Analysis of cardiac troponin-T stability in point of care testing: is delay okay?

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Objectives: Acute coronary syndrome (ACS) is the leading cause of mortality worldwide, accounting for more than 9 million deaths in 2016. With rapid diagnosis and treatment, many of these deaths are preventable, although in rural communities this becomes increasingly more difficult. The introduction of point of care testing analysers measuring cardiac troponin-T, such as the Abbott i-STAT has greatly decreased morbidity and mortality rates worldwide. However, samples have a short stability of only 30 minutes following collection, making it difficult to abide by in rural practices. The objective was to analyse the sample stability of cardiac troponin-T when tested up to 4 hours following collection, to determine if samples received outside of the 30-minute timeframe can still be accepted.
Methods: Venous blood samples from in-patients at Dunstan hospital, who presented with symptoms suggestive of ACS, were collected into lithium heparin tubes. Within 30 minutes and at 1 hour, 2 hours and 4 hours, 17 µL of blood was dispensed into an Abbott cTnI i-STAT cartridge and the results recorded
Results: Twenty venous blood samples that matched the criteria were collected throughout the duration of the study. All samples had very little variability between timepoints with a correlation of 0.998 for 60 minutes, 0.996 for 120 minutes and 0.997 for 240 minutes when compared to samples tested less than 30 minutes after collection.
Conclusion: There was no significant change in cTnI concentration between samples tested less than 30 minutes after collection and 4 hours after collection. If more datapoints are collected this could introduce a change in protocol where samples received more than 30- minutes after collection can still be accepted for testing.

Determining the diagnostic accuracy of the white precursor cell channel on the Sysmex XN-20 automated haematological analyser

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Objectives: The purpose of the study was to determine the diagnostic accuracy of the white precursor cell channel of the Sysmex XN-20 Automated Haematological Analyser. The XN20 Analyser has an added parameter for the white precursor cell channel, designed to further distinguish samples originally flagged in the XN differential channel as 'blast/abnormal lymphocyte?'. Abnormal cells are classed as lymphocytes indicating potential malignancy.
Methods: Full blood counts flagged as 'blast/abnormal lymphocyte?' were further investigated using the DI60 Sysmex cell imaging software and light microscopy to see if the reflex testing in the white precursor cell channel had classified them correctly.
Results: The reflex testing in the white precursor cell channel classified 19 blasts, 42 abnormal lymphocytes and 50 negative flags (including 17 atypical lymphocyte flags which were counted as negative and non-malignant). Of the 100 flagged samples, 64% were correctly classified either as containing abnormal lymphocytes, blasts, or negative. Four samples could not be verified microscopically due to extremely low WBC counts.
Conclusion: The channel showed poor specificity (47%) but had high sensitivity (97%). A highly sensitive flagging algorithm is critical to ensure all abnormal cells are detected in reflex testing. Given the serious consequences of a false negative, it is acceptable in this case for the test to be more sensitive than specific. Although the calculated diagnostic accuracy overall (67%) was low, the sensitivity of the reflex white precursor cell channel test means that staff can be confident the test will not miss a malignancy if negative and can use this channel to this channel to reduce slides generated and therefore overall costs.

Clinical validation of the BD MaxTM system for detection of parvovirus B19 DNA

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Objectives: Parvovirus B19 (B19V) is a small, non-enveloped, single-stranded DNA virus that causes a range of symptoms in children and adults. A laboratory-developed real-time PCR assay for B19V is established on the integrated BD MaxTM platform. The aim of the analysis is to determine the performance of BD MaxTM in detecting B19V DNA when using DNA-3 or DNA-1 Extraction Kit, in comparison to the current in-house assay at Canterbury Health Laboratories (CHL) which utilises NucliSENS easyMAG extraction and ABI7500 amplification.
Methods: Plasma and molecular grade water dilution series were performed to compare analytical sensitivity of the BD MaxTM B19V assay with DNA-3 and DNA-1 Extraction Kit to current assay. To determine clinical sensitivity and specificity, thirty-one previously-tested samples were analysed on the BD MaxTM to determine detection of B19V DNA.
Results: Results from the plasma dilution series using the DNA-3 extraction kit show that the current in-house assay is 10-fold to 100-fold more sensitive. However, analytical sensitivity of the BD MaxTM assay improved upon switching to DNA-1 Extraction Kit. Clinical analysis using the BD MaxTM B19V assay with DNA-1 Extraction Kit showed a 95.5% sensitivity and 100% specificity.
Conclusion: Based on the results, the performance of the BD MaxTM B19V PCR assay is comparable to the current in-house B19V assay at Canterbury Health Laboratories. By considering the fully automated capabilities of the BD MaxTM, B19V assay with DNA-1 extraction kit can be validated and used for testing clinical samples as this improves the laboratory's workflow and turnaround time.

