Abstracts for the Semester 1, 4th year Otago BMLSc student research projects

Analysis of platelet use over a one-month period at Wellington Hospital

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Objectives: The aim of this study was to analyse patterns of platelet usage in Wellington Hospital (Capital and Coast) and to encourage further study into certain aspects of platelet use. The usage of platelets was also assessed with regard to the New Zealand transfusion guidelines to determine if the indications for platelet use were met.
Methods: Data was collected from Wellington Blood Bank of all platelet issues from the 17th of January 2019 until the 17th of February 2019. Repeat issues where a unit was returned and then reissued to a different patient were discounted, to make the data more reflective of actual platelet use. The pre-transfusion count was assessed with regard to patient condition to determine if the request for a transfusion was appropriate according to the NZ guidelines. A range of other factors were also assessed to give a more holistic view of platelet use.
Results: There were 187 issues of platelets which could be analysed within the specified time period. Twenty-seven units were used for cardiac surgery patients and there was not enough information available to determine if these issues were appropriate. Of the units that could be assessed, 80% were deemed to have been used appropriately. This is consistent with previous studies. The mean platelet increment pre and post transfusion was 12 x10^9/L. Of the platelets used, 48% were given to Haematology patients; 24% to Intensive Care/ Haematology patients and 14% were used for Cardiac Surgery indications. The remaining 14% were used by other departments within the hospital. The study was limited by the fact that not all indications for using platelets could be assessed.
Conclusion: Seventy-nine per cent is a good proportion of appropriately used platelets when factoring in the incomplete analysis of cardiac surgery and some other cases; the true value is likely to be significantly higher.

Clinical stability of K-EDTA plasma ammonia, stored at -20°C and then transported to a central laboratory for analysis

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Objectives: Due to in vitro deamination, the stability of ammonia samples is frequently questioned. Smaller laboratories are often unable to measure ammonia, and instead separate, freeze, and transport samples to a larger laboratory for analysis. The aim of this study was to determine if peripheral laboratory EDTA ammonia samples are clinically stable when they reach the central laboratory.
Methods: A mixture of 25 inpatient and outpatient samples were used to assess the stability of ammonia. Each sample was centrifuged, with the plasma being divided into 7 Hitachi cups. One cup was used to obtain a baseline measurement. The other 6 were stored at -20°C for 24, 48, or 72 hours, after which they were left to stand in an ammonia transportation container for either 1 or 5 hours. Ammonia concentrations were measured on the Cobas c 502 analyser, using an enzymatic method. Bias was calculated by comparing each measurement to the baseline value. Clinical significance was determined by comparing the bias to assay performance criteria established by the Royal College of Pathologists of Australasia.
Results: The average bias did not exceed the allowable limit of performance after 24, 48, and 72 hours of storage at -20°C, for both 1 and 5 hours of transportation. No clear trend existed between the storage time at -20°C and the average bias.
Conclusion: Peripheral laboratory EDTA ammonia samples are clinically stable for up to 72 hours of storage (-20°C) and then, for up to 5 hours of transportation.

Determining the origin of contaminating DNA using QF-PCR: A case report

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Objectives: Genetic testing that cannot be undertaken in New Zealand is referred to overseas reference laboratories. A female sample sent to an international laboratory for whole exome sequencing showed an abnormally large number of variants (n = 46,704 variants) compared to previously observed exome studies ($n \approx 39{,}000$). A large number of variants can be indicative of chimerism, mosaic polyploidy or sample admixture. **Methods:** Chimerism, polyploidy and sample admixture can all be elucidated by quantitative fluorescence- polymerase chain reaction (QF-PCR). QF-PCR was performed by amplification of microsatellite markers using fluorescent-labelled primers, followed by quantitative analysis of the allele peaks on a genetic analyser. A multiplex of 22 primer pairs for loci on each of chromosomes 13, 18, 21, X, and Y was used (Q-STRplusv2, Elucigene, UK).
Results: QF-PCR analysis showed the original DNA sample stored at Canterbury Health Laboratories had a normal chromosome complement, ruling out chimerism or polyploidy as disease mechanisms. Further, the stored DNA was not contaminated, eliminating the DNA extraction process as a source of the potential DNA admixture. The contaminated sample held by the overseas laboratory was repatriated for further analysis, and appeared to have alleles from at least three individuals (of both female and male origin). Sixteen additional samples were sent away to international laboratories the same day this sample was dispatched. These samples were also analysed by QF-PCR and all of them had unique alleles which were not shared with the admixed sample. **Conclusion:** A thorough review of the Sendaway and DNA extraction processes within our laboratory was undertaken and we concluded that sample admixture was unlikely to have occurred at Canterbury Health Laboratories. In the event that a similar case may arise in future, a protocol for investigating potential sample contamination has now been established within our laboratory.

