Otago BMLSc 4th Year Student Research Project Abstracts Semester 1, 2021

Comparison of histological staining techniques for copper

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Objectives: The aim of this study was to trial two stains previously unused by Canterbury Health Laboratories Histology Lab (CHL) for copper. An underperforming copper stain was suspended from use at CHL since 2018, due to inconsistent and unreliable results. A standardised and safe stain for copper with reproducible results is required.
Methods: Formalin-fixed, paraffin wax embedded (FFPE) sample tissue, donated by LabPLUS Auckland, was cut using a rotary microtome onto white adhesive slides. Tissue was obtained from an anonymous patient positive for Wilson's disease. Stains trialled included: the original suspended dimethylaminobenzylidene rhodanine stain (modified Lindquist's rhodanine technique, CHL 1990); Victoria Blue technique (Newcomer 2021); and the Abcam Copper Stain Kit (rhodanine technique, Abcam, 2018). Validation of the successful method was carried out using results provided by LabPLUS for 4 positive cases and 4 negative cases of copper accumulation.
Results: The Abcam Kit method showed successful staining that was safe, standardised, reproducible, and validated using patient results supplied by LabPLUS Auckland. Victoria Blue was unsuccessful, only showing diffuse nonspecific staining and introducing more health and safety risks, so was rejected for use at CHL.
Conclusion: This study identified the Abcam Kit as a successful stain to be used by CHL for the investigation of copper accumulation in routine tissue specimens. This allows the diagnosis of patients with Wilson's disease and other hepatic abnormalities and eliminates the need to send specimens to LabPLUS Auckland for investigation of copper accumulation

Evaluation of fresh walnut versus commercial walnut allergen for skin prick testing

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Objectives: Skin prick testing is a test used to aid in the diagnosis of IgE-mediated allergy. We compared the results (wheal and flare) obtained for skin prick testing using crushed fresh walnut as the allergen versus commercial walnut allergen.
Methods: Fresh walnut and commercial walnut allergen skin prick testing were performed on 20 patients with suspected cases of walnut allergy. Patients with dermatographism, or were unable to stop antihistamine medication, were excluded from the test. The wheal and flare were measured (in mm) 15 minutes after skin prick testing were performed.
Results: The fresh walnut had a higher maximum wheal (14mm) than commercial walnut allergen (7mm). The fresh walnut also had a higher mean wheal compared to commercial walnut allergen (7mm and 1.5mm respectively). The correlation coefficient of fresh walnut against commercial walnut allergen was determined to be 0.487, while the coefficient of determination is 0.237. Bland-Altman analysis revealed that there was a bias of +5.5mm, with a standard deviation of 4.03mm, when comparing fresh walnut to commercial walnut allergen. The fresh walnut gave 16 positive results and 4 negative results, while commercial walnut allergen gave 9 positive results and 11 negative results.
Conclusion: The fresh walnut and commercial walnut showed poor correlation in results. Determination of sensitivity and specificity, negative predictive value and positive predictive value may guide the selection of one allergen over the other.

Influence of storage time on stability of routine coagulation parameters (INR, APTT, and fibrinogen) at room temperature

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Objectives: Extending the maximum acceptable specimen age for testing/retesting some routine coagulation parameters has many benefits (e.g., reagent evaluation, fibrinogen add-on for DIC, or addressing sample delay during snow in Otago/Southland). This study assessed such a possibility, where stability of INR, APTT, and fibrinogen results were examined in relation to storage time.
Methods: From each participating individual (50 total), 4 citrate tubes were collected. A baseline tube was centrifuged and tested for INR, APTT, and fibrinogen at time of arrival. The other three tubes were kept as whole blood. After 24h, 48h, and 72h from time of collection, 1 tube was taken for centrifuging and tested for INR, APTT, and fibrinogen. In addition, centrifuged tubes were retested for INR and fibrinogen after 24h, 48h, and 72h (centrifuged fibrinogen after 96h) from time of collection. All specimens were kept at room temperature.
Results: The mean within-individual biological variations of INR, APTT, and fibrinogen at after 24h, 48h, and 72h (centrifuged fibrinogen after 96h) were calculated and compared with specific allowable limits of performance (ALP). For both centrifuged and uncentrifuged specimens, INR variations up to 72h all passed RCPA ALP (±0.3); fibrinogen variations up to 72h (centrifuged fibrinogen up to 96h) all passed EFLM ALP (±10.7%). APTT had a clear increasing trend, and all of its variations failed the EFLM ALP (±2.7%).
Conclusion: Evidence supports the extension of maximum acceptable age of uncentrifuged specimens for INR and fibrinogen tests to 72h. Changing the maximum allowable age of APTT is not recommended. For centrifuged specimens, the acceptable age for INR can be extended to 72h and for fibrinogen, to 96h.

Distinguishing between foreign pigmented lesions of the oral cavity using histological methods

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Objectives: Foreign pigmented lesions are common in the Oral Pathology Centre, often appearing similar. This study aimed to determine a histological staining profile that allows for easier discrimination between the foreign pigmented lesions; Amalgam Tattoo and Melanotic Macule.
Methods: Formalin-fixed, paraffin-embedded wax blocks were collected from patients who were biopsied at the University of Otago Dental School and were diagnosed with an amalgam tattoo or melanotic macule. Ten slides per diagnosis were produced from the biopsy wax blocks. These were stained using Perl's Prussian Blue reaction for ferric iron and five slides per diagnosis were stained using Masson's Fontana method for melanin. Select specimens from each group were stained using automated immunohistochemistry for S-100 and Vimentin. One specimen from each category was taken for scanning electron microscopy investigation. Retrospective data were gathered from the final histopathology reports of all samples to compare the haematoxylin and eosin morphologic characteristics.
Results: The Masson's Fontana stain showed increased positivity in melanotic macules. The Perl's Prussian Blue only stained positively on two samples; therefore, it was shown to have little diagnostic value. Immunohistochemistry for S-100 demonstrated increased positivity in melanotic macules. Vimentin is a marker for melanocytes and mesenchymal cells which both reside in the basal layer, making this difficult to

interpret. The Scanning Electron Microscope made visualizing the amalgam tattoo obvious as it appeared much brighter and more defined. The retrospective data analyzed showed each diagnosis had its own characteristic profile on haematoxylin and eosin staining.
Conclusion: Routine haematoxylin and eosin staining of tissues remains the preferred, most cost-effective method to discriminate between these lesions. However, a pathologist is needed to ensure results are accurate.