Evaluating a screening test for the replacement of the discontinued Bio-Rad heparin/PF4 antibody test used in the diagnosis of heparin induced thrombocytopenia (HIT)

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Objectives: The study evaluated the HemosIL Acustar HIT-IgG, and the Stago STic Expert methods against the discontinued Bio-Rad Particle gel immunoassay (PaGIA), as replacement assays to screen for HIT and vaccine-induced thrombotic thrombocytopenia (VITT).
Methods: Twenty samples were used from community and hospital patients. Due to the rarity of HIT, several Quality Assurance Programme (QAP) samples were used. Additionally, six VITT QAP samples were tested. Correlation studies were performed using the three different methods. Results were statistically evaluated using Cohen Kappa statistics; and calculation of the Sensitivity, Specificity, Positive Predictive values (PPV), and Negative Predictive Values (NPV).
Results: Kappa statistics generated values of 0.76 for Stago,

and 1.00 for HemosIL, against Bio-Rad. The Stago value of 0.76 is interpreted as moderate agreement, whereas the hemosIL value of 1.00 is interpreted as almost perfect agreement. The HemosIL assay generated 100% sensitivity, specificity, PPV, and NPV. The Stago method yielded 92% sensitivity and 100% specificity along with 100% PPV and NPV. Stago and HemosIL tested negative for all five of the positive VITT samples.
Conclusion: The HemosIL assay was the best performer in terms of sensitivity and Kappa values and in combination with cost, turn-around time and accessibility, is considered a potential replacement method at WSCL. An additional aspect for consideration is both the difficulty and subjective nature of interpreting the Stago results where a lateral flow reader could be of use. Both Stago and HemosIL were found insensitive for the detection of VITT and therefore require requests be sent to a referral lab for enzyme-linked immunosorbent assay (ELISA).

Optimising dual staining of CK5 and P5O4S on prostate tissue

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Objectives: The aims of this project consisted of optimising a protocol for dual staining of CK5 and P5O4S on prostate tissue, to achieve a faster turn around time for diagnosis and a reduced workload for pathologists and laboratory workers.
Methods: Appropriate test tissue was selected based on its ability to express both CK5 and P5O4S silmutaneously; this was found to be cancerous prostate. A patient's radical prostatectomy resulting from cancerous prostate core biopsies were sectioned, and had multiple different protocols run; adjustements were made on antigen retrieval time, primary or secondary antibody application time, or a mix of these variables. The two best protocols in relation to the project's aims were then selected and run on prostate core biopsies.
Results: Antigen retrieval time saw optimal results at 24 minutes, with 16 minutes seeing incomplete staining from both CK5 and P5O4S, and 32 minutes seeing increased bleeding from CK5. CK5 bleeding occurred throughout most protocols but was reduced if applied for 12 minutes. P5O4S had appropriate staining abilities ranging from 32-48 minutes.
Conclusion: An antigen retrieval time of at least 24 minutes must be applied to have appropriate staining reaction, yet a number of protocols differing in antibody application time for either CK5 and P5O4S were found to yield optimal results. In conclusion, the application of CK5 and P5O4S as a dual stain on a single section helps pathologists diagnose prostatic adenocarcinoma faster; reduces amount of patient tissue used, and frees up space on the immunostainer for other tests to be run.

Method comparison of the Elecsys FT4 IV assay kit with the Elecsys FT4 III assay kit on the Roche Diagnostics Cobas e801 analyser

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Objectives: Thyroxine (T4) is one of two main hormones (the other being Triiodothyronine, T3) produced by the thyroid gland, and is present in the circulation either bound to serum proteins, inactive T4, or as biologically active free T4 (FT4). At Taranaki Pathology Services (TPS) FT4 is currently measured using the Roche Diagnostics Elecsys FT4 III assay/kit which will be replaced by the new Elecsys FT4 IV assay. The aim of this study was to compare the two assay kits and to confirm whether the current methodology for FT4 quantification would be suitable for the new version of the assay.
Methods: Forty samples with validated FT4 results previously tested with the FT4 III kit were selected and tested with the FT4 IV kit. Of these samples, 15 were less than the FT4 reference interval (RI) for adults, 16 were within the RI and 9 were above the RI. At TPS, FT4 is measured by the Roche Diagnostics Cobas e801, an automated analyser for chemistry immunoassays, which uses a competitive test principle and electrochemiluminescence technology for FT4 quantification in serum/plasma. The results obtained from each kit were analysed.
Results: The results indicated that a strong positive correlation exists between the current FT4 III assay and the new FT4 IV assay given the correlation coefficient, r, of 0.997. Conclusion: **Conclusion:** This study was able to validate that the method used for the current FT4 III assay would be applicable for the updated FT4 IV assay intended for diagnostic use, meaning that the methodology for FT4 testing may remain the same once the kit transition is completed.

Testing immunohistochemistry on frozen sections

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Objectives: Frozen sectioning is the rapid production of tissue sections stained with Haematoxylin and Eosin using fresh tissue received from a patient undergoing surgery. A specific diagnosis cannot be confirmed by this procedure alone. Immunohistochemical staining on formalin fixed, routinely processed tissue targets specific antigens on abnormal cells to provide a prognosis and a definitive diagnosis for the patient.
To determine the procedure that would yield similar if not better immunohistochemical staining results from frozen sectioned tissue.
Methods: Several fresh tissue samples were bisected, with one half undergoing routine processing and immunohistochemistry staining using validated antibody protocols; CKAE1/3, S100, CD45 and Vimentin (panel for carcinomas of unknown primary and expressed in normal tissue) on the Roche BenchMark ULTRA. The other half was frozen and sectioned using a cryostat so that 12 slides were produced per sample and divided into 3 groups; fixed in Neutral Buffered Formalin (NBF), acetone or air-dried (no fixative). Frozen section slides were then stained using the BenchMark ULTRA. For antibody validation, four antigen retrieval incubation times were tested for each antibody for each group.
Results: All four antibodies had at least weak positive reactions to every combination of tissue, fixation method and retrieval time. Over half of the slides fixed in acetone had poor background staining. The quality of antibody staining of the samples fixed in NBF was of equal if not better quality compared to the routinely processed slides. Slides that were air-dried with no fixation produced sub-optimal staining results.
Conclusion: Frozen sectioned slides can produce good quality immunohistochemistry staining when fixed in NBF and each antibody protocol validated to optimal retrieval times. Combining both procedures could produce a diagnosis within several hours instead of days.