Verifying EUCAST rapid antimicrobial susceptibility testing (RAST) method direct from positive blood cultures

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Objectives: In November 2018 the European committee on antimicrobial susceptibility testing (EUCAST) released a novel rapid antimicrobial susceptibility testing (RAST) method directly from positive blood culture bottles. The aim of this project was to ratify the RAST method and evaluate the potential for implementation into the laboratory.
Methods: Fifteen patient isolates of *Staphylococcus aureus, Escherichia coli* and *Streptococcus pneumoniae* were inoculated (100–200cfu/mL) into blood culture bottles containing fresh human blood. Bottles were sampled following positive signals from the BD BACTEC system and inoculated onto appropriate media for the disc susceptibility test. Growth inhibition zones were read at 4, 6 and 8 hours with recording fields left blank if zones were unreadable and the retrospective data compared to standard disk diffusion. QC evaluation with 11 staff members and three control microorganisms was also carried out.
Results: The QC evaluated 11 participating staff results, with a passing rate (within the target range) at 4 hours incubation ranging from 47.3-72.7%, 86.4%-100% at 6 hours and 96.6-100% at 8 hours incubation. The categorical agreements for the retrospective study ranged from 35-75% at 4 hours and 90-100% for both 6 and 8 hour incubations, with no very major errors and 5 major errors (2.2%).
Conclusion: The performance of this method improved with longer incubation, with the poor performance of 4-hour incubations partly attributed to the organism growth being too light to accurately read zones. The implementation of this method at 8 hours would provide a lowered turn-around time of 10 hours with the highest degree of accuracy, potentially reducing the likelihood of treatment failure and mortality in sepsis patients. However, careful consideration regarding workflow is recommended before implementation, particularly with the requirement of rapid identification in tandem with this method.

Mismatch repair proteins: A comparison of immunohistochemistry staining quality between Ventanna and Dako manufacturers

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Objectives: The mismatch repair protein panel (MMR panel) is an immunohistochemical staining panel used for the diagnosis of Lynch syndrome. As a disorder characterised by the loss of mismatch repair genes, it is responsible for 15% of colorectal cancer cases worldwide. Cells with malfunctioning mismatch repair genes have an increased chance of proliferating uncontrollably without being able to repair DNA replication errors. The aim of this study was to analyse the staining pattern of two manufacturer's antibodies in the immunohistochemical panel screening for this disorder, Ventanna and Dako.
Methods: Antibodies included in this panel were MLH1, MSH2, MSH6 and PMS2, stained on positive control tissues including tonsil, appendix and colorectal adenocarcinoma. Two slides were assigned to each antibody – a low risk adenocarcinoma with normal MLH1 and PMS2 antigen expression and a high-risk adenocarcinoma with low antigen expression. The quality of the stains was assessed based on intensity of nuclear staining, specificity of staining and presence of sub-optimal staining features. A qualitative score between 1-5 was assigned to each slide from 5 different individuals (pathologists and scientists). **Results:** The results showed that the average score for Dako antibodies was higher than the corresponding Ventanna ones. The difference of average score between Dako and Ventanna MSH2 was low, with n=4.2 and 4.4 for Dako and n=4 and 4.4 for Ventanna. This shows that the staining quality between these 2 antibodies is very similar on average.
Conclusion: The conclusion of the study was that Dako antibodies were superior for MLH1, MSH6 and PMS2, whilst differences in quality in MSH2 was negligible.

