is still acceptable to replace the reference assay with GGT2 as bias can be due to the assay not being used in the format recommended by the manufacturer. Upon careful consideration, Abbott Diagnostics have recommended that the correlation factor of the new assay should be adjusted to allow compensation for the negative bias.

Evaluation of Point-of-Care Testing for glucose and haemoglobin at Wairau Hospital

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Objectives: This study evaluated three point of care analysers (RAPIDPoint 500e, ABL90 Flex Plus, and Hemocue Glucose 201) for measuring glucose (mmol/L) and total haemoglobin (g/L). ABL90 Flex Plus used in one ward was compared to Hemocue Glucose 201 in another ward for glucose measurements, and also to ABL90 Flex Plus used in the laboratory. Total Haemoglobin (tHb) levels were analysed on both the laboratory XN1000 system and the ABL90 Flex Plus on a ward.
Methods: Ten blood gas samples were analysed on two ABL90 Flex Plus, the laboratory RAPIDPoint500e blood gas analyser and XN1000 haematology analyser. A further twenty blood gas samples were run on two HemoCue 201 Point-of-Care and the ABL90 Flex Plus. Statistical analysis established the R^2 value, linear intercept, and difference comparison. RCPA allowed limits of performance (ALP) were applied.

Results: Glucose: RAPIDPoint 500e vs ABL90 Flex Plus comparison yielded a coefficient of determination (R^2) value of 0.99. Comparison between the methods were within the quoted RCPA ALP ranges. ABL90 Flex Plus vs HemoCue comparison yielded an R^2 value of 0.99 in the paediatric ward and 0.98 in the maternity ward. Two samples in the maternity ward and four samples in the paediatric ward had results outside the ALP. Total haemoglobin: RAPIDPoint 500e vs ABL90 Flex Plus yielded with an R^2 value of 0.99, with one sample outside the ALP. The XN1000 vs ABL90 Flex Plus yielded an R^2 value of 0.99, again with one sample outside ALP.
Conclusion: Both of the ABL90 Flex Plus (ward and in the Laboratory) along with the RAPIDPoint 500e proved accurate for glucose and haemoglobin testing in Wairau hospital compared to the XN1000. Glucose level measured by HemoCue 201 showed some variations against ABL90 Flex Plus.

A managed variant list for Canterbury Health Laboratories' clinical exome sequencing project

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Objectives: Create a comprehensive catalogue, or Managed Variant List (MVL), of molecular variants previously identified by CHL Molecular Pathology, that could be used to assist variant curation in the future.
Methods: Variants previously identified by CHL's Customised Variant Testing (CVT) service were added into an MVL by the student, using Agilent Technologies' online software, "Alissa Interpret". These variants were curated using the ACGS 2020 and ACMG/AMP 2015 guidelines, which were also used to assist completion of the project. (1) The MVL created will be used in a pipeline/triage variant exclusion feature, designed in Alissa for the upcoming Clinical Exome Sequencing Project at CHL. Before inclusion in this pipeline, each variant added to the MVL by the student was approved by a Scientific Officer. Activity on Alissa associated with this project, including edits and updates to variants, was tracked using Alissa's inbuilt audit tracing technology. Patient details were omitted from MVL entries except for clinical features, and CHL accession number identification, in order to connect multiple variants identified in the same patient.
Results: Variants for which classification changed since inclusion in the MVL were updated accordingly. Software errors were corrected by email contact with representatives from Agilent. The pipeline was tested against validation cases for which the variants identified were known. This process showed successful functioning of the MVL as part of the pipeline. Issues that arose surrounding transcript differences were noted but not fully resolved.
Conclusion: By completion of this project, an MVL has been successfully set up for use in CHL's future Clinical Exome Project, and has been populated with variants that will help to reduce curation workload.

Immunohistochemical expression of proliferation markers in central and peripheral giant cell granulomas

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Objectives: Giant cell granulomas are lesions that are relatively common in the oral cavity with an aetiology that is uncertain. Central giant cell granuloma (CGCG) arises within the mandibular or maxillary bone while peripheral giant cell granuloma (PGCG) arises in the gingival soft tissue. Histologically they comprise active fibroblasts and multinucleated giant cells in a vascular fibrous stroma. Ki-67 and PCNA are proliferation markers with different temporal expressions within the cell cycle. The aim of this study was to determine the expression of Ki-67 and PCNA in CGCG and PGCG using anti-Ki67 and anti-PCNA.
Methods: Ten blocks of formalin fixed paraffin embedded tissue of confirmed CGCG and PGCG were selected for each group from the archives of the Oral Pathology Centre, University of Otago. Slides were produced and stained using the Leica Biosystems Bond-III autostainer. They were examined using light microscopy and the number of positive cells were determined in each group. T tests were used to determine the difference in antigen expression between the two groups.
Results: Scattered mesenchymal mononuclear cells stained positive within the stroma of both CGCG and PGCG. The multinucleated cell nuclei were negative for both Ki67 and PCNA while their cytoplasm was $Ki67^-$ and $PCNA^+$ in some cells. There was no statistically significant differences in the expression of Ki67 and PCNA between the two groups.
Conclusion: The lack of significant difference in proliferative activity of CGCG and PGCG suggests they are similar lesions but are just found in different areas.