Assessing the possibility of implementing a sieving protocol to improve diagnostic stewardship in urinalysis

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Objectives: Urinary tract infection (UTI) is a highly prevalent cause of bacterial infection and urologic disease. Asymptomatic bacteriuria is common and presents without pyuria (<10 WBC

x10^6/L). Sieving protocol uses defined clinical details for samples without pyuria to indicated that they must be cultured. This retrospective study was conducted to assess the possibility of implementing a sieving protocol for urinalysis in the Invercargill laboratory to improve diagnostic and antibiotic stewardship.

Methods: A total of 236 urines were analysed between 29th June and 6th July, 2022, and of these, 83 samples had <10 WBC x10^6/L. A variety of collection techniques including voided midstream urine, catheter urine etc. were accepted for this study. Manual urine microscopy was performed on each specimen and all were cultured onto Blood/Chromogenic Orientation Agar. Any significant growth was analysed by a Senior Scientist, recorded and the causative agent identified. Clinical details that would meet the culture criteria were also recorded. Microsoft excel was used for data analysis and other statistical calculations.

Results: Of the 83 urine specimens with <10 WBC x10^6/L, 11% had significant growth. 34% of urines met the culture criteria and, of the 66% that did not, 11% had significant growth. The margin of error for <10 WBC x10^6/L urine samples that did not meet the culture criteria but had significant growth was ± 5.5% (95% confidence interval) and for total urine specimens received, it was ± 2%.

Conclusion: Implementing the sieving protocol for urinalysis in the Invercargill laboratory will improve diagnostic and antibiotic stewardship, be more cost effective, reduce the harm of over interpreting and over reporting urine cultures and generally enhance patient care.

Method evaluation and automation of bioMérieux kit detecting antibodies to Streptococcal DNAse B

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Objectives: Detection of infection from Group A streptococcal bacteria (such as Streptococcus pyogenes) is crucial for preventing illness and later sequelae such as post-streptococcal glomerulonephritis and acute rheumatic fever. Infection is determined serologically, with a high enough titre of antibodies to either Streptolysin O or DNAse B indicating infection. The production of the Siemens Antibody Inhibition of Dnase B Enzyme kit has ceased, so an alternative is required to continue the ability to test. The selected option is the Anti-Streptodornase B (ASD) Antibody Testing kit manufactured by bioMérieux and used by Labtests in Auckland.

Methods: A range of patient samples and RCPA samples that had been tested via the Siemens kit were collected over 2022, with age and clinical details recorded. Samples were run using the bioMérieux kit both manually and via
the automated DSRie platform, then compared to the previous results on the Siemens kit.

Results: The new bioMérieux kit had 100% specificity and 42% sensitivity compared to Siemens, and concordance was 66%. The kappa concordance was 0.37, which indicates a low level of agreement. A paired two tailed T test was performed; there was a significant difference between the interpretation of the kits ($p<0.0003$) and medium effect size (Cohen's d = 0.7425).

Conclusion: There was inadequate comparability between the two kits. The bioMérieux kit was far less sensitive in comparison to the Siemens kit. When looking at just RCPA mean results, bioMérieux was concordant with 8/8 results, and Siemens was concordant with 7/8. While not comparable to the previous kit, bioMérieux is an acceptable alternative.

Investigation of D-Dimer stability with INNOVANCE D-Dimer assay

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Objectives: The present study aimed to examine whether the D-dimer stability time can be extended from 4 hours to 6 or 8 hours at room temperature to account for late arrival samples and add-on test requests received after 4 hours.

Methods: Three citrate blood tubes were collected from 25 patients. For each patient, a baseline tube was centrifuged and tested for D-dimer upon arrival (within 4 hours). This baseline tube was then repeatedly tested at 6 hours and 8 hours post-sample collection. The other two tubes were stored horizontally at room temperature until 6 hours and 8 hours post-sample collection, at which they were centrifuged and tested, respectively. All tests were analysed with the INNOVANCE D-Dimer assay.

Results: The D-dimer levels at baseline ranged from less than 190 µg/L FEU to 10512 µg/L FEU. The mean percentage changes at 6 hours and 8 hours were all below the clinically acceptable cut-off of 10% compared with the baseline. Specifically, for centrifuged samples, a decrease of 2.39% and 2.23% were found after 6 and 8 hours, respectively. For uncentrifuged samples, a decrease of 2.83% and 1.93% were observed at 6 and 8 hours, respectively. Results from each tested condition all met the allowed limit of performance in EFLM (European Federation of Clinical Chemistry and Laboratory Medicine). Further analysis using Passing-Bablock regression showed excellent agreement between D-dimer levels at baseline and D-dimer levels after 6- and 8-hours storage.