An evaluation of the QBC Star dry haematology analyser for point-of-care use

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Objectives: QBC Star is a point-of-care haematology device designed to give results for nine common blood parameters on a venous or capillary whole blood sample. These include Haematocrit, haemoglobin, total white cell count, MCHC, granulocyte count (absolute and %), lymphocyte + monocyte count (absolute and %) and platelet count. The aim of this study was to assess the comparability between QBC Star and Sysmex XN analysers for eight of these parameters. The effects of platelet clumps, alpha thalassaemia and lipaemia on QBC star performance was also investigated.
Methods: Comparability of the QBC was assessed by comparing 33 K2EDTA samples on both the Sysmex XN analysers and QBC Star, Results were analysed using Bland-Altman and Passing-Bablok statistical analysis.
Results: The results showed that the two methods were comparable for the haematocrit, haemoglobin and platelet parameters. The lymphocyte + monocyte absolute count was not comparable. Two samples with platelet clumps which were run on the XN analysers and subsequently tested on the QBC Star showed normalisation of the platelet count suggesting that the QBC Star was not affected by clumps to the same degree as the XN analysers. Alpha thalassaemia and lipaemia did not appear to affect the comparability of the two analysers. Imprecision for QBC Star was assessed and was adequate for the platelet count. Total white blood cell count precision was above the manufacturer's stated range.
Conclusion: The QBC Star dry haematology analyser provides excellent comparability with the Sysmex XN analysers for the haematocrit, haemoglobin and platelet parameters. It appears to provide a more accurate platelet count in samples with platelet clumps when compared with Sysmex XN analysers. Lipaemia and thalassaemia did not appear to affect comparability of QBC Star results but the sample size was small. Patients requiring an accurate WBC count and differential would benefit from testing on Sysmex XN analysers.

Instrument comparison of the B-type natriuretic peptide (BNP) assay between the Abbot Architect and the Abbot Alinity analysers

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Objectives: At Canterbury SCL the immunoassay analyser, Abbot Architect i2000SR was being replaced by an updated immunoassay analyser, Abbot Alinity i-series. The aim of this study was to evaluate the analytical performance of the BNP assay on the Alinity by comparing it against the established instrument, Architect.

Methods: Sixty-three BNP samples were collected 3 weeks before the comparison. A broad range of BNP samples were collected across the clinically significant range of 2.9 - 1000 pmol/L. The samples were stored and frozen. Before the comparison, the samples were thawed at room temperature, vortexed, and centrifuged. The samples were then manually ordered on both instruments and statistical analysis was performed on the Analyse-it programme in Excel.
Results: The Alinity ran BNP controls (3 levels) multiple times and had a coefficient variation (CV) of 3.27 (1st level), 4.76 (2nd level) and 1.46 (3rd level). SCL Architect vs SCL Alinity had a correlation of y = -4.569 + 0.8856 x. The instruments had a mean difference of -20.28 and a standard deviation of 26.58. SCL Alinity and 2 CHL Architect comparisons were performed. The correlation was y = -2.354 + 0.9971 x and y = -5.853 + 1.049. The mean difference was -1.42 and -2.61. The standard deviation was 9.30 and 4.25.
Conclusion: The Architect BNP controls were running erroneously in the lead up to the comparison. The comparison was done knowing that the Architect was producing abnormal controls, to ensure it was not just a QC problem. The statistical analysis proved that not only was it a QC problem, but it also affected patient samples. The pathologist did not sign off the first comparison as the reference method (SCL Architect) was producing erroneous results. The pathologist and the biochemistry head of department decided that we should compare our Alinity BNP assay against two of the Canterbury Health Laboratory (CHL) Architects as a reference method. The comparison was much improved, therefore, the Alinity was authorised by the pathologist for clinical laboratory testing. A few weeks later, Abbot determined that there was an issue with multiple lot numbers of calibrators and controls that were utilised on the SCL Architect during the instrument comparison. Abbot requested an urgent Medical Device Recall for specific lots of the Architect BNP calibrators and controls due to a time dependent, stability drift in patient and control results that has been identified.