Optimisation of the PRAME antibody on the Ventana Benchmark ULTRA

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Objectives: PRAME (Preferentially expressed Antigen in Melanoma) expression has been associated with increased metastatic risk of melanoma and numerous other nonmelanocytic malignant growth, therefore it is a useful prognostic biomarker and a target for development of therapeutic treatments. The aim of this study was to optimise the PRAME antibody to enable adoption of qualitative immunohistochemical identification of PRAME at Taranaki Pathology Services (TPS).
Methods: An antibody dilution of 1:100 was performed on a concentrate of PRAME [EPR20330] Rabbit Monoclonal Antibody (Biocare Medical). Known positive control tissue (testis) from both TPS and Wellington Southern Community Laboratories (WSCL) were run on the Ventana Benchmark ULTRA using the OptiView DAB IHC detection kit, and the stains were examined. Heat induced epitope retrieval- using ULTRA Cell Conditioning #1 (CC1), and was modified, while antibody incubation remained constant, to obtain the desired result.
Results: All test slides utilised a 32 min antibody incubation time, while HIER time was altered. In all instances positive staining was demonstrated. Initial testing of tissue provided by WSCL following the recommended protocol by the manufacturer: 64 min HIER time and 32 min antibody incubation, exhibited appropriate staining intensity. The stain was repeated on TPS control tissue and evaluated in agreement with the pathologist to produce a valid protocol. For comparison, a section was stained following parameters practiced at WSCL. However, the stain result was unfavourable.
Conclusion: After close assessment of the results, it was concluded that 64 min HIER time and 32 min antibody incubation time achieved optimally stained tissue sections, consistent with the manufacturer's recommendation. Findings from this study indicated the applicability of the PRAME antibody for in vitro diagnostic use at TPS.

DNA extraction of Gram-Positive Bacteria for MinION sequencing

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Objectives: To design, optimise, and test the efficacy of extraction methods for bacterial DNA, to provide a large yield of high-quality DNA for Oxford Nanopore MinION sequencing. This will contribute to development of a procedure for Multilocus Sequence Typing (MLST) of bacterial isolates in the Wellington region.
Methods: Various DNA extraction methods were tested using *Staphylococcus aureus* isolates to find the most optimal. Pre-extraction variables involved combinations of freezing suspended bacterial colonies, enzymatic digestion, and bead-beating. DNA extraction methods included MagNAPure 96 Automated extraction, and High Pure manual extraction. The concentration of DNA extracted was quantified using the Qubit Fluorometer. Each extract was sequenced on the MinION device, producing data on the mean length of DNA fragments (N50), overall quality, and total bases read.
Results: After testing combinations of the above variables, the most promising was to use bead-beating without any further extraction steps. The efficacy of this method was then tested using multiple samples from the following species: *Staphylococcus aureus, Klebsiella pneumoniae, Clostridium difficile,* and *Enterococcus faecium.* From the limited results, bead-beating showed no improvement in either average read length (N50) or total bases read over the existing method. Encouraging data was seen in a test on combining freezing and beat-beating isolates but this was not able to be tested further.
Conclusion: The bead-beating extraction method needs more testing across multiple sequencing runs before it can definitively be called to be better or worse compared to the control method for the purposes of MinION sequencing. However, the data collected during the initial stages of this experiment both ruled out and opened new avenues of investigation into the optimal extraction technique for MinION sequencing.

Influence of storage time on the stability of reticulocytes

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Objectives: Reticulocytes are immature red blood cells that can indicate the functionality of erythropoiesis. Testing of this parameter is often additionally requested after initial analysis, so determining its maximum storage time has many benefits. This study assessed the possibility of extending the acceptable specimen storage time for the analysis of reticulocytes to 72 hours from initial analysis, whilst ensuring reticulocyte stability.
Methods: 200 venous blood samples were collected into K2EDTA tubes and using the Sysmex XN analyzer, the initial reticulocyte count was determined. Samples were kept at room temperature for 8 hours and retested before being put in the refrigerator (2-8°C). Sample reticulocyte counts were then subsequently analysed at 24, 48 and 72 hours respectively. The significance of the difference in variation of reticulocyte counts at each time interval was determined using the Royal College of Pathologists Australasia's (RCPA) analytical performance specifications (APS). Microsoft Excel statistical software was used for Bland-Altman and other additional statistical analyses.
Results: The mean coefficient of variation (CV %) of reticulocyte counts from the initial analysis to 8, 24, 48 and 72 hours were calculated and compared to the RCPA APS. The mean CV% values compared to the initial reticulocyte at 8, 24, 48 and 72 hours were 3.17%, 3.57%, 3.75% and 3.11% respectively. These results were all within RCPA APS (+/- 10%) and therefore all passed.
Conclusion: Evidence supports the extension of the maximum acceptable age of EDTA venous blood specimens for the analysis of reticulocytes to 72 hours.