Conclusion: Our study demonstrated that the stability of D-dimer at room temperature can be reliably extended to 8 hours for both centrifuged and uncentrifuged whole blood samples.

Assay Validation of the Updated Elecsys FT3 III Assay

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Objectives: In April 2022, Roche informed SCL's Southland Hospital laboratory of the launch of an updated Elecsys FT3 III assay. To ensure that the new assay could be implemented without significantly changing the results obtained, assay validation was required.

Methods: Forty-five samples were analysed; 18 were collected from the Royal College of Pathologists of Australasia (RCPA) Quality Assurance program, and 27 were retrospectively collected from past patient samples. The samples were analysed on the Cobas Pro e801 analyser, first using the reference assay and then analysed again using the updated assay. The two sets of results were compared and analysed using XLSTAT and Analyse-It software to measure the degree of method agreement and the presence of any significant differences between the assays. RCPA Analytical Performance Specifications were used to determine whether the difference between the assays was considered significant.

Results: Analysis revealed that the results obtained from the new FT3 assay did not vary significantly from the results from the reference FT3 assay. Passing-Bablok analysis revealed no systemic bias and small levels of proportional bias. The Bland Altman graph identified that, on average, the reference assay produced FT3 results that are 0.151 pmol/L higher than the new assay. The difference between the results from the new FT3 assay compared to the reference assay were below the limits set by the RCPA Analytical Performance Specifications, meaning the differences were insignificant.

Conclusion: This assay validation assures us that when SCL's Southland Hospital laboratory starts using the updated Elecsys FT3 III assay, there will be no significant change in the results or how free T3 levels are reported.

Validation of an X-Chromosome Inactivation Analysis Method for use at Canterbury Health Laboratories

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Objectives: X-chromosome inactivation (XCI) is a process in females where one X-chromosome is inactivated by methylation. XCI can contribute to, and protect from, disease. Canterbury Health Laboratories has been undertaking functional XCI studies in a research capacity, however this method was not fully validated or accredited. Due to increasing demand for XCI testing, an accredited method is required. This study determined the suitability of a method that determines XCI status at the androgen receptor (AR) gene.
Methods: Female DNA samples extracted by two methods (Kurabo and Qiagen) were analysed following restriction enzyme digestion using HpaII, PCR using a fluorescent FAM-labelled primer to the AR locus, and fragment analysis on an ABI3500 genetic analyser. Digestion time, and inter-run and intra-run variability were assessed. GeneMapper software was used for data analysis. Patients (n=4) with skewed XCI were analysed and compared to previous methodology.
Results: 1h, 4h and overnight DNA digestions were indistinguishable. The AR locus was found to be highly informative (heterozygosity was detected in 24/28 females). DNA extraction method did not affect the assay, however DNA concentration was critical. Inter-run and intra-run variability was assessed in two samples with no evidence of skewed XCI. The samples, run in triplicate, and repeated three times, yielded an inter-run variability of 49.41% XCI ±2.11% and 52.96% XCI ±5.88%, and an intra-run variability of 48.5-50.53% XCI ±1.49-2.69% and 48.92-59.97% XCI ±0.2-5.06% respectively.
Conclusion: This method was easy to perform and implement, and suitable for XCI analysis. The inter-run/intra-run variabilities showed that it is reproducible and reliable. Further validation using additional known skewed XCI samples and another X-chromosome locus are planned.

Assessment of Elecsys assay updates for the Roche Cobas 601

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Objectives: Increased levels of serum biotin in patients who take multivitamins or biotin therapy have caused interference in Streptavidin-biotin complex-based assays for the Cobas 601 analyzer. In response to this, Roche is beginning to update their electrochemiluminescence principles to include a highly specific anti-circulating biotin antibody. The purpose of this assessment is to validate the changes made to the follicle stimulating hormone (FSH) and free- triiodothyronine (FT3) assay kits used in the Timaru Medlab South Biochemistry laboratory.
Methods: Method comparison sample pools were made from stored patient samples, 30 samples for the FSH assay and 40 samples to compare the FT3 assay kits. The samples were then tested, and the results linked to sample number for comparison. Data was analysed for suitability using Passing-Bablock linear regression and Bland-Altman mean difference graphs.
Results: The FT3 assay Passing-Bablock results had an intercept of –0.09625, the confidence interval including zero, the slope of 0.9507, the confidence interval including 1.0. The Bland-Altman comparison calculated a mean difference of –0.031, the limits of agreement containing 0.0. The FSH Passing-Bablock value for intercept was 0.002609, the confidence interval included 0.0, the slope value of 1.048 has a confidence interval of 1.023-1.065. The Bland-Altman mean difference of 0.940 had limits of agreement of –2.719 and 4.599.
Conclusion: The results obtained through method comparison analysis suggest that both the updates to the assay kits have not had any major effect and will not require changes to the method of interpretation. The updates FT3 and FSH assays are deemed fit for use within the Timaru Med Lab south biochemistry department.