The optimisation and validation of NKX3.1 prostate antibody using immunohistochemical techniques

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Objectives: NKX3.1 is a prostate specific androgen-regulated homeobox gene located on chromosome 8p. The loss of function results in the initiating event of prostate carcinogenesis. NKX3.1 is a differentiating antibody used to aid and confirm the diagnosis of prostatic origin in primary and metastatic carcinomas of unknown origin by employing immunohistochemical techniques. The optimisation and validation procedures establish the clinical validity of the antibody as an adjunct tool, ensuring the antibody's performance and characteristics meet acceptable quality limits and standards.
Methods: The NKX3.1 antibody was optimised and validated on the Leica Bond III automated immunohistochemical system facilitating the Bond polymer refine detection kit. The variables of: the antibody dilution; antigen retrieval solution; and the incubation period of the primary antibody determined the staining result. The optimisation protocol was run on a positive control, a negative control, and a patient prostate chip biopsy.
Results: Of the 10 protocols tested, the 1:100 dilution factor in combination with ethylenediamine tetraacetic acid (EDTA), pH 9 based antigen retrieval solution and an incubation period of 30 minutes at 100°C yielded the best results. This combination produced a strong, nuclear staining intensity in cells expressing the antigen of interest. In addition, the positive and negative controls proved the protocol to be valid.
Conclusion: The validation of the performance parameters proved that the optimisation of the NKX3.1 antibody produced a precise, accurate, specific, and reproducible result. However, further testing of the sensitivity of the antibody is required to develop a protocol that is optimal and validated, providing scientists and pathologists confidence within the testing procedures and the results obtained .

Evaluation of potential carry over risk in the misdiagnosis of tumour markers, Ca 19-9 and hCG on the Abbott Alinity and COBAS-8000 analysers

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Objectives: In the diagnostic laboratory, sequential analysis of specimens occurs routinely. There is potential for contamination by previous specimens when analysing in sequence, particularly if the same sample probe is used. Diagnostic analysers use specific mechanisms to reduce carryover risk, including instrument flags and specific probe washes to limit carryover altering results. Tumour markers such as Ca 19-9 and hCG are more prone to carryover risk, and therefore clinical errors and misdiagnosis, due to the large concentration range and low clinical detection levels. The efficacy of probe wash stations is currently unknown in the Dunedin laboratory and has not been well characterised in the literature. This study aimed to report the effectiveness of probe wash stations on the Abbott Alinity and Roche COBAS-8000 analysers, using previously characterised tumour markers Ca 19-9 and hCG.
Methods: Clinically positive Ca 19-9 and hCG patient samples were run on specified analysers followed by negative patient samples for the tumour marker of interest. A deliberate contamination step was performed, and sequential testing was rerun, with initial and final values noted.
Results: The results indicated minimal carry over. Any change to results did not alter the overall diagnosis. hCG yielded clinically insignificant results. The P-value for hCG was undetermined as the difference between results was zero. The P-value for Ca 19-9 (0.0007^{1}) indicated statistically significant findings that are not clinically significant when compared with the RCPA Analytical Performance Specifications.
Conclusion: This study revealed the probe wash mechanisms on the Abbott Alinity and Roche COBAS-8000 analysers were sufficient in preventing contamination and therefore adequate for producing reliable clinical results.

Evaluation of need for sample preincubation in rheumatoid factor analysis

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Objectives: At Canterbury Health Laboratories, samples are currently preincubated at 37°C before rheumatoid factor (RhF) analysis. This is a historical step thought to prevent potential false rheumatoid factor concentration results caused by precipitation of cold-reactive rheumatoid factors, found in conditions such as mixed cryoglobulinaemia, due to lower sample temperature. This study was performed to determine the necessity of sample preincubation in RhF analysis.
Methods: Serum samples (116), with known detectable RhF, had RhF concentrations measured using endpoint nephelometry with a Siemens BNII Nephelometer and Siemens N Latex RF Kit. Samples were first analysed after thawing at 4° C, then reanalysed after incubation at 37°C for an hour. The significance of the difference between RhF results was determined using the Royal College of Pathologists Australasia's (RCPA) analytical performance specifications (±12 difference for less than 60IU/mL, ±20.0% for 60IU/mL or more). MedCalc statistical software was used for Passing-Bablok regression and Bland-Altman statistical analysis.
Results: Only two patient samples (1.72%) showed significant measured RhF concentration variation between methods. These patients both had Sjögren's syndrome antibodies. Bland-Altman plot limits of agreement (LoA) and 95% confidence intervals (CI) were within RCPA specifications. LoA for samples measuring less than 60 IU/mL: -9.77 IU/mL (CI: -11.90 to -7.64 IU/mL) and 5.58 IU/mL (CI: 3.45 to 7.71 IU/mL). LoA for samples measuring 60 IU/mL or more: -13.48% (CI: -15.69 to -11.27%) and 8.49% (CI: 6.28 to 10.71%). Passing-Bablok showed no significant systematic or proportional difference between methods (intercept: -0.7419 (CI: -2.0 to 0.1684); slope: 0.9901 (CI: 0.9717 to 1.0)).
Conclusion: This study suggests that sample preincubation for RhF nephelometric analysis may be discontinued to reduce RhF testing time. Further investigation into RhF analysis for patients with Sjögren's syndrome is recommended.

Verifying a Radiometer® Hemocue WBC Diff device

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Objectives: The Hemocue WBC Diff is a point of care device (POC) that provides a total leukocyte count (WBC) and a 5-point differential count of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. This verification study was performed to verify a Hemocue WBC Diff's fitness for purpose within the scope of its intended use, as required by the SCL Quality Assurance program. The reference method for this study was the Sysmex XN 2000 automated haematological analyser.