Optimisation of CD68 staining of the bone marrow: Comparing clones KP1 and PG-M1

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Objectives: In SCL's Dunedin Histology laboratory, the CD68 antibody clone KP1 is used in immunohistochemistry staining. An alternative antibody clone, PG-M1, may be more useful for bone marrow. The purpose of this research was to compare these antibody clones with the aim of optimising CD68 staining of the bone marrow.
Methods: Four retrospective bone marrow blocks requiring CD68 staining were extracted from the archives. Each slide that was cut contained a section of bone marrow, tonsil (control), and brain tissue (control). Roche's BenchMark ULTRA system was used to stain slides with the OptiView DAB IHC protocol. For each case, one slide was stained with the current protocol using KP1 (RTU), one slide with the PGM1 antibody clone (1:50 dilution), and then slides were stained with PG-M1 at increasing dilutions; 1:100, 1:200, and 1:500. Finally, the detection kits were changed from OptiView to UltraView to investigate any staining differences. One UltraView slide used the KP-1 antibody clone, the other PG-M1 (both at a dilution of 1:50). A pathologist and a haematologist compared these slides under light microscopes.
Results: Both observers decided that the OptiView PG-M1 1:200 slides showed optimal staining of CD68 in the bone marrow. There was less background noise, and more specific and distinct staining of the target macrophages and monocytes.
Conclusion: This study suggests that the CD68 antibody clone PG-M1 provided better diagnostic information than KP1 when used for bone marrow. This was due to the improved definition of cells and increased specificity of macrophage staining. The optimal dilution of PG-M1 was 1:200, producing a higher signal: noise ratio while maintaining distinct positive staining of macrophages in the bone marrow.

Immunoglobulin D quantitation: Method comparison between radial immunodiffusion and nephelometry

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Objectives: Currently at Canterbury Health Laboratories, immunoglobulin D (IgD) is quantitated using radial immunodiffusion (RID). Because RID plate production is discontinuing, an alternative IgD quantitation method is required. This method comparison study between RID and nephelometry aimed to determine the suitability of IgD quantitation on the Siemens BNäII nephelometer as a replacement method.
Methods: Serum samples (33) underwent IgD quantitation on the Siemens BNäII nephelometer. IgD concentrations were compared to the samples' previously determined RID IgD concentrations. The Royal College of Pathologists Australasia's Analytical Performance Specifications (RCPA APS) for IgD (±0.04g/L up to 0.2g/L, ±20% >0.2g/L) were used to determine significant differences between IgD measurements. Bland-Altman analyses and Passing-Bablok regression were completed using MedCalc statistical software.
Results: Nine samples showed significant IgD concentration variation (27%). The lower limit of agreement (LoA) in the Bland -Altman plot for IgD concentrations up to 0.2g/L (-0.01g/L [95% confidence interval (95%CI)=(-0.034,0.005g/L)]) was within RCPA APS, whereas the upper LoA (0.06g/L [95%CI= (0.044,0.083g/L)]) exceeded RCPA APS. The Bland-Altman

plot for IgD concentrations >0.2g/L showed that samples outside of RCPA APS had low IgD concentrations (between 0.617-1.21g/L). This was due to imprecision (%CV 18.8%) and a propensity for overcalculation among low concentrations in the RID method. Samples with IgD concentrations >1.21g/L were within RCPA APS. Passing-Bablok regression showed no proportional difference between methods. However, a significant systematic difference was detected (intercept: -0.025 [95%CI=(-0.040,-0.008)]; slope: 0.928 [95%CI=(0.856,1.001)]).
Conclusion: This study suggests that IgD quantitation using the Siemens BNäII nephelometer is comparable to RID, rendering it a suitable replacement method. The observed differences in measurements were not clinically significant.

Incorporation of COVID-19 variant testing within a community laboratory using the TaqMan SARS-CoV-2 Mutation Panel

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Objectives: Corona Virus Disease 2019 (COVID19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first identified in Wuhan, China in December 2019 and the first case was reported in New Zealand on 28th February 2020. The World Health Organisation declared the outbreak a pandemic on 11th March 2020. COVID-19 has the ability to overwhelm an already over-stretched, under-staffed, under-funded national health system, therefore the ability to determine the variant of a positive result as quickly as possible can determine the variants prevalent within the community. Currently, genome-wide sequencing is our only resource which is expensive and time consuming. A genotyping panel within the testing laboratory may better serve the community as part of an initial screen for positive COVID-19 samples. Saliva samples from known non-Omicron B1 COVID-19 individuals were selected to determine whether they were delta or B2 – the two known variants, alongside Omicron B1, within the community during January – May 2022.
Methods: COVID-19 positive saliva samples (400) were processed using the TaqMan SARS-CoV-2 Mutation Panel for the S.L425R.U_R mutation, the mutation that distinguishes Delta from B2, using the QuantStudio 7. Results were analysed on QuantStudio Design and Analysis v2.5.0.
Results: Five out of 400 samples were positive for S.L425R.U_R mutation.
Conclusion: The TaqMan SARS-CoV-2 Mutation Panel can easily be incorporated into the general laboratory workflow enabling the determination of COVID-19 variant, with appropriate primers, within 2 hours of positive identification.

Sysmex XN WDF and WPC channel Q-flags: tools for detecting abnormal or reactive lymphoid populations

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Objectives: Detecting aberrant lymphoid populations is one of the most vital roles of the diagnostic haematology laboratory. The aim of this study was to assess the value of the WDF and WPC channel Q-flags on the Sysmex XN-2000 analyser as tools to identify abnormal or reactive lymphoid populations.
Methods: Samples (221) were processed on the Sysmex XN-2000 analyser, in the WDF and WPC channels which utilise flow cytometry measurement principles. Samples with low white cell counts (<4.0 x10'9/L) were excluded. The lymphoid population of each sample was assessed microscopically. Counts from the WDF and WPC channel flagging systems were evaluated in conjunction with microscopic results, using XL-Stat analytical software to generate Receiver-Operator Characteristics (ROC) and associated analytical data.
Results: All except the WPC "blasts" Q-flag were found to have a significant relationship with abnormal or atypical lymphoid populations, as demonstrated by a ROC Area Under the Curve (AUC) of ≥ 0.5. AUC for the WDF "blasts/abnormal lymphocytes" Q-flag :- 0.828 (95% CI: 0.723-0.923). AUC for the WPC "atypical lymphocytes" Q-flag :- 0.572 (95% CI: 0.310-0.833). AUC for the WPC "abnormal lymphocytes" Q-flag :- 0.807 (95% CI: 0.632-0.983) AUC for WPC "blasts" Q-flag :- 0.348 (95% CI: 0.244-0.453).
Conclusion: The results of this study suggest that the WDF Q-flag "blasts/abnormal lymphocytes" and the WPC Q-flags "atypical lymphocytes" and "abnormal lymphocytes" are useful as tools to identify abnormal or reactive lymphoid populations. The WPC Q-flag "atypical lymphocytes" had a weak relationship with these populations. The WPC Q-flag "blasts" had no significant relationship to the presence of blasts in a sample – however this Q-flag may be useful in the further detection of abnormal lymphoid populations.