Assessing the effect of delay between incubation and interpretation on the reliability of Lewis Leb phenotyping performed using Immulab Epiclone Anti-Leb antisera

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Objectives: Reliable antisera is essential to accurately phenotyping red cells. Dunedin NZBS Blood-Bank found one batch of Immulab Anti-Leb antisera produced ambiguous gritty reactions, leading to a false-positive Leb phenotype reported to clinicians. The aim of this study was to assess the effect of delay between incubation and interpretation on the reliability of Leb phenotyping performed using Immulab Anti-Leb antisera.
Methods: Four Le(b+) and four Le(b-) samples were phenotyped using Lorne Anti-Leb antisera. Positive and negative controls were selected from Grifols Perfect Screen 3 cell panel. Samples and controls were phenotyped using Immulab Anti-Leb antisera, tube method. Incubation times represented increasing delay: T=15 minutes (validated incubation time), T=16, T=18, T=20, T=22 and T=24 minutes. Samples were centrifuged immediately and reaction strength interpreted using the 0=negative to 12=positive grading scale. Grades 3 and 5 are weak positive.
Results: The T=15 incubation with immediate interpretation produced accurate phenotypes for all samples and controls. Reaction strengths of Le(b+) and Le(b-) samples and positive control trended upwards with increased incubation time, whilst accuracy of Le(b-) phenotyping decreased. At T=16, two Le(b-) samples graded 3. At T=22 all Le(b-) samples graded 3 or 5. At T=24 one Le(b-) sample graded 8, a positive reaction, and the negative control graded 3.
Conclusion: The results suggest Immulab Anti-Leb antisera is only reliable at a strict 15-minute incubation, with immediate centrifugation and interpretation. Delays of 1 minute or more produced results of varying accuracy, which could cause inaccurate Le(b-) phenotyping in blood banks. Care should be taken by scientists to time the incubation carefully and eliminate delay when performing centrifugation and interpretation.

Method validation of the Roche Cobas u411 analyser

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Objectives: The Roche Cobas u411 is a semi-automated, reflectance photometry-based urinalysis device designed to determine various urine analytes, using Roche dipsticks. These semi-quantitative measurements are useful in the screening and diagnosis of patients for kidney and genitourinary diseases. This study aimed to validate the analytical performance of the Cobas u411 for use at Wairarapa Laboratories.
Methods: This method validation involved an investigation into the precision and accuracy of the Cobas u411. Two levels of the Bio-Rad Liquichek Urinalysis control material were used to determine within-run and between-run precision. Accuracy was

evaluated in a comparison conducted against the Roche Urisys 1100 analyser with patient samples (n=60). As the laboratory solely acts on abnormal leukocyte content, a subset of urine samples (n=12) was used to further explore the accuracy of the Cobas u411 against the Sysmex XN-Lite analyser.
Results: The coefficients of variation for within-run and between-run precision ranged from 0 – 26% and 0 – 13.5%, respectively. The level of discordance between the Cobas u411 and the Urisys 1100 was ≤ 5% for all parameters, demonstrating a strong agreement between analysers. Further investigation into leukocyte content showed strong correlation between the Cobas u411 and the Sysmex XN-Lite analyser, with a Spearman's correlation coefficient value of 0.8456.
Conclusion: The results of this method validation indicate that the Cobas u411 analyser demonstrates satisfactory analytical performance and has the potential to greatly increase both efficiency and consistency of urinalysis procedures. Thus, this analyser can be confidently recommended to Wairarapa Laboratories to replace the current Urisys 1100.

Evaluation of the effects of centrifugation on samples for complete blood count analysis

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Objectives: A complete blood count is an important routine haematological test in the assessment of a patient's general health. Current laboratory protocol calls for the rejection of spun specimen samples intended for complete blood count analysis. Therefore, the aim of this study was to evaluate the effects centrifugation has on whole blood, focusing on MCV, Hb, WBC and Plt and whether it was suitable for analysis and reporting after centrifugation had taken place. Morphology was also looked at to see if there was a discernible difference.
Methods: Whole blood specimens with reported normal, high and low MCV, Hb, WBC and Plt were selected. The specimens were subsequently centrifuged, then re-suspended by rocking gently back and forth and re-analysed on the XN analyser. The pre and post centrifugation values were compared and evaluated for differences using statistical analysis.
Results: In most cases the difference in pre and post centrifugation values were statistically significant (p-values <0.05). The MCV, WBC and Plt values decreased and Hb value increased post-centrifugation. Although the differences were statistically significant, they were not clinically significant.
Conclusion: Contrary to current laboratory protocol, specimen centrifugation does not clinically affect MCV, Hb, WBC and Plt levels and would not change the overall management of a patient's condition. Therefore, laboratory protocol that calls for the rejection of spun whole blood specimens intended for complete blood count analysis should be revised. Re-suspension of centrifuged whole blood samples can be processed for complete blood count analysis without compromising patient results.

Comparison of D-dimer controls for diagnostic use

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Objectives: The purpose of the investigation was to investigate the validity of the Bio-Rad LiquidChek D-dimer controls and compare their performance to the Siemens D-dimer control material currently used, to help maintain the quality standards across both Taranaki Pathology Services and Wellington SCL.
Methods: The two levels of Siemens D-dimer controls were run every day for two weeks alongside the four levels of Bio-Rad D-dimer controls. The setup of these controls was performed following the laboratory protocol. There are 2 different levels of the Siemens controls and 4 levels of the Bio-Rad controls. The performance and the price of the two controls was assessed.
Results: The Bio-Rad D-dimer controls were found to have the best performance, having low variability and more clinical significance. However, Siemens D-dimer controls were found to be significantly more cost effective.
Conclusion: Performance and cost are not the only important things to consider when comparing controls. Other factors which influence which of these D-dimer controls will be better for TPS includes, controlling clinically relevant values, handling requirements, stability, and the peer group size of others who are using the same controls for troubleshooting and comparison. Another factor to consider is that the Siemens controls are assayed controls, while the Bio-Rad controls are not. Overall, the D-dimer control which is most suited to TPS depends on the priority of the laboratory. It mostly comes down to cost versus performance, as considering all other factors results in an equal comparison of the two controls.