Comparison of culture techniques for diagnosis of prosthetic joint infection

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Objectives: The aim of this study was to compare the sensitivity, specificity, and speed of culture techniques for diagnosis of prosthetic joint infection (PJI). Traditional techniques using agar plates and cooked meat broth were compared to an alternative culture technique using blood culture bottles.
Methods: Periprosthetic tissue and fluid specimens were sampled from patients undergoing revision surgery for suspected PJI, and inoculated onto agar plates, into cooked meat broth, and into aerobic and anaerobic blood culture bottles. Media were incubated for a set number of days and monitored for growth, with blood culture bottles incubated in and monitored by the BD BACTEC blood culture system. All microorganism(s) were identified from direct agar plates or from sub-culture onto agar plates by MALDI-TOF-MS. Infectious Diseases Society of America (IDSA) criteria for PJI were used as the gold standard for true infection, to determine the sensitivity and specificity of each media type.
Results: Over the 50-day period, at Wellington Southern Community Laboratories, 17 patients met the study criteria. Seven were determined to have a true infection by IDSA criteria. The sensitivity of blood cultures was 67%, and specificity 86%. The sensitivity of direct plates and cooked meat broth was 58%, and specificity 86%. Blood culture bottles demonstrated a median of two days for microorganism(s) to be observed on agar plates, and the same for direct plates and cooked meat broth.
Conclusion: The blood culture method had increased sensitivity for culture of bacteria causing PJI with no loss of specificity compared to direct plating and cooked meat broth. This method may be attractive to laboratories as it does not require daily reading of multiple plates and manual subculture of enrichment broth.

Effects of storage on spun citrated blood sample prothrombin times and international normalised ratio test results

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Objectives: The logistics of transporting off-site samples creates unavoidable delays in the testing of these samples that could affect the accuracy and precision of any coagulation test results produced. This study was performed to review the accuracy and precision of delayed coagulation tests. **Methods:** Fifty random citrated blood samples were obtained over a three-week period. Ten INR tests with Innovin were performed on each sample over a period of three days. An initial prothrombin time and international normalised ratio was obtained within 4 hours of sample collection. The samples were stored upright at room temperature from the initial test to the last. Samples were not resuspended but were stored as centrifuged samples. Subsequent delayed results were obtained at 5, 6, 8, 26, 29, 32, 50, 53 and 56 hours after the sample collection time.
Results: The mean INR difference after 56 hours was 0.05 INR units (2.5%) and no INR result exceeded the analytical performance specifications set by the Royal College of Pathologists of Australasia within the three days of testing. Prothrombin times had a few results outside of allowable limits within the lower numbers.
Conclusion: There was no clinically significant change in INR results when testing was delayed for three days if the Royal College of Pathologists of Australasia guidelines were used. However, when expected precision standards set in-house were used for comparison, the results exceeded the limits set by the stricter guidelines.

The use of automated complete blood counts to screen for myelodysplastic syndrome

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Objectives: Myelodysplastic syndrome involves the proliferation of a clonal myeloid lineage, causing ineffective haematopoiesis (pancytopenia). Recent literature has recommended installing an MDS-CBC screening score on automated haematology analysers, providing a decisive method to stratify patients with high probability of having the disease. The objective of this study was to validate the Myelodysplastic syndrome-complete blood count (MDS-CBC) screening score established in: Boutault R, Peterlin P, Boubaya M, et al. A novel complete blood count-based score to screen for Myelodysplastic syndrome in cytopenic patients. *British Journal of Haematology* 2018; 183: 736–746.
Methods: Retrospective evaluation of 109 samples (patients n=22) diagnosed with myelodysplastic syndrome from January to March 2019 were compared to a 'normal' cohort (n=53). Confidence intervals of 95% from the normal results were used for interpretation. MCV, neutrophil count and Ne-WX values were used to calculate each score; from this, the screens' ability to differentiate patients with myelodysplastic syndrome from normal results was determined.