Methods: To verify this device 50 venous samples were selected based on the following criteria: (1) white cell total count, (2) lack of abnormal cell flags, and (3) time elapsed since sample collection. WBC count values were between 1-30x10^9/L. Samples were measured by the Hemocue within 4 hours of sample collection. Blood films of the samples were examined to identify possible morphological interferences.
Results: Bias for WBC, neutrophils, and lymphocyte counts were -0.610x10^9/L, -0.576x10^9/L, and 0.162x10^9/L. These results were statistically significant; p-values were ≤0.05.
Precision values for low and high precision check samples were within the Royal College of Pathologists Australasia (RCPA) quality assurance programme (QAP) analytical performance specifications (APS) for Hemocue POC devices.
WBC, neutrophil, and lymphocyte counts showed strong correlation with the reference method. Adjusted R^2 values of 0.995, 0.992, and 0.903 for WBC, neutrophil, and lymphocyte counts were obtained.
Monocytes, eosinophils, and basophil counts had CV values exceeding 20%, indicating poor precision.
Conclusion: This Hemocue WBC Diff POC device provides clinically relevant and accurate total WBC, neutrophil and lymphocyte counts in the context of acute medical care. In the absence of a differential count being generated accurate WBC counts can still be obtained. Confirmation with automated haematological analysis is recommended.

Evaluation of the effect of high-speed centrifugation on haemostasis specimens using the Heraeus Pico 17 centrifuge and the Stago STart Max analyser

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Objectives: Increased sample turbidity interferes with optical clot detection on the Sysmex CS-5100 (Siemens) automated analyser. One method to remove turbidity is high-speed centrifugation of the plasma at 17,000g for five minutes and re-analysis of the infranatant. If there is interference with the optical method after high-speed centrifugation, the STart Max (Stago) provides an alternative mechanical clot detection method. Previous in-house evaluation of high-speed centrifugation found the Activated Partial Thromboplastin Time (APTT) was shorted by up to 2.4s (mean value 1.0s) with re-analysis on the CS-5100 (Siemens). This study aimed to evaluate the effect of high-speed centrifugation on Prothrombin Time (PT), International Normalised Ratio (INR), APTT and fibrinogen results when re-analysed on the STart Max (Stago).
Methods: Sodium citrate samples were initially centrifuged at 3195g for five minutes in the Thermo Scientific Megafuge 16 (Thermo Fisher). An aliquot of each sample was then centrifuged at 17,000g for five minutes in the Heraeus Pico 17 high-speed centrifuge (Thermo Fisher). Plasma centrifuged at each speed was then tested for PT/INR (*n=40*) for APTT (*n=40*) and fibrinogen (*n=20*) on the STart Max (Stago) analyser and results were compared. The laboratory Measurement of Uncertainty (MU) was used in assessing the results, as well as statistical analysis performed using Bland-Altman plot, Passing-Bablok regression.
Results: In comparison of results from 3195g and 17,000g centrifuged plasmas, PT results showed a mean bias of -0.7s, INR a mean bias of -0.05 and fibrinogen a mean bias of +0.13g/L, which are not statistically significant differences. However, APTT results showed a statistically significant mean bias of -3.0s, with differences in results up to 8.7s. 50% were outside the APTT MU (8.7%).
Conclusion: This study found the effect of high-speed centrifugation on STart Max (Stago) APTT results to be significant, and is greater than the previous CS-5100 (Siemens) study. There was no significant difference in PT, INR and

The future of Rubella IgM testing at Canterbury Health Laboratories

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Objectives: This report aimed to identify whether the Euroimmun Anti-Rubella Virus Glycoprotein ELISA (IgM) kit is an acceptable replacement for the Siemens Anti-Rubella-Virus/IgM kit (World Health Organisation recommended) which stopped production at the end of 2020. The World Health Organisation is yet to recommend a new kit for Rubella IgM testing. Rubella is a viral infection spread through respiratory droplets, producing a speckled rash on patients. It has an incubation period of two to three weeks prior to symptoms (rash, fever, headache, enlarged lymph nodes, etc.) after which anti-rubella IgM is detectable.
Methods: Stored samples previously tested at Canterbury Health Laboratories using the Siemens Anti-Rubella-Virus/IgM kit were retested using the new Euroimmun kit. Other samples from The Royal College of Pathologists of Australasia were also tested on the Euroimmun kit and compared to results from numerous laboratories involved (using different methodologies) throughout Australasia as a form of external kit validation. Cross-reactive agents specified on the Euroimmun kit insert were also tested to identify any kit limitations.
Results: The results from this research project demonstrated that the Euroimmun kit sufficiently reports results comparable to the World Health Organisation recommended Siemens kit (and other kits used across Australasia) while suspected cross-reactive agents do not appear to interfere with results. Besides reporting, the efficiency, comprehensibility and accessibility of the Euroimmun kit make it a step up from the Siemens kit rather than just a substitute.
Conclusion: It can be concluded the Euroimmun Anti-Rubella Virus Glycoprotein ELISA (IgM) kit is an acceptable replacement for the Siemens Anti-Rubella-Virus/IgM kit, with greater sensitivity and specificity essential for diagnostic use.

Expanding the range of tests for the Stago STart Max coagulation analyser and standard operating procedure production

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Objectives: The Wairau hospital laboratory has a Stago STart Max point of care coagulation device. The STart Max software is difficult to navigate and use but it is a useful backup analyser to the Sysmex CS2500. The primary aim of this project was to calibrate the fibrinogen assay on the STart Max and ensure the assay was clinically viable. The secondary aim was to ensure the STart Max thrombin clotting time assay was operational and clinically viable. The tertiary aim was to produce standard operating procedure for these two STart Max assays, as well as the STart Max prothrombin time and activated partial thrombin time, which were created and handwritten prior to this project.
Methods: A calibration curve was produced for the fibrinogen assay and the process recorded for reproducibility. The thrombin clotting time, not requiring a calibration, was made available on the list of tests on the STart Max, allowing it to be used. Thirty sample comparisons were performed for both assays on the STart Max and the CS2500. The thrombin clotting time required an additional 16 sample comparisons after statistical analysis. Standard operating procedure was typed up and refined with supervisor feedback.
Results: The 30-sample comparison for the fibrinogen assays produced a correlation coefficient of 0.990 and a regression graph demonstrating a slight positive bias. The thrombin clotting time had an initial correlation coefficient of 0.780 but, with the

addition of 16 more samples, which provided a broader range of results, the correlation coefficient became 0.954. There was no significant bias in the thrombin clotting time. The standard operating procedures were adequate.
Conclusion: The Wairau hospital laboratory has clinically viable backup methods for fibrinogen and thrombin clotting time assays. They also have clear and easy to follow standard operating procedure for each of their STart Max assays.