Evaluation of the AusDiagnostics 16-well Viral and Syphilis panel for use at SCL Dunedin

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Objectives: Recently, there has been a worldwide monkeypox outbreak. In response, AusDiagnostics has developed a 16-well Viral and Syphilis PCR panel which includes monkeypox and other similar-presenting pathogens, such as syphilis and enterovirus. The aim of this study was to verify and validate targets of interest on this panel and validate extraction-free testing to evaluate the feasibility of using this panel at SCL Dunedin.
Methods: Forty-four samples were selected to detect targets of interest; of particular interest were samples of sexually-transmitted infections. Each sample was run extracted and unextracted. Samples were extracted on the MT-Prep. Paired unextracted samples were run using the Viral and Syphilis panel on the High-Plex, a multiplex tandem PCR system. Results of extracted and unextracted runs were compared to diagnostic testing to assess concordance.
Results: There was strong concordance between diagnostic testing and extracted samples on the 16- well panel. This concordance was less when comparing diagnostic testing and unextracted samples, mostly because all known syphilis samples had an inhibited SPIKE sequence. Take-off values were generally lower for extracted samples compared to diagnostic testing and unextracted samples. However, the viral panel currently used for diagnostic testing is run unextracted, and diagnostic testing and unextracted samples had similar take-off values.
Conclusion: Our findings highlighted the advantages of using syndromic panels in testing for monkeypox, as known monkeypox-negative samples returned positive results for enterovirus and syphilis. While the targets were able to be verified and validated, we were unable to establish whether extraction-free testing could be performed. Further testing on the effect of universal transport media on SPIKE inhibition, particularly on syphilis samples, is planned to assess this suitability.

Validation of the Xpert MRSA SA/BC Assay on GeneXpert and comparison to culture-based method

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Objectives: *Staphylococcus aureus* is one of the most common organisms responsible for bloodstream infections in hospitals and

the community. In 1959, the drug methicillin was first introduced as an agent to treat staphylococcal infections. Consequently, methicillin-resistant *S. aureus* strains (MRSA) were identified in 1960. Numerous nosocomial and community-acquired infections are caused by MRSA, which results in significant morbidity and mortality. This research project aimed to validate a new Xpert-MRSA-SA-BC assay on the GeneXpert and compare results with current culture-based methods.

Methods: A total of 40 positive blood cultures containing gram-positive cocci were collected, this included 10 MRSA, 10 methicillin-susceptible *S. aureus* (MSSA), 10 coagulase-negative methicillin-susceptible Staphylococcus spp., and 10 coagulase negative methicillin-resistant Staphylococcus spp. Blood cultures for this research were obtained by either in-patient collections or mock (spiked) samples. Whole blood samples were processed on the GeneXpert, a real-time PCR instrument that detects the presence of MRSA genes mecA and spa. The GeneXpert MRSA assay reports the results one hour following sample loading onto the analyser.

Results: The GeneXpert MRSA SA/BC assay correctly identified all methicillin-resistant and susceptible strains of *S. aureus* and did not provide false positive results for any coagulase negative staphylococci.

Conclusion: The GeneXpert MRSA SA/BC assay can rapidly and accurately detect the presence of MRSA and MSSA in blood cultures. Implementation of this assay would significantly benefit the turnaround time of results and improve patient treatment regimens to overall positively impact patient outcomes.

Method comparison of faecal elastase-1 test between Schebo and Liaison

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Objectives: Faecal elastase-1 is commonly recommended for the diagnosis of pancreatic exocrine insufficiency due to its high sensitivity and specificity. It is currently performed by Schebo ELISA kit in the Wellington immunology department. We are looking to transfer this test to the Dunedin immunology department and run it on the Liaison analyser. The correlation and agreement between these two methods should be checked.

Methods: Stool samples were supplied by Wellington laboratory. Sixty-two frozen samples were extracted by Liaison Q.S.E.T. device plus method and the faecal elastase-1 levels were re-tested by Liaison analyser at Dunedin Immunology department. The linear relationship and agreement between the results from Liaison and Schebo were analysed by Pearson correlation, Passing-Bablok regression and Altman-Bland percentage plot analysis.

Results: The faecal elastase-1 results from Liaison and Schebo were statistically different. However, the r-value from Pearson analysis was 0.91, which indicates a good linear relationship between the results from the two methods. Passing-Bablock regression analysis demonstrated good comparability within the investigated ranges. The results from LIAISON were generally 0.98% lower than those from Schebo, but the Altman-Bland percentage plot analysis demonstrated an excellent agreement. The lowest agreement occurred in the group of mild to moderate pancreatic insufficiency.

Conclusion: The faecal elastase-1 results from Liaison correlated well with Wellington Schebo's results. It is suitable to introduce the faecal elastase-1 test to the Dunedin immunology laboratory.