Mysterious metastases - an examination of anomalous metastatic bodies in mice injected with tumour cell lines

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Objectives: Current research at Otago University aims to examine the tumour suppressor protein p53 and its isoforms, specifically how the murine p53 delta-122 isoform changes the development of tumours vs the wild-type. During this research, mice were injected with tumour cell lines subcutaneously. Upon slide examination prior to the start of this project, 6 mice presented with metastatic bodies within the liver, and an increased quantity of eosinophils around these metastases. This project aimed to categorise the immune cells present within the metastatic regions.
Methods: Of 129 mice included in the initial study, 6 were selected for further examination. Five staining procedures were performed on tissue slides made from a variety of tissues from each mouse. Slides were stained with H&E, Giemsa, and immunohistochemistry for CD3, CD31, and PDGFR. Staining enabled the quantities of white blood cells within tissue to be determined alongside positive, negative, and variable results for immunohistochemistry.
Results: Slide examination of the 6 selected mice provided negative result for metastases in 3 of the mice, these slides were excluded. Of the remaining 3, examination reported increased eosinophils, CD3+ T cells, and CD31 negative metastatic tissue. A potential explanation for increased eosinophils is tumour expression of IL-5, an alternative would be the CD3+ T helper cells producing IL-5, upregulating the production of eosinophils which continue to produce IL-5 upon activation.
Conclusion: Multiple anomalous results were obtained through examination. More research into these results is required as to the mechanism of increased eosinophils and the prognostic implications. Future investigations should involve a larger sample size and other stains specific for white blood cells.

Method evaluation for the Roche Fructosamine assay

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Objectives: Fructosamine is often used as an indicator for glycated protein levels to monitor short term glycaemic control. Serum fructosamine levels can therefore be used in the diagnosis and management of diabetes mellitus. This report covers a method evaluation of the Roche Fructosamine assay on the Roche Cobas c501 analyser at Wellington Southern Community Laboratories (WSCL).

Methods: Method evaluation of the Fructosamine assay involved investigation into the precision, accuracy, and linearity of its implementation at WSCL. Normal and pathological levels of Roche quality control (QC) material were used to determine intra-precision and inter-precision. Accuracy was investigated through the correlation of external QC in addition to a comparison of Fructosamine results from patient samples (n=23) between Northland Pathology and WSCL. Linearity was evaluated using a two-fold serial dilution of the pathological QC material.
Results: For normal and pathological QC, the coefficients of variation for intra-precision were 2.07% and 2.64% and for inter -precision, 2.43% and 1.42%, respectively. The comparison of patient samples demonstrated a Pearson's correlation coefficient of 0.9515, suggesting excellent correlation between results produced from Northland Pathology and WSCL. The external QC results demonstrated satisfactory accuracy to the desired results with a correlation coefficient of 0.9978. The dilution of the high QC level showed strong linearity with a correlation coefficient of 0.9998.
Conclusion: The results of this method evaluation proves that the Roche Fructosamine assay provides consistent and reliable results. Hence, this assay can be confidently recommended to WSCL as a method of glycated protein detection in patient samples.

Validation of a direct immunofluorescence buffer system for staining of renal biopsies

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Objectives: Direct immunofluorescence (DIF) in the Waikato DHB Histology laboratory had been performed using a phosphate-buffered saline (PBS) wash with tris-buffered antibody diluent. The purpose of this study was to compare methods using a single buffering agent throughout the procedure to achieve DIF results that were bright, clean and reliable.
Methods: The buffer systems were: (i) PBS wash + tris-buffered diluent (Leica "Bond" antibody diluent), (ii) PBS wash + phosphate-buffered diluent (Cell Marque "Emerald"), (iii) TBS wash + tris-buffered diluent (Cell Marque "Diamond"), and (iv) TBS wash + tris-buffered diluent ("Bond"). Three renal biopsies representing different disease states were stained with FITC-labelled antibodies against human IgA, IgG and C3, using each buffer system. Fluorescence images were compared subjectively, and brightness of positively stained and background regions of selected images were measured using the Zeiss Zen Blue imaging application.
Results: Fluorescence results were similar with all four buffer systems; positive for some antibodies and negative for others, according to pathology of the case. However, in some cases, all-phosphate and all-tris buffered protocols gave slightly brighter images than the existing protocol and, in others, they were slightly less bright. These observations were confirmed by statistical analysis of quantitative measurements.
Conclusion: All methods produced results that were consistent with previous clinical diagnoses. Minor differences in brightness of fluorescent signal were associated with either the buffer system, antibody/antigen, or specimen. These factors were difficult to disentangle, but no staining protocol was clearly better than any other. Since acceptable results were achieved with the tris-buffered system with "Bond" diluent, this amended protocol has been adopted for ongoing DIF tests in our laboratory.