An evaluation of the Hemosure Accureader A100 *Helicobacter pylori* assay

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Objectives: The Hemosure Accureader A100 is a colorimetric analyser now available for use in New Zealand clinical laboratories. This report evaluates this analyser, specifically the *Helicobacter pylori* assay. This assay was evaluated and contrasted against the CerTest *Helicobacter pylori* card-based assay; a highly accurate testing kit currently used by multiple laboratories in New Zealand.
Methods: Seventy faecal samples were selected during standard laboratory function and run through both testing methods to compare results. Inconclusive results were retested. Samples with repeated discrepant results were sent to Dunedin SCL to be tested on the Liaison XL immunological analyser as a confirmatory test. Both CerTest and Hemosure utilise latex immunochromatography based lateral flow assays to provide results.
Results: Testing revealed the CerTest assay was more accurate, specific, and had a higher positive predictive value than the Hemosure assay (91.4%, 95.6%, and 90.9% against 68.5%, 56.5%, and 52.4% respectively). For twenty samples sent for confirmatory testing, CerTest presented with higher accuracy against Hemosure (85% and 10% respectively). From the confirmatory testing, Hemosure presents with a high rate of false positives with values being reported from 61ng/ml to 257ng/ml, however this may be limited due to the analysers' adjustable cut-off values.
Conclusion: Due to the low accuracy, the suboptimal positive predictive value, and the high rate of false positives, the Hemosure Accureader A100 *H. pylori* assay cannot be recommended for use inside Wellington SCL at this time. Further advice is needed from the manufacturer to improve the performance of this test at Wellington SCL.

Is the BioMérieux ASD-Kit a suitable replacement for the Beckman Coulter IMMAGE in the determination of anti-streptodornase B in test serum?

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Objectives: To determine if the BioMérieux ASD-Kit is a suitable replacement for the Beckman Coulter IMMAGE for the detection of anti-streptodornase B in test serum.
Methods: Through the application of the BioMérieux ASD-Kit, semi-quantitative analysis of test serum determined the titre of anti-streptodornase present in serum and therefore the clinical significance of this titre. The test utilises a single dilution of test serum and increasing quantities of Streptodornase B in a strip of wells. Operating under the principle of inhibition, a dye colour change from blue to pink illustrates the absence of anti-streptodornase B in test serum and therefore the inability to neutralise streptodornase B .
Results: Patient samples with known clinically significant anti-streptodornase titres and quality control samples, with semi-quantitative titres previously determined by BioMérieux ASD-Kit, were analysed. Patient samples with a previous clinically significant titre of anti-streptodornase B, indicating current or recent Group A Streptococcal infection, were determined using the BioMérieux ASD-Kit to yield a clinically significant titre at a 100% success rate. Furthermore, 100% of previously negative patient samples were determined as having clinically insignificant titres of anti-streptodornase B indicative of no current, or past, Group A streptococcal infection. Additionally, 100% of quality control samples provided by The Royal College of Pathologists of Australasia and Waikato District Health Board Quality Assurance Programmes yielded titre results identical to their prior established values.
Conclusion: The BioMérieux ASD-Kit has been established as a suitable replacement for the Beckman Coulter IMMAGE in the determination of anti-streptodornase B antibody titre and therefore the clinical significance of antibody titre.
Acknowledgement: The author thanks staff at Waikato District Health Board Laboratories, Hamilton for supervision of this project.

Establishing key performance indicators for faecal pathogen PCR and BRAF mutation assays

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Objectives: Key performance indicators (KPIs) are objective measures for quality assurance and performance monitoring within the diagnostic laboratory and are required as part of the IANZ ISO15189 standard. The aim of this study was to establish meaningful key performance indicators for the turnaround times of faecal pathogen PCR and BRAF mutation assays.
Methods: All faecal pathogen PCR requests from the 01/01/2019 to the 31/03/2021 and all BRAF mutation requests, from the start of testing (July 2019) to 31/12/2020, were extracted from the laboratory information system. This data was used to determine the turnaround time KPI for faecal pathogen PCR and BRAF mutation assay results. Turnaround time targets were developed based on clinical performance and contractual requirements. Performance acceptance criteria were determined based on known operational procedures and workflow.
Results: Two KPIs for faecal PCR testing were established. A clinical KPI to report 95% of results within 3 days and operational KPI to report 97% of results within 4 days. For BRAF mutation testing one KPI was determined with the aim to report 90% of results within 3 days was set.
Conclusion: Turnaround time KPIs were developed for the faecal pathogen PCR and BRAF mutation assays. These key performance indicators are now able to be used by the Molecular Pathology department to continually monitor performance.

Comparison of an immunochromatographic test with a chemiluminescence immunoassay on faecal samples for the detection of *Helicobacter pylori* antigen in active infection

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Objectives: *Helicobacter pylori* (*H. pylori*) causes gastritis, peptic ulcers and is associated with gastric adenocarcinoma. Accurate diagnosis and efficient treatment can eliminate *H. pylori*, resolving these conditions and reducing cancer risk. SCL Dunedin intends to adopt the LIAISON® chemiluminescence immunoassay (CLIA) to diagnose an *H. pylori* infection.
Methods: The diagnostic accuracy of the CLIA was determined using the CerTest immunochromatographic lateral flow test as reference standard. The results from the CerTest are subjective; antigen presence initiates a colorimetric reaction, indicating a positive result. These results were reported on the

ULTRA database and used for comparison. Forty-two patients with symptomatic queried *H. pylori* infection provided stool samples. These samples had been tested by the CerTest and subsequently frozen. These were defrosted to room temperature and faecal *H. pylori* antigen was quantified by chemiluminescent immunoassay, examined on the Diasorin XL LIAISON® Analyser. Antigen concentration is proportional to relative light units which are converted to an index value. Index values range from 0.01 to 50; <0.90 is negative, 0.90-1.10 is equivocal and >1.10 is positive. Study results were reported as qualitative results for comparison to the CerTest.
Results: There was 67% concordance and a moderate agreement between the LIAISON® and CerTest (κ = 0.50). The LIAISON® achieved a sensitivity of 59.1% and specificity of 78.9% when compared to the CerTest.
Conclusion: This study showed poor agreement between the LIAISON® and CerTest. This may reflect methodical issues, subjective CerTest interpretation or sample issues. Additional analysis is required before the LIAISON® can be adopted as an *H. pylori* diagnostic method in SCL.