Validation Performance of the Roche Free T4 IV Assay

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Objectives: In Southern Community Laboratories, Dunedin, free T4 patient measurements are performed using the Electrochemiluminescence Immunoassay which utilizes a Free T4 Assay. The free T4 assay refers to the measurement of the unbound T4, for the assessments of thyroid disorders. Recently, Roche has released a new generation of the Free T4 Assay which is noted to have an increased tolerance to biotin.

Objectives: The objectives of the study were two-fold: (1) evaluating the correlation in Free T4 results between the Roche FT4 III and FT4 IV assay and; (2) evaluating the precision in Free T4 results using the FT4 IV assay

Methods: For the correlation study, patient samples with free Thyroxine results were selected. The 42 samples with previous free T4 results were rerun with the new Free T4 IV assay. Both parametric and non-parametric statistical tests were used to assess if there was any significant difference in free thyroxine results between the assays. Biorad 1+ and 3+ controls were rerun 15 times each to generate the coefficient of variation for assessing the ability of the Free T4 IV assay to produce precise free Thyroxine results.

Results: For correlation analysis, both parametric and non-parametric tests performed showed there was a significant difference in free thyroxine results between the assays. For precision analysis, coefficient of variation results showed the assay executed precise results.

Conclusion: Based on the correlation results, there was a significant difference in reporting free thyroxine results. However, the difference was not clinically significant according to the Royal College of Pathologist Australasia guidelines. Therefore, the assay was validated for routine use, as it also produced precision results satisfactory to the laboratory standards.

A comparison of the HIL check performed on the ACL TOP 750 LAS analyser with a manual method for routine coagulation tests

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Objectives: To determine if the set haemolysis icterus lipaemia (HIL) check on the ACL TOP 750 LAS analyser is in line with the current manual method using the Plasma Haemoglobin comparator so that the use of the HIL check can be validated for routine coagulation tests.

Methods: Samples of varying haemolysis were run on the ACL TOP 750 LAS analyser using optical-based methods to assess when the HIL alert would trigger to determine a sample as unsuitable for testing. The results were compared to the Plasma Haemoglobin comparator, a manual method used by Pathlab, New Zealand. A lipaemia icterus haemolysis (LIH) check was performed on the Beckman Coulter AU5800 analyser using optical-based methods to confirm the validity of the results.

Results: Overall, the HIL check on the ACL TOP 750 LAS analyser is more accurate at interpreting haemolysis than the manual method using the Plasma Haemoglobin comparator. The manual method determined 17 samples as being unsuitable for testing compared to both the HIL check and LIH check which determined 7 of those 17 samples as being unsuitable for testing, with the ACL TOP LAS analyser and Beckman Coulter AU5800 analyser having the same haemolysis interpretations. Some mildly haemolysed samples did not trigger the HIL alert despite the manual method interpreting them as being unsuitable for some tests. Icteric and lipaemic samples were not included in this study as all samples lacked significant levels of icterus and

lipaemia.
Conclusion: The HIL check on the ACL TOP 750 LAS analyser was valid for use for routine coagulation tests. The validity of the ACL TOP 750 LAS analyser was confirmed by the results of the Beckman Coulter AU5800 analyser. The manual method was more likely to interpret samples as grossly haemolysed.

Diabetic foot wounds and *Corynebacterium striatum*: a look into laboratory protocol

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Objectives: To investigate outcomes of a protocol change to no longer anaerobically culture diabetic foot swabs in a diagnostic microbiology laboratory. Secondly, to determine if reports of *Corynebacterium striatum* from tissue biopsies was increased in 2022.
Methods: Since diabetic foot swabs were not cultured anaerobically after December 2021, tissues received 6 months before and after the change were compared to determine if the number taken from diabetic feet had altered. Secondly, reports of *C. striatum* isolated from tissues in 2021 and 2022 were compared to see if there was a statistically significant increase following the swab protocol change.
Results: There were 961 total tissues taken in 2021, 167 (17.4%) from diabetic patients and 50/167 (29.9%) from diabetic foot wounds. In 2022, the overall tissues taken increased to 1056, and tissues from diabetic patients increased to 213 (20.2%). Tissues from the feet of diabetics also increased to 96/213 (45.1%) in 2022. In terms of *C. striatum*, there were 10 reported in 2021, 5 of which were in diabetic tissues. There were 25 reports of *C. striatum* in 2022, with 11 occurring in diabetic tissue biopsies.
Conclusions: Following the protocol change, the number of tissues taken from diabetic foot wounds significantly increased ($P<0.05$). This is the desired outcome of the protocol change as tissues provide superior anaerobic cultures to swabs. Reports of *C. striatum* in diabetic tissues was not significantly increased in 2022, but reports from all other tissues were increased ($P<0.05$). This means that the increase in *C. striatum* was likely not due to the increase in diabetic tissues received, but from another cause. Further investigations are required to determine if this may be clinically significant.