Validation of a commercial sodium bisulphite conversion kit method for Prader Willi syndrome and Angelman syndrome testing

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Objectives: The purpose of this study was to validate the Qiagen EpiTect sodium bisulphite kit for use at Canterbury Health Laboratories (CHL) for methylation studies. The kit was validated by performing Prader Willi and Angelman syndrome testing using high resolution melting analysis.
Methods: The bisulphite conversion was undertaken following the manufacturers recommended protocol. Three Prader Willi Syndrome (PWS) and three Angelman Syndrome (AS) DNA samples were converted alongside controls of different concentrations extracted by varying DNA extraction methodologies available at CHL. Amplification of the methylation specific PWS/AS locus was then undertaken using 2mL of converted DNA.
Results: All PWS samples produced a melting curve showing hypermethylation and all AS samples produced a melting curve showing hypomethylation. Three of the four control samples produced a melting curve showing the expected result, however, the control at 100ng total converted DNA did not amplify reproducibly. The DNA extraction method investigations showed that Kurabo and Qiagen extraction methods produce consistent results, whereas samples below 100ng total DNA, those extracted by the Biomek, did not amplify consistently.
Conclusion: The Qiagen EpiTect bisulphite conversion kit results in expected conversion patterns for Prader Willi and Angelman syndrome samples. Therefore, it is able to be used for diagnostic analysis of methylation. From the DNA extraction method investigations total converted DNA concentrations lower than 100ng do not consistently amplify when performing melting curve analysis without optimising the DNA input for PCR. This indicates that while DNA conversion is efficient for low concentrations, the PWS/AS melting curve analysis requires more input DNA.

Evaluation of Rheumatoid Factor assay on a Roche Cobas c503

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Objectives: Rheumatoid factor (RF) is an autoantibody used for the diagnosis of rheumatoid arthritis (RA). Taranaki Pathology Service (TAR) is planning to start testing RF instead of referring specimens to SCL Wellington (WEL). The purpose of this study was to evaluate the analytical performance of the RF assay of the chemistry analyser in TAR.
Methods: Two studies were done. One was a comparison study. Sixty patient samples that were tested for RF at WEL were tested in TAR using a Roche Cobas c503 analytical unit. The results in UI/mL from the two laboratories were compared using concordance correlation coefficient (r) and Bland-Altman analysis to assess the agreement between the two laboratories. Another study was a carryover study that assessed the sample-to-sample carryover effect of the Cobas c503 unit. Firstly, samples containing low RF levels were tested following the samples with high RF levels. Secondly, samples were tested in the opposite order to examine the potential contamination.
Results: Comparison study: r was 0.994 which indicated high concordance between the two laboratories. The Bland-Altman plot showed 3.33%, which is 2 out of 60 samples, were outside the limit of agreement. Carryover study: there was no evidence of carryover effect.
Conclusion: RF levels from TAR show excellent agreement with ones from WEL. However, there might be inconsistency in RF level around the diagnostic cut-off of 14IU/mL.

Evaluation of Diasorin Direct Renin assay on Liaison XL analyser

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Objectives: Renin is secreted in response to sodium depletion and decreased blood volume and pressure. Therefore, it has an important role in the homeostasis of water and electrolyte balance and in the regulation of arterial pressure. The renin-aldosterone ratio is a vital component in diagnosis of primary aldosteronism, among many other clinically significant disease processes. Renin has very short stability which has been noted to have poor reproducibility. The aim of this assay evaluation was to minimise pre-analytical variables of renin testing, and in light of previous findings, improve method agreeability between the two Diasorin Liaison analysers in Dunedin Southern Community Laboratories and Auckland's LabPlus
Methods: The Liaison direct renin assay uses chemiluminescence immunoassay technology for quantitative determination of renin in human ethylenediaminetetraacetate acid (EDTA) plasma. A small sample size of 20 EDTA patient samples were collected with a greater focus on pre-analytical errors. The 20 samples were run in parallel on both Auckland's LabPlus and Dunedin's SCL Diasorin Liaison analysers.
Results: Deming fit (y=38.34 + 0.2591x) Showed a significant negative bias, approximately 74%, for comparison of SCL Dunedin to LabPlus Auckland. The RIQAS acceptable limit of performance for this assay is 24.7%, making these assay results statistically significant. The Bland-Altman plot showed a statistically significant difference (mean difference -22.29 µIU/ mL; 95% LoA -242.3417 - 197.76µIU/ mL) between results obtained from LabPlus and SCL Dunedin.
Conclusion: The results obtained for renin from LabPlus, Auckland and SCL, Dunedin were statistically non-comparable. Pre-analytical variable were most likely responsible for the lack of agreement rather than the assay itself. Further evaluation of these variables is required before the assay can be implemented within SCL, Dunedin.