The validation of the laboratory-developed human adenovirus real-time PCR on the BD Max platform

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Objectives: The objective of this study was to validate a real-time PCR assay on the BD Max platform for detecting human adenovirus DNA in combination with a sample process control based on the modification of the current in-house assay.
Methods: The validation of the BD Max system was compared to the in-house assay
used in Canterbury Health Laboratories. The fully automated BD Max system combines DNA extraction and PCR amplification for improved workflow and reduced labour requirements preventing many of the manual errors associated with PCR. The current in-house assay for detecting human Adenovirus involves a three-step process, where the DNA extraction is completed on the NucliSENS EasyMag instrument, and the Light Cycler 480 instrument achieves PCR amplification.
Results: All samples from the 2020 external proficiency panel were correctly identified by the BD Max system. For the clinical validation, 39 specimen samples from specimen types such as nasopharyngeal swabs, nasal swabs and eye swabs were tested. These specimen samples had been previously analysed with either the in-house assay or the respiratory multiplex commercial assay for the detection of human Adenovirus. From these samples, one presumptively positive sample was undetected by the BD Max system and the in-house assay when retested, all negative samples were correctly identified as undetected by the BD Max system. The BD Max system detected the PCR signals 2 to 7 crossing threshold (Ct) values earlier than the in-house assay or the respiratory multiplex assay.
Conclusion: The fully automated BD Max assay showed improved workflow with superior analytical sensitivity and an excellent detection of human Adenovirus in the various specimen types.

Pre-analytical time delay of five hours on platelet function as measured by PFA-200

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Objectives: Platelet function analyser (PFA) provides a simple, rapid, qualitative and precise automated assessment of platelet adhesion and aggregation as it assesses for any inherited, acquired, or drug induced platelet dysfunction, especially von Willebrand disease. It is also used to monitor desmopressin therapy in both, and possibly anti-platelet therapy. The aim of this study was to investigate platelet function by comparing the closure times of within 1 hour and 5 hours after sampling to see if the pre-analytical time delay influenced platelet function. This will enable us to formulate a possible extension for our PFA turnaround time.
Methods: Citrate tubes from 20 participants were delivered immediately (within the first hour in the laboratory) for analysis using a PFA-200 microprocessor-controlled unit by measuring the closure time (CT) in seconds. Each donor samples was re-processed after five hours for a data comparison to see if there was a significant change under the influence of pre-analytical delay.
Results: The mean CT in two different time points for both agonists demonstrated a significant difference as it showed a 20% and 22% increase for both epinephrine and ADP respectively after 5 hours. The CT of the two different time points correlated positively in both agonists. The mean of the 20 samples in 1 hour and after 5 hours after sampling for both agonists reached significance ($p<0.05$).
Conclusion: There was a systematic and proportional difference between the two means of the different time points in both epinephrine and ADP. The pre-analytical delay of 5 hours does affect platelet adhesion and aggregation and therefore we can exclude the idea of extending the PFA turn-around time PFA-200 for Waikato DHB.

Comparison of 37°C indirect antiglobulin test incubation times in tube

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Objectives: The New Zealand Blood Service currently performs routine tube indirect antiglobulin tests (IAT) using a standard 60 min 37°C incubation time. However, the method recommended by Lorne Laboratories Ltd for their anti-human globulin reagent suggests a 15 min incubation time. Introduction of a shorter method would be of significant benefit due to the decrease in time taken to process samples. The aim of this project was to investigate the significance of reduced incubation times in terms of the resulting reaction strengths. It also acted as a preliminary test for the validation process that is required for the implementation of a shorter IAT incubation time.
Methods: Three quality control specimens with known red cell antibodies were used to perform antibody screens using the tube IAT technique. Each specimen was tested with three different 37°C incubation times – 15 min, 30 min, 60 min. The presence of agglutination was measured optically according to standard tube technique grading and reaction strengths were recorded.
Results: Positive reactions were observed for each incubation time length, as was expected according to the known red cell antibodies of the specimens and known screen cell phenotypes. For each specimen, the 30 min incubation resulted in the strongest reaction strength when compared to the grades of both the 15 min and 60 min incubations. **Conclusion:** The results suggest that the optimal tube IAT incubation time is 30 min. However, a more extensive investigation would be required to confirm these findings. A 30 min incubation time should be included in the official validation process .

A comparative analysis of the Sysmex XN-20 to the manual microscopy method for schistocyte counts

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Objectives: Schistocytes are the product of red cell fragmentation and important in the diagnosis of microangiopathic haemolytic anaemia. At Canterbury Health

Laboratories, scientists will perform manual schistocyte counts on a sample, when requested by a clinician. The aim of this study was to determine if the Sysmex XN-20 can replace the need for a manual count by comparing automated counts with manual counts performed by scientists of varying levels of experience.
Methods: 20 venous blood samples positive for schistocytes were analysed by the XN-20 over a 2 month period. A blood film created from each sample was analysed by three scientists (expert, competent and trainee) manually via microscopy. Analyser counts were compared to the expert's results, which were considered to be the gold standard.
Results: The results of the Sysmex XN-20 and scientists were compared using linear regression and achieved coefficients of determination of 0.18, 0.98 and 0.99, respectively. Bland-Altman difference plots were produced from the same data set, demonstrating a bias of 0.99 between the XN-20 and expert scientist. The competent and trainee scientists demonstrated a bias of 0.90 and 1.9, respectively, in comparison to the expert scientist. Reproducibility tests demonstrated a coefficient of variation of 38.4% for the Sysmex XN-20 and the expert, competent and trainee scientist achieving 21.3%, 29.2% and 18.6%, respectively.
Conclusion: The analyser was found to poorly correlate with the gold standard and demonstrated erroneous results. Reproducibility was also found to be poor, thus confirming that the analyser should not be used to replace manual methods. The manual counts, despite the level of experience, demonstrated a high correlation with the expert, however demonstrated low reproducibility.