Implications of one-point calibration assay on amphetamines samples

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Objectives: Amphetamines are psychostimulant drugs used to increase electrical impulse velocity between the brain and the peripheral nervous system. Amphetamine assays are required for the court system and workplaces. This research project aimed to investigate the implications of installing one-point calibration assay and how it affects the patient results when compared to a semi-quantitative assay or six-point calibration. The reagents and calibrators are the same but only the number of calibration points used was different.
Methods: 30 random amphetamine samples were collected from freezer M. The primary urine samples were thawed and centrifuged for 5 minutes. A generous amount of urine is aliquoted into the labelled test tube. The samples are loaded on a urine rack and the appropriate test is selected. AM3Q2 is the one-point calibration and AM3S2 is the six-point calibration. The positive results are sent to LCMS for confirmation. A random amphetamine patient sample was run 10 times on one- and six-point calibration to investigate precision.
Results: The analysis depicted a strong parallel between the quantitative and qualitative assays. The qualitative and quantitative assays have a sensitivity of 75% and a specificity of 100%. Both assays can detect 75% of the amphetamine patient sample but the remaining 25% are undetected leading to false negatives. The precision CV of 6-point calibration is 12.5%- and 1-point calibration is 1.96% indicating better precision for the one-point calibration.
Conclusion: From the findings, there are no negative implications of installing a one-point calibration assay since both qualitative and quantitative assays share the same specificity and sensitivity. Consultation with clinicians is required on how the assay is interpreted.

Clinical validation of a real-time PCR for HLA typing of Coeliac disease

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Objectives: Coeliac disease has a studied relationship with the HLA alleles DQB*02 and DQB*03:02, with the absence of both allowing Coeliac disease to be ruled out during diagnosis. Validation of a high-throughput qualitative real-time PCR will allow for fast and sensitive screening of patients for these HLA markers. Our objective is to validate this real-time PCR for clinical use on a new analyser, transitioning it from an outdated system.
Methods: A qualitative real-time PCR using SYBR green melting curve analysis was performed for 53 patient samples. Each was run on the previously validated Roche LightCycler 2.0 and the Roche LightCycler 480 using separate probes and cycles conditions for the two alleles. A dilution series of positive controls was performed to compare the limits of detection for the PCR on both analysers.
Results: The Roche LightCycler 480 was found to have the same limit of detection and level of sensitivity as the previously validated Roche LightCycler 2.0 method using the same PCR programme and reagents. The PCR showed 100% sensitivity and specificity in detection of HLA alleles DQB*02 and DQB*03:02.
Conclusion: Transition of the PCR to the Roche LightCycler 480 will not result in a decrease in sensitivity, specificity or limit of detection compared to the Roche LightCycler 2.0 and is suitable for clinical use. In addition, the number of samples that can be handled in a single run is increased from 36, on the LightCycler 2.0, to 96 on the LightCycler 480. Thereby reducing turnaround times and making the workflow more efficient.

Methodological comparison of enhancement using various tube techniques for weak/low reverse blood groupings

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Objectives: ABO typing is a simple serological test to determine an individual's blood group, requiring the reverse group to confirm the ABO forward grouping. Anomalous reactions may occur in the reverse group when a weak/missing reaction is seen and can be caused by patient factors or technical errors. The aim of this study was to determine the best and quickest tube method to enhance the ABO reverse groupings of low grading reverse groups found by Grifols Erytra Eflexis.
Methods: The main enhancement method currently used by NZBS is leaving the samples at room temperature for between 15 minutes and one hour. Twenty patient samples were flagged by Grifols Erytra Eflexis as requiring further testing and validation of weak or missing reverse group grading. Manual microcolumn

cards were tested on 18 samples to determine whether Grifols Erytra Eflexis was at fault. Methods of tube techniques used for enhancement were increase in centrifugation time, cold incubation and increase in plasma concentration.
Results: All three tube techniques gave good enhancement gradings compared to results from Grifols Erytra Eflexis. Increased centrifugation and increased plasma concentration gave better results than cold incubation.
Conclusion: The tube method is the gold standard of transfusion medicine and is a valuable technique for resolution of blood grouping anomalies observed in column technologies. All three tube techniques were good methods for enhancement of low reverse groupings. Increase in centrifugation and increase in plasma concentration provided best results.

Evaluation of CHROMagar and Mannitol Salt Agar for MRSA detection

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Objectives: The MedLab South Microbiology Department at Nelson hospital uses mannitol salt agar (with 4 µg/m oxacillin) for methicillin resistant *Staphylococcus aureus* (MRSA) screening, while most other laboratories around New Zealand use a chromogenic agar. This study aimed to compare the recovery of MRSA on mannitol salt agar with a MRSA chromogenic media.
Methods: A total of 12 known MRSA samples were provided and then re-confirmed with cefoxitin resistance. A 0.5 McFarland standard was prepared and then diluted 1:100 to be inoculated onto both the mannitol salt and chromogenic agars. A colony count was then done after 24 and 48 hour incubation to compare MRSA detection.
Results: From the 12 MRSA samples, 1 was deemed non MRSA as it was sensitive to cefoxitin but it was still inoculated which it showed no growth on both plates. Of the other 11 samples, 5 were identified on both plates within 24 hours of incubation, the remaining 6 were identified on the chromogenic media but not the mannitol salt. These 6 samples were later detected after 48 hours of incubation. A non-diluted 0.5 McFarland was then also inoculated for these 6 samples but only 1 of these samples was detected after 24 hours while the other 5 still only after 48 hours on mannitol salt.
Conclusion: The present study highlights that MRSA recovery was improved with a chromogenic agar compared to mannitol salt and from this study, Nelson microbiology has already begun transitioning to using the chromogenic agar for improved detection of MRSA.