An evaluation of the Biorad ammonia quality control material

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Objectives: In patients with advanced liver disease, ammonia accumulates in systemic circulation because of hepatocyte dysfunction or portosystemic shunts. Hyperammonaemia is highly associated with hepatic encephalopathy. The ammonia quality control material currently performed is supplied by Roche Diagnostics and is run on the Roche c502 analyser. International Accreditation New Zealand recommends that laboratories should use third-party quality control preferably. In this project, we aim to evaluate the accuracy, stability and concentration we need for an ammonia quality control material supplied by BioRad and compare it with the Roche material.
Methods: A blank calibration with saline and a two-levels of Roche quality controls were run before the analysis of BioRad materials. Seven replicate measurements on both refrigerator and freezer BioRad materials were then run on a Cobas c502 analyser with the same procedure as for running patient samples. Results were plotted on the Levey-Jennings chart. The accuracy of BioRad quality control was verified by comparing it with target values. Stability was compared with Roche materials.
Results: The BioRad quality control results show good accuracy because the observed means were within the preassigned acceptable range. The results are precise during a period of six weeks according to the coefficient of variation (< 5%). The BioRad material is more stable than the Roche material.
Conclusion: The BioRad quality control material has high accuracy and is stable when kept in the freezer. All three levels of BioRad quality control are needed to cover the clinical diagnostic points and increase the probability to detect erroneous results. BioRad could be an alternative for Roche material.

Osteoclastic activity in central and peripheral giant cell granuloma of the oral cavity

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Objectives: Giant cell granulomas (GCGs) are lesions that are relatively common in the oral cavity. They may arise within mandibular or maxillary bone where they are known as central (CGCG) or in gingival soft tissue (peripheral [PGCG]). Histologically, they mostly comprise active fibroblasts and osteoclasts in a vascular stroma. Receptor Activator of nuclear factor kappa-B ligand (RANKL) and Osteoprotegerin (OPG) have been implicated in osteoclast development and progression of these lesions. The objective of the study was to assess and compare osteoclastic activity in CGCG and PGCG by using immunohistochemistry staining with anti-RANKL and anti-OPG antibodies.
Methods: Formalin fixed paraffin embedded (FFPE) blocks sent for diagnostic purposes to the Oral Pathology Centre which had informed consent from the patients and confirmed diagnoses of either CGCG and PGCG were used. 10 blocks were selected for each group. Two sections were cut from each block for immunostaining with anti-RANKL and anti-OPG antibodies, then placed onto charged adhesive slides and stained using an autostainer. The slides were examined microscopically to determine the expression of RANKL and OPG quantitatively and qualitatively. T test was used to determine whether there was a significant difference in osteoclastic activity between the two lesions.
Results: There was no statistical difference in anti-RANKL and anti-OPG expression between CGCG and PGCG.
Conclusion: Both types of lesions had the same osteoclastic activity demonstrated by RANKL and OPG staining, adding strength to the argument that PGCG is the soft tissue counterpart to CGCG.

A comparison between conventional and automated techniques for antibody panels

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Objectives: The aim of this study was to compare the advantages, disadvantages, and the results of the conventional manual technique with the automated technique performed by the Grifols Erytra Eflexis analyser to determine the effectiveness of both techniques for antibody panel interpretations and to validate the use of the automated technique for routine use.
Methods: Patients (sample size = 11, 100% female) from Palmerston North Hospital were subjected to testing by the conventional manually performed indirect antiglobulin technique in Grifols DG Gel cards and the automated indirect antiglobulin technique in Grifols DG Gel cards on the Grifols Eflexis analyser. This study took place over the course of two months from the 28th of March 2022 to the 17th of May 2022.
Results: Overall, patients were shown to have the same antibody panel interpretation results in both the manual and automated technique. In some patients, there were minor differences in the reaction grades (0 – 4; 0 = negative, 1 - 4 = positive) given to a positive result and some discrepancies in very weak positive results (w+ or 0.5) which were not detected by the automated technique. Some antibodies were not able to

be identified solely using the conventional or automated technique and were determined using other methods as per the appropriate standard operating procedures.
Conclusion: The automated technique is highly consistent with the conventional manual technique and is valid for use in routine testing. The exception to this may be patients who present with weak reacting antibodies which may not be detected using automated techniques. Further studies using a larger sample size are needed in order to further validate this study.

The validation and optimisation of H3.3 G34W antibody for giant cell tumour of the bone diagnosis

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Objectives: Giant cell tumour of the bone is a neoplasm that is typically found in the epiphysis and metaphysis of long bones in the mature skeleton. The condition can be difficult to diagnose as it may mimic other benign giant cell-rich lesions of the bone. However, H3.3 G34W antibody can be utilised with immunohistochemical staining to positively identify giant cell tumours of the bone. H3.3 G34W is a substitution mutation specifically found in mononuclear neoplastic cells in giant cell tumours of the bone. Validating and optimising an antibody specific for this condition will provide pathologists with a reliable adjunct diagnostic tool.
Methods: H3.3 G34W antibody was validated and optimised for use on the Leica BOND-III, to be run using the BOND polymer refine detection kit. Using a positive control, tests were carried out to determine the optimal primary antibody dilution, as well as the preferred antigen retrieval solution and incubation time required. A negative control test using tissue from colon, appendix, skin, and tonsil was performed to evaluate any non-specific staining.
Results: Optimal dilution of the antibody was found to be 1:100, as it produced strong, nuclear staining in the mononuclear neoplastic cells. Sections incubated for 30 minutes at 100°C in citrate pH 6 buffer were preferred as they produced less background staining than those incubated in ethylenediaminetetraacetic acid (EDTA) pH 9 buffer. No staining was observed in the negative control, proving the antibody to be specific.
Conclusion: The optimisation process determined a protocol for H3.3 G34W antibody that gives specific and reproducible results. Validation means the antibody is tested on a range of cases with known positive/negative results to see if the stain is reproducible.