A comparison between two Siemens' thrombin clotting time (TCT) reagents: Test Thrombin and Thromboclotin®

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Objectives: The aim of the study was to compare the Test Thrombin reagent, which is currently used for TCT at CSCL, with another TCT reagent, Thromboclotin®.
Methods: To test different aspects of the two reagents, 136 platelet-poor plasma samples (PPP) with a wide range of clotting times were selected. A number of these samples were diluted to achieve low fibrinogen levels (i.e. <1.0 g/L). TCT was performed using both reagents, results were statistically analysed using Analyse-It, and sensitivity and specificity were calculated for Thromboclotin®. Additionally, the precision and reference interval provided by the manufacturer were validated. Sample cost was calculated and compared for both reagents.
Results: There was 87.5% consistency of result interpretation between the two reagents. Despite some discrepancy, in 12.5% of the samples tested, statistical analysis showed no significant overall difference between the two reagents. Most of the discrepancy occurred in samples with very low fibrinogen, as Test Thrombin appears to be more sensitive in detecting low fibrinogen concentrations in PPP samples. In this study, precision was calculated for Test Thrombin to be 0.72%, compared to 1.9% by the manufacturer, while it was 0.86% for Thromboclotin®, compared to 1.3% by the manufacturer. Thromboclotin® had 62.5% sensitivity and 95.2% specificity compared to Test Thrombin. Thromboclotin® is slightly cheaper, but if other local factors are taken into consideration, e.g. reagent storing, aliquoting, and possible wastage, then the Test Thrombin will be overall more economic to use under CSCL's conditions.
Conclusion: There is no significant difference between the two reagents, however, the Test Thrombin appears to be more suitable and practical to use at CSCL, considering its local conditions.

Mechanical versus optical INR detection: A comparative investigation

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Objectives: Both mechanical and optical methods are used to determine prothrombin time, however these methods have different reference ranges and thus the unitless ratio INR is necessary to compare results across methods. The INR should be consistent across these methods. However, this was not the case for a sample analysed by the Stago CM2 and Sysmex CS2500 analysers used in Taranaki labs. Thus, this investigation was carried out to assess analyser agreement.
Methods: Twenty-five samples were run on both analysers to obtain INR values for comparison. The samples gathered over a four-week period to obtain a wide range of INR values and were frozen together until analysis to maintain viability. The Sysmex analyser utilised Dade Innovin reagent, and the Stago utilised Stago INR Neoptimal 10 reagent. Both analysers passed QC checks and were up to date with maintenance and calibration prior to analysis.
Results: The two INR results showed strong correlation (r=0.9772) but the Stago analyser was observed to have a mean difference of -0.17 compared to the Sysmex analyser. The 95% limits of agreement for the Stago analyser were broad: the upper limit 0.93 above and the lower limit 1.27 below the Sysmex. Adjusted for an increasing difference with magnitude the limits of agreement for the Stago compared to the Sysmex analyser were between 22% lower and 20% higher results.
Conclusion: Due to the broad 95% limits of agreement, it is implied that these two analysers may not agree sufficiently to be used interchangeably. This is because an INR value variation of up to 20% above or 22% below during monitoring may be significant enough to result in erroneous alteration of patient coagulative treatment.

Evaluation of the criteria for the identification of beta-haemolytic *streptococci* Lancefield groups A, C and G

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Objectives: Group A *streptococci* (GAS) are an important cause of bacterial pharyngitis and a range of skin infections. Severity of GAS infections ranges from mild to life threatening invasive infection. Group C and G *streptococci* (GCS/GGS) are genetically similar to GAS, but clinical significance of isolation is debated. Rapid and accurate identification of b-haemolytic streptococci (BHS) is important, especially in sterile sites. This study aimed to determine an effective criterion that demonstrates reliable species identification of BHS using matrix -assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS), Lancefield latex grouping and the PYR test.
Methods: A total of eighty-five BHS isolates were obtained and identified using the Bruker MALDI TOF MS. All isolates were then Lancefield grouped against all three groups (A, C and G) using the ProLab Diagnostics Prolex Streptococcal Grouping Latex Kit. All Isolates were also tested for PYR enzyme activity using the Key Scientific Products PYR test kit.
Results: MALDI TOF MS correctly identified eighty-three/eighty -five (97.6%) BHS isolates. Two discrepancies were seen among isolates with a spectral score of <2.00. Of the isolates that received a spectral score of ≥ 2.00, Eighty/eighty (100%) were correctly identified. Lancefield latex grouping correctly identified eighty-two/eighty-five (96.5%) BHS isolates, with three discrepancies seen. The PYR test correctly identified

100% of isolates, all forty-four GAS isolates were PYR positive and all forty-one GCS/GGS were PYR negative.
Conclusion: MALDI TOF MS proved to be the most effective method for identification of GAS and GCS/GGS. The current spectral score accepted for reliable MALDI TOF MS identification of ≥ 2.30 is effective, but over half of isolates would require additional latex grouping. Lowering the acceptable score for reliable identification would maintain accuracy of identification and reduce the need for additional latex grouping, in turn reducing laboratory costs.