Results: The MDS-CBC score had a sensitivity of 81% and specificity of 100% regarding the recommended threshold (0.2). High specificity identifies truly negative results in a mixed population, positive scores are probably caused by myelodysplastic syndrome and should be referred for further investigation. Falsely negative scores increase consequently; it is, however, a slow progressing disorder and will likely become positive in time without risk of rapid morbidity/mortality.
Conclusion: The MDS-CBC score is a useful tool when screening for myelodysplastic syndrome against normal samples, prioritizing specific investigation to patients with a higher probability of having the disease. This could improve the rate and efficiency of diagnosis, treatment, and monitoring, thus enhance patient outcome.

Evaluation of the cut off value used in the identification of antibodies to extractable nuclear antigens

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Objectives: Screening for antibodies to extractable nuclear antigens (ENA) is used to assist in the diagnosis of connective tissue diseases and systemic autoimmune rheumatic diseases. The ENA screen is performed on all patients with an antinuclear antibody (ANA) titre ≥160. Samples above the ENA cut off value of 20 units undergo ENA identification testing with an enzyme immunoassay (EIA). Currently further confirmatory line blot testing is needed if there are no positive antigens detected by the EIA identification. The aim of this investigation was to assess whether ENA screen results between 20 and 50 units with a negative ENA identification EIA will require a confirmation line blot test
Methods: Thirty patient samples were selected, the majority being between the current cut-off of 20 and 50 for the ENA screen. All samples were screened for ENA antibodies with the Bio-Flash chemiluminescence assay followed by the confirmatory Biorad EIA and Euroimmun line blot.
Results: In the confirmatory testing for the samples with an ENA screen value between 20 and 50, there were 15 negatives in both confirmation tests. There were seven confirmation positives and four samples which were negative in the Biorad enzyme immunoassay but positive in the Euroimmun line blot. None of these four positives were of clinical significance.
Conclusion: From this data collected it has been shown that a negative ENA identification EIA with a positive screen between 20 and 50 would not need confirmatory line blot testing.

Verification for qPCR Adenovirus DNA detection in plasma samples

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Objectives: To ascertain whether the Adenovirus R-gene® commercial kit was suitable for diagnosing Adenovirus disseminating disease and the monitoring of antiviral treatment. Methods: A standard curve was set up, as recommended by the manufacturer, in triplicate to ascertain precision and accuracy for quantitative PCR. External quality control samples from Quality Control for Molecular Diagnostics were run in triplicate to ascertain intra-assay and inter-assay performance. Statistical analysis was performed in R-studio.
Results: Percentage of error of the standard triplicates ranged from -3.2% to 2.4%. Coefficient of variances of the Quality Control for Molecular Diagnostics samples ranged from 0.15 to 2.22%. The standard curve reliably predicted copies/mL of Adenovirus DNA within a sample. Comparison ofour results toother laboratories showed that our results closely matched the laboratories using the Adenovirus R-gene® commercial kit or other commercial kits.
Conclusion: Based upon the results of this study, the Adenovirus R-gene® commercial kit will be suitable for the detection of adenovirus DNA from clinical samples such as plasma and to monitor antiviral treatment related to systemic disseminating disease.

ImmunoCard STAT! EHEC: Improved Screening for Shiga toxin-producing *Escherichia coli* in patient faecal specimens at Canterbury Health Laboratories

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Objectives: This study was undertaken to assess the performance of the ImmunoCard STAT! EHEC (ISE) assay for the improved screening for Shiga toxin-producing *Escherichia coli* (STEC), in patient faecal specimens at Canterbury Health Laboratories.
Method: Retrospective and prospective studies were undertaken, with an overall of 57 tests performed on 42 patient faecal samples. Samples were accepted for testing after visualisation of mauve colonies on CHROMagarTM STEC (STCA) which were identified as *E. coli* by MALDI-TOF. Due to specific laboratory requirements, multiple methods were used. Methods included the manufacturer stated GN (Gram negative) broth and two altered plate culture methods utilising STCA and a blood agar plate (BA). This involved the direct inoculation of specimen onto a STCA plate, where colonies were further sub-cultured on BA for testing. Results were recorded and discrepant samples retested.
Results: Retrospectively, the test performed with a specificity of 100%. However, results varied greatly dependent on whether the GN broth, STCA or BA method was used, with a sensitivity of 33.3%, 75% and 100%, respectively. During the prospective study, the GN broth had a sensitivity of 0% with a specificity of 100%. The BA method performed best with an incomparable result of 100% for both specificity and sensitivity.
Conclusions: It can be concluded that the ISE is a rapid, qualitative, assay which is a clinically beneficial screening test for Shiga toxin producing *E. coli* in patient stool samples. With a specificity of 100% and negative predictive value of 93.3%, this test device accurately reports a negative test result. The test also identifies toxins from a wide variety of serotypes. The BA method was determined to be the most favorable method due to its high sensitivity and specificity. Because of the limited timeframe and number of specimens available, the clinical sensitivity of this test may be greatly underestimated in this study. Further continuation of this study would be valuable.