Use of reticulocyte haemoglobin content in identifying iron deficiency in anaemic patients with concurrent acute or chronic inflammation.

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Objectives: Serum ferritin is the most sensitive iron deficiency indicator currently routinely used. This acute-phase protein is increased during inflammation making it an unreliable parameter. This study was to confirm if the recommended <29pg mean reticulocyte haemoglobin content measurement is an appropriate cut-off value for use in identifying iron deficiency in patients with iron deficiency anaemia, a raised CRP or inflammatory condition, and ferritin levels between 15-150 µg/L.
Methods: Retrospective patient data included mean reticulocyte haemoglobin content, ferritin, and CRP or evidence of concurrent inflammatory disease collected from 2020-2022. There were 118 patients in total: 38 non-anaemic controls, 40 iron deficiency anaemia patients, and 40 iron deficiency and raised CRP or inflammatory condition patients. A haemoglobin value of <115 g/L for women, and <130 g/L for men was taken to indicate anaemia.
Results: Ret-He values were significantly lower in both the iron deficiency anaemia and acute or chronic inflammation and iron deficiency group compared to the non-anaemic control group (iron deficiency anaemia 15.62pg (+/- 2.82), acute or chronic inflammation and iron deficiency 20.42pg (+/-4.11), control 33.51pg (+/- 1.69)). A Ret-He cut-off value of <29pg was shown to have a sensitivity of 97.5% for identifying iron deficiency in acute or chronic inflammation and iron deficiency patients. Specificity could not be determined.
Conclusion: Following the guidelines in the British Journal of Haematology (2021), using a cut-off value of reticulocyte haemoglobin content <29pg has good sensitivity in identifying iron deficiency in patients whose ferritin levels have been confounded due to the presence of inflammation. Further work will be needed to assess the specificity of this cut-off value for iron depletion.

Optimisation of the Sysmex XN-2000 atypical lymphocyte Q flag criteria

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Objectives: Abnormal flags generated by automated analysers which prompt manual blood film review have a factory set threshold which must be optimised to better suit individual laboratory needs. The objective of this study was to optimise the atypical lymphocyte Q flag threshold in the Sysmex XN-2000 haematology analyser, to reduce film rates and improve laboratory work flow efficiency.
Methods: Data was collected from 120 routine blood samples flagged with the suspect IP message for atypical lymphocytes. Manual white blood cell differential counts of 200 cells were performed by an experienced haematology scientist and a second examiner. The optimal threshold was determined by analysis of positive predictive values, efficiency, sensitivity, specificity, and the distance to corner of a receiver operating characteristic curve, calculated at each Q flag threshold. The optimised threshold was applied retrospectively to film review data for calculation of a revised film review rate.
Results: Application of the optimal threshold improved the positive predictive value of the atypical lymphocyte flag from 17% at the factory default threshold, to 92% at the optimal threshold of 130. Efficiency also increased from 55% to 70%, with the flagging threshold at 130 showing the smallest distance to corner at 0.4245. The retrospectively calculated film review rate showed an approximate reduction of 2%.
Conclusion: Through a comprehensive review of statistical measures which evaluate analyser performance, the optimal threshold was determined to be 130. The optimised Q flag threshold resulted in a reduction in film rates, improving laboratory workflow by reducing the number of films reviewed unnecessarily.

Significance of the Sysmex XN-20 left shift flag

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Objectives: The Sysmex XN-20 full blood count analyser provides an accurate, precise and rapid white blood cell differential with a flow cytometry-based method. This new generation analyser is also equipped with a set of 'Q flag' algorithms to provide screened detection of likely pathological samples that would benefit from morphological examination. The objective of this study was to determine whether left shift flagged samples show signs of pathology that needed blood film examination.

Methods: This was a retrospective sample study of 31 patient samples flagged for left shift and no other white cell flags by the XN-20 analyser. Each sample had a full blood count and microscopic examination accompanied by a 200 cell manual differential to determine if any clinically important pathology was present. Results from the XN-20 analyser were also used for comparison.
Results: Microscopic examination of the 31 samples showed a wide range of reactive features including toxic granulation in 29 samples and the presence of Dohle bodies in 9. Dysplastic neutrophils were also noted in 2 samples. The differential revealed a variable level of band neutrophils ranging from 0.5-20% of cells counted. None of the samples showed unusual presence of non-band immature granulocytes during examination or automated counting.
Conclusion: The presence of reactive changes in 30 of the 31 samples shows strong evidence that the XN-20 left shift flag was indicating pathology in the samples it triggers on. Toxic granulation, Dohle bodies, >10% band neutrophils and dysplasia may be indicators of infection and sepsis. Blood film examination of samples flagged for left shift must remain protocol if these significant indicators of pathology are to be detected in the laboratory.