Developing a SNP profiling panel for the tracking of whole-exome sequencing studies

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Objectives: Single nucleotide polymorphism (SNP) panels that uniquely identify samples are useful for genetic research. Previously developed SNP panels are composed of a high number of SNPs that target mostly intragenic regions. With increasing interest in whole-exome sequencing (WES) at Canterbury Health Laboratories, we aimed to develop a practical, rapid and inexpensive method for genotyping eight SNPs in unknown DNA samples for identity verification.
Methods: A panel of eight SNPs had been identified which exhibit a minor allele frequency of approximately 50%, and a selection of random samples were genotyped for these SNPs using three different methods; Sanger sequencing, high resolution melting (HRM) analysis and ABI SNaPshot multiplex reaction kit. Genotype data was extracted from exome sequences using Agilent Alissa Interpret software. The probability that any individual of European descent would match another was calculated to be 0.4%, assuming no family relations.
Results: A panel of eight SNPs was not sufficient to differentiate two random samples, as two genotypes of the ten samples tested were an exact match; more SNPs are required to achieve sufficient discriminatory power. The ABI SNaPshot multiplex was the most efficient method for genotyping and Agilent Alissa Interpret software was useful in genotyping SNPs in WES data. Sanger sequencing was the most reliable method, while HRM only worked for 1 of the SNPs.
Conclusion: A SNP panel provides a simple, yet powerful, method for the assignment of highly discriminatory identifiers to genetic samples. However, minimising the number of SNPs used while still allowing sufficient redundancy requires further investigation.

Optimization of kappa and lambda light chains on the Ventana Benchmark ULTRA

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Objectives: All immunoglobulins are made up from two identical heavy chains and two identical light chains. Heavy chains define the immunoglobulin class and subclass (IgG, IgM, IgA, IgE and IgD). While the light chains can either be kappa or lambda, the distribution of kappa and lambda differs for different classes of immunoglobulins. In immunohistochemistry, kappa and lambda are B cell specific antibodies used for plasma cell identification and investigation of lymphoid cell related clonality/ neoplasia in a tissue. However, studies have shown that kappa and lambda assay results are challenging due to their low pass rate and low optimal score rate. Thus, the aim of this project was to optimize the kappa and lambda immunohistochemical protocol to produce optimal staining results on lymphomas.
Methods: Kappa and lambda polyclonal antibodies from Dako were used for this project. Five factors of the immunohistochemical protocol were considered for optimization. These were the Ventana detection kit (Ultraview DAB and Optiview DAB), the antibody dilution, the pH of the antibody retrieval solution (CC1 or CC2), the time of antibody retrieval (HIER) and the antibody incubation time.
Results: The original protocol used a Optiview DAB detection kit, an antibody dilution of 1:8000, CC1 solution for 32 min and an incubation time of 40 min. Following the research project, the optimized protocol consisted of an Ultraview DAB detection kit, an antibody dilution of 1:10000, CC1 solution for 20 min and the incubation time dropped to 24 min.
Conclusion: Optimization was through modifications of detection kits, antibody dilutions, incubation time, pH solution and retrieval time. As a result, a pathologist validated diagnostic test protocol was achieved.

Investigating the correlation and agreement of a newly installed coagulation analyser

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Objectives: Medlab Central uses two coagulation analysers interchangeably to produce patient results that have clinical implications. The newly installed analyser had to show adequate correlation and agreement with the pre-existing analyser before it could be used routinely.
Methods: Citrated whole blood samples of both in- and out-patients were used to produce a pair of results for statistical investigation using correlation coefficient and Bland-Altman plots.
Results: The correlation coefficients for INR, APTT, Fibrinogen, and D-dimer were 1.00, 1.00, 0.99, and 1.00 respectively. The Bland-Altman plots showed acceptable agreement within the clinically relevant ranges for all assays tested. Greater difference was seen in the clinically insignificant ranges. The INR showed good agreement with minimal difference of ±0.1 in the range of 0.8-1.8. A positive bias was noted in the INR as the value increased, differing by no more than 0.4 and no less than 0.1. The new analyser showed a negative bias for APTT with most results differing by ± 1 second in the range of 18-50 seconds. At extremely prolonged APTTs, the difference was greater but insignificant. Fibrinogen showed good agreement in the range of 0-2 g/L. The D-dimer assay showed good agreement with the same conclusions drawn 24/26 times.
Conclusion: Clinical relevance had to be considered when interpreting the results. The INR, APTT, Fibrinogen, and D-dimer assay showed good correlation and agreement within their clinically relevant range. Further improvements include subdivision of samples into physiologically- matched groups, collection of more data covering the D-dimer range of 475-525 ug/L, the inclusion of frozen/thawed samples, the inclusion of haemolysed, lipemic, and icteric samples to assess the effect of interfering substances and investigation into all available parameters.

A comparison of Columbia and Tryptic-Soy Base Sheep Blood Agar, and CHROMagar™ StrepA Agar for detection and isolation of β-haemolytic *Streptococcus* species

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Objectives: This project aimed to compare the haemolytic properties of β-haemolytic streptococci (BHS) on Sheep Blood Agar with Columbia Base (CB) against a Tryptic Soy Base (TSB). This project also determined the effectiveness of CHROMagar™ StrepA, a chromogenic agar designed for screening of Group A streptococci (GAS).
Methods: One hundred and eleven patients' throat swabs were streaked onto one CB, one TSB, and one half of a CHROMagar™ StrepA plate each (all media from Fort Richards

Laboratories). The plates were incubated at 35°C with 5% CO_2 for 2 days. The plates were observed after one and two days of incubation. Growth characteristics, morphology, and the appearance/size of haemolysis were compared. Suspect pathogens were identified by catalase testing, Lancefield latex agglutination, or MALDI-TOF MS.
Results: Of the 111 throat swabs, 26 contained BHS, or a member of the *Streptococcus milleri* group. Differences in growth, morphology and/or haemolysis were observed among 19/26. In 7/26, BHS were identified on the TSB plate before the CB plate. Only 1/26 was identified on CB before TSB. Three swabs containing a *S. milleri* group species were not isolated on the CB plate. Of the 12 swabs that contained GAS, it took 2 days for red colonies to appear on the CHROMagar™ plate for 3/12, and 1 day for 6/12. No red colonies appeared for 3/12 swabs.
Conclusion: β-haemolysis is more evident on TSB than on CB and may allow for faster identification of throat pathogens on the former. CHROMagar™ StrepA does not reduce the turn-around-time of results and therefore, does not allow for identification of GAS within one day of incubation.