PorA Opa confirmation test for *Neisseria gonorrhoeae*

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Objectives: *Neisseria gonorrhoeae* is an oxidase positive, Gram negative diplococcus which causes a sexually transmitted infection, commonly referred to as gonorrhoea. If a sample tests positive and is from a child (≤13-year old) or involved in a medico-legal case, the result must be verified in-house. This is done using the PorA and Opa confirmatory test which currently does not have an incorporated extraction control. The objective was to evaluate the DNA Process Control Kit (Roche) for the

PorA Opa test to provide the assurance that the extraction of the patient's DNA has occurred correctly with no inhibition.
Methods: The Roche LightCycler 480 (LC480) Probe Master (Roche) master-mix was tested against the DNA Extraction Control Kit master mix, to explore which concentration of primer is the most effective, as well as which annealing temperature is the best overall for the three primer concentrations. The best combination of primer and annealing temperature was then tested against the patient samples. A comparison of results was cross referenced with the current LC480 Probe Master. The analytical sensitivity was also compared between the two master mixes.
Results: The study results showed the patient outcomes as well as analytical sensitivity was comparable between the original master mix and the trial master mix.
Conclusion: Test results indicate that a changeover from the LC480 Probe Master to the DNA Process Control Kit will be of equal positive result reliability with the additional security of adding negative result confirmation, and improved sensitivity for low concentrations of the target gene.

Immature platelet fraction as an indicator for platelet recovery in acute myeloid leukaemia patients who received chemotherapy

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Objectives: Thrombocytopenia is a common feature post extensive chemotherapy, which can lead to a patient being at high risk of bleeding. In terms of internal bleeding prevention, prophylactic platelet packs can be administered to the patient. However, unnecessary platelet transfusion may induce side effects such as alloimmunization and transfusion-induced infections. The aim of this retrospective study was to identify whether the immature platelet fraction (IPF% and IPF#) could be used as a predictive indicator for platelet recovery in post-chemotherapy patients.
Methods: This study focused only on acute myeloid leukaemia patients and compared the IPF values around the point of platelet nadir and platelet recovery. IPF was measured using the PLT-F channel of Sysmex XN20 instruments.
Results: Platelet nadir had a median of IPF% of 0.7% (0.3% to 2.4%) and platelet recovery was 4.2% (2.4% to 9.2%). For IPF#, the median was 0 (0 to 0.2) at platelet nadir and 1.3 (0.8-1.8) at platelet recovery. As this study was performed in a short time frame, the sample population was only six patients with nine cycles of chemotherapy. Hence, this study can only provide an indication of the usefulness of IPF in predicting platelet recovery in post chemotherapy patients, and further prospective studies need to be done to determine true cut-off points for IPF values in patients at platelet nadir and platelet recovery.
Conclusion: This study showed a potential for using IPF as means for predicting platelet recovery in chemotherapy patients.