Utility of Plasma Separation Cards for the quantification of Hepatitis C viral load against PPT tubes

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Objectives: Hepatitis C infection causes inflammation of the liver and is a major health issue worldwide. For patients with poor access to health services, Plasma Separation Cards (PSCs) offer an alternative that is less expensive, easy to transport, store and require less blood volume than plasma preparation tubes (PPT). The efficacy, stability, and limit of detection (LOD) of PSCs were tested with comparison to traditional venipuncture for patients where collection factors may be difficult.
Methods: Six Hepatitis C positive patient EDTA samples were spotted onto PSCs in various volumes (50uL, 100uL and 140uL) and extracted to determine the minimum volume to quantify viral load against 400uL PPT plasma when run on Roche Cobas® 4800. A serial dilution of two patient samples made in HCV negative EDTA blood was used to determine LoD for each volume. Extraction of PSCs was done at varying time points (24 hours, 72 hours, 1 week and 20 days) to determine stability.
Results: The $\log_{10}$ values of all three volumes showed good concordance with 400uL PPT results. 100uL and 140uL volumes best demonstrated Hepatitis C detection to a dilution of 10e3 and 10e4 respectively. PSCs appeared to be stable at various time periods prior to plasma extraction, with little difference in $\log_{10}$ values seen between different dilutions and volume.
Conclusion: PSCs showed great efficacy and stability over a long period of time. PSCs require less blood volume, appear to be stable long term, have easy storage and transportation, proving a good alternative to PPT tubes for Hepatitis C detection in circumstances where venipuncture is difficult.

Evaluation of a BIO-FLASH chemiluminescent analyser for the detection of anti -DFS70 antibodies

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Objectives: The BIO-FLASH chemiluminescent analyser at Dunedin Southern Community Laboratories (SCL) was evaluated for the detection of anti-DFS70 antibodies. The aim of this study was to determine whether anti-DFS70 antibodies can be assayed in-house instead of sending samples away for testing.
Methods: The experiment was conducted by sampling 35 patients' sera that were tested by the same instrument at Wellington SCL. There were 12 negative, and 23 positive samples all of which presented dense fine speckled patterns in the immunofluorescent assay. An interlaboratory comparison determined the suitability of the BIO-FLASH for the anti-DFS70 assay.
Results: The qualitative results of 97% of samples (34/35) were consistent. In positive cases, the mean value between the two laboratories did not significantly differ. The positive results were, on average, 16.7 units lower than those detected by the BIO-FLASH analyser in Wellington. In negative cases, all results were in agreement. Some possibilities that cause low antibody concentration: (1) the aging of samples, and (2) repeated freeze-thaw cycles. It poses an issue with sera having weak positivity because they can become negative. Thus, it is suggested to duplicate the anti-DFS70 assay in patients who have results close to the cut-off value. A new BIO-FLASH is being set up in the Dunedin laboratory and will be further evaluated before the assay is officially performed.
Conclusion: Although the DFS70 antibody concentration appeared to be low when samples were assayed in Dunedin, the qualitative results were in agreement. The consistency of qualitative interpretation (positive and negative) is the most important aspect of this study. It indicates that the anti-DFS70 assay could be brought in-house and analysed by the BIO-FLASH analyser at the Dunedin laboratory.

Comparing a 30 and 60 minute incubation time for the Indirect Antiglobulin Test

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Objectives: The current NZBS protocol for the indirect antiglobulin test (IAT) advises a 45-60 minute incubation to detect IgG antibodies. This study aimed to investigate whether or not a reduced incubation time to 30 minutes has a significant effect on the reactivity strength of an IAT test. This test utilises anti-human globulin (AHG) reagent and the manufacturer (Immulab) for the reagent used during this study suggests a 30 minute incubation in their protocol for an IAT test.
Methods: Quality control cells and patient samples with known antibodies were tested. Antibody screening was carried out at both a 30 minute and a 60 minute incubation in a tube IAT to compare reactivity strengths.
Results: Out of the 6 samples tested, 3 samples produced identical reaction strengths at both 30 and 60 minute incubations. Two samples showed an increased reaction strength at 60 minutes compared to 30 minutes. The final sample demonstrated a reaction at 60 minutes which was not present after a 30 minute incubation.
Conclusion: A 30 minute incubation was able to detect most of the antibodies tested but the results were suggestive that a 60 minute incubation supports better reactivity strength and this is critical to the sensitivity of the test as some antibodies will require the full incubation time to be detected.

Verifying a thrombin clotting time reference interval for the West Coast District Health Board laboratory

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Objectives: Previously, the only coagulation tests run in the Greymouth laboratory have been prothrombin time, activated partial thromboplastin time, and fibrinogen. With the upgrade of the coagulation analyser from the Stago Satellite to the Stago Compact Max, it was decided to introduce thrombin clotting time as part of the coagulation screen. This research examines the process undertaken to verify a thrombin clotting time reference interval for this laboratory.

Methods: Selected samples were aliquoted, frozen, and sent in two batches to Greymouth by Canterbury Health Laboratories in Christchurch. The samples were then thawed and run on the Stago Compact Max. The initial samples were compared with fresh Christchurch samples, while the subsequent group compared frozen aliquots which were thawed and run concurrently in both locations. For each sample, the difference and mean between the two obtained values were calculated and overall trends plotted on a scatterplot and Bland-Altman plot.

Results: Of the fifty-nine samples sent over, fifty-five were used in the final analysis. Overall, the results showed a good correlation between the two coagulation analysers, with an R2 value of 0.9565. This correlation improved when comparing two frozen aliquots as opposed to a fresh Christchurch sample against a frozen Greymouth aliquot (R2 values of 0.9965 and 0.9398 respectively). Normal results were comparable, while the Greymouth analyser tended to have a greater prolongation of abnormal values.

Conclusion: There was enough similarity between the Christchurch and Greymouth coagulation analysers to verify their reference range. However, the results highlighted the importance of removing the fresh versus frozen variable when comparing the performance between two analysers.

Acknowledgement: The author thanks staff at the West Coast District Health Board Laboratory, Greymouth for supervision and assistance with this project.

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