Method comparison between tube and microcolumn techniques for the indirect antiglobulin test

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Objectives: In routine blood bank serology, antibody identification is more commonly performed through an indirect antiglobulin test using commercial microcolumn cards. However, tube techniques, including the tube indirect antiglobulin test, still remains the "gold standard" for serological testing. This study was done to compare the sensitivities of the two methods.
Methods: Plasma samples were taken from a total of 10 patients that had a positive red cell antibody screen during their Group and Screen tests. Each sample was tested against a set of panel cells (Grifols Perfect Panel 11) twice; once with the microcolumn method using the DG Gel Coombs card, and once with the conventional tube method. Results between the two methods were compared. The identity of the antibody(s) in each sample was known.
Results: Out of 48 reactions with cells that were positive for the corresponding antigen, the microcolumn method had three false negative reactions, while the tube method had 25. This resulted in the tube method having a lower sensitivity (48%) compared to the microcolumn method (94%). Out of 62 reactions with cells that were negative for the corresponding antigen, there was one false positive reaction in the microcolumn method which lowered the specificity to 98%. The tube method had no false positives and therefore had 100% specificity.
Conclusion: When performing the indirect antiglobulin test, the microcolumn method was found to have significantly higher sensitivity for the detection of antibodies compared to the tube method. This suggests that the microcolumn method is more suitable for routine antibody identification. However, laboratory scientists should follow laboratory protocols and use whichever method is most appropriate at the time.

Evaluation of the performance of the albumin bromocresol purple reagent

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Objectives: The aim of the evaluation was to critically analyse the performance of the Sentinel Diagnostics albumin bromocresol purple reagent on the Beckman Coulter AU680 analyser.
Methods: In this evaluation, a series of tests were performed on the Beckman Coulter AU680 analyser to analyse the precision and accuracy of the method, along with the linearity of the test and stability of the reagent. The reagent was tested using quality controls over 19 days to evaluate the stability. Pooled high and low patient serum was tested both with mixing three times weekly and without, in order to evaluate both the precision and accuracy, and the effect of sedimentation on the reagent. A single bottle of quality control was tested with the routine albumin bromocresol purple reagent to evaluate the stability of the quality control material. Precision was checked at 2 levels using pooled high and low patient serum. Linearity was assessed using a serial dilution of patient samples. Additionally, 2020 external quality control data was assessed.
Results: The albumin bromocresol purple reagent was found to be stable within 2 SD over the 19 day period. The quality control material was stable over the period tested. As found previously, unmixed reagent results drifted. Intra-batch precision was found to be similar to that stated by the manufacturer. The reaction was linear down to about 6 g/L albumin.
Conclusion: The albumin bromocresol purple reagent performed satisfactorily for the requirements of the laboratory. Both the reagent and quality control materials were stable for the manufacturer claimed period of use and the linearity was sufficient for medical purposes.

Verification of the Pathogen 200 v3.1 protocol on the Roche MagNA Pure 24 Diagnostics for the extraction of DNA from faecal samples with comparison to the Pathogen 200 hp protocol

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Objectives: Acute gastrointestinal illness (AGI) creates a significant burden of disease in New Zealand. Pathogenic causes of AGI are explored using Multiplex Tandem (MT)-PCR at Wellington SCL. The quality of nucleic acid extracts has significant effects on the ability of PCR to detect targets within samples. This study aimed to verify an updated extraction protocol (Pathogen 200 v3.1) by comparing its performance against the current extraction protocol (Pathogen 200 hp) used for faecal samples.
Methods: Take-offs were generated using the Faecal Pathogen and Parasite (12-well) assay by AusDiagnostics on nucleic acid extracts from twenty-three faecal aliquots using the current and updated protocols on the Roche MagNA Pure 24 system. Samples were selected to produce a data range that detected each nucleic acid target in the MT-PCR assay at early (≤5 cycles) and late (18-20 cycles) take-offs.
Results: A strong correlation between take-offs produced by extracts from the current and updated protocol was observed ($r = 0.99$, $R^2 = 0.98$). There was no statistically significant difference between protocols ($p = 0.70$). No trend in take-off differences by target was observed.
Conclusion: The updated protocol showed statistically comparable results with the current protocol during MT-PCR analysis to generate take-offs for each pathogen. No change in extraction time was observed. Limitations such as insufficient samples size and extraction failures meant not all targets could be verified for both take-off ranges. Other stated benefits of the updated protocol were not observed. Therefore, there is sufficient but limited evidence to recommend changing the faecal extraction protocol used by WSCL.

Evaluation of DiaSorin aldosterone assay

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Objectives: Aldosterone is a steroid hormone secreted by the adrenal cortex which regulates body fluid volume. Southern Community Laboratories (SCL) Dunedin is utilising a new DiaSorin plasma aldosterone assay on a Liaison analyser to

test patient samples. Currently, all aldosterone tests are sent to Canterbury Health Laboratories (CHL), which uses an IDS-iSYS aldosterone assay. The aim of this research was to evaluate the agreement between DiaSorin aldosterone assay results and IDS-iSYS aldosterone assay results and discover any potential reference interval changes needed.

Methods: The DiaSorin aldosterone assay on the Liaison analyser uses a chemiluminescent competitive immunoassay. In this research, two DiaSorin quality controls (lower 184.2pmol/L; higher 678.7pmol/L) were tested 11 times. Also, 52 patient plasma specimens were collected and analysed by both laboratories with their own aldosterone assays.

Results: Student's t-test (mean difference 45.33pmol/L; 95% confidence interval 17.26-73.39pmol/L; p=0.0021) indicated a statistically significant mean difference between the results obtained at SCL Dunedin compared to CHL. Passing-Bablok fit (y=31.77+0.8696x) showed a negative bias (approximately 13%) for SCL Dunedin results compared to CHL results, which is clinically insignificant when compared to the RCPA Analytical Performance Specification. The SCL Dunedin results were precise according to the coefficient of variation (CV) of QCs (DiaSorin Lower: 10.19%, DiaSorin Higher: 7.43%).

Conclusion: The mean of SCL Dunedin aldosterone assay results was statistically different from the mean of CHL results. However, the SCL Dunedin results had good precision and were clinically comparable to the CHL results with a downwards bias of approximately 13%. Thus, the DiaSorin results and IDS-iSYS results have acceptable agreement and the reference interval does not need to be changed .