Validation and comparison of Biomedica's NT-proCNP ELISA kit

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Objectives: C-type natriuretic peptide (CNP) is an upcoming biomarker of myocardial dysfunction. It is rapidly degraded within the circulation making it hard to detect. Instead a presumably non-bioactive fraction of CNP, NT-proCNP is measured. There are different methods available for the measurement of proCNP. Biomedica's NT-proCNP ELISA kit is a clinical research kit that measures the end terminal of proCNP. The aim of this project was to test the analytical performance of Biomedica's second generation ELISA kit for measurement of NT-proCNP.
Methods: The ELISA kit was validated through the testing of four parameters. These were sensitivity, specificity, precision and accuracy. The kit was also compared to another proCNP method, the processing-independent assay (PIA), via comparison with 254 patient samples to see if there was a correlation between the two methods. Each parameter was assessed using an ELISA immunoassay plate that uses the sandwich principle. The sensitivity was determined via the production of 6 standard curves in order to find the limit of quantification. The specificity was assessed by a cross reactivity study with animal samples. The accuracy was assessed by a serial dilution and a spiking experiment. The precision was calculated by the use of controls present on each plate.
Results: Following the completion of the validation and comparison of the kit, the functional sensitivity was determined to be <10% between the range of 16-128 pmol/l of NT-proCNP. Also, the kit was seen to have accuracy. The precision and comparison aspect failed to show expected results and only a very small correlation was seen between the two methods. The comparison of the ELISA to the PIA showed a modest correlation (R^2 = .01245)
Conclusion: Overall, the ELISA for NT-proCNP measurement performed very well on measures of functional sensitivity, accuracy and precision.

Fragmented red cells (FRC) of the Sysmex XN-3000® cell analyser is a useful parameter for screening thrombocytopenic patients for red cell fragments in blood films

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Objectives: Red cell fragments (schistocyte) evaluation is an important initial workup for primary thrombotic microangiopathy (TMA) syndromes diagnoses. The lack of standardisation in counting schistocytes by microscopy has prompted the investigation of automated methods. The objective of this study was to determine: (i) a reference interval for schistocytes and; (ii) the usefulness of the fragmented red cell (FRC) parameter of the Sysmex XN-3000® cell analyser in detecting schistocytes during routine haematological screening.
Methods: The FRC reference interval was determined from 51 patients with a normal complete blood count. FRC stability and within-run precision were assessed. FRC-positive samples (50) were analysed for schistocytes using cell analyser and by a standard microscopy method. Blood film results were quality controlled by four qualified medical laboratory scientists.
Results: The FRC reference interval was determined to be 0 to 0.148%. This parameter was stable for up to 24 hours. High Hypo-He resulted in spuriously high FRC (r_s=0.904, P<0.001), therefore samples with Hypo-He > 2% were excluded (n=6). A positive correlation (r_s=0.729, P<0.001) and good agreement (P=0.158) between the two methods were found. At 0.12% FRC cut-off to indicate schistocyte occurrence, the sensitivity and negative predictive value were both at 100%.
Conclusion: FRC may be useful for screening schistocyte occurrence at 0.12% cut-off, but due to its high imprecision at < 1.48% and lack of a true gold standard method, counting on blood films is still recommended.

Acknowledgement: AY conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, wrote the paper. BB provided the project idea. RP provided guidance. Richard Hosking, Katherine Lyon, Caity Dalley and Lauren Eddington have contributed to the skill verification process of schistocyte counting.

Assessment of red cell ABO antibody avidity using chaotropic test methods

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Objectives: The purpose of this project was to test blood donor serum/plasma for haemolysins using a chaotropic ion test method and identify cases where chaotropic results do not match the New Zealand Blood Service-assigned haemolysin status of the donor. This test method has been developed by previous BMLSc students as part of an ongoing project.
Methods: Donor serum/plasma samples that had already undergone routine haemolysin screening were tested for haemolysins using chaotropic ion solution phosphate-buffered potassium chloride and A_1B red cells. Samples were determined to be haemolysin positive or negative through grading the agglutination using a 0-12 scale. Grades of 8 or higher signified the presence of haemolysins, while 5 or lower gave the donor a haemolysin negative status.
Results: Of the 394 samples tested, 13.29% of donors given a haemolysin negative status by current New Zealand Blood Service test methods may be haemolysin positive. Conversely 4.98% of haemolysin positive donors gave haemolysin negative results using chaotropic ion methods.
Conclusion: This project found that a significant proportion of donors (8.63%) may be assigned the wrong haemolysin status by current New Zealand Blood Service testing methods. This presents an opportunity for further confirmatory testing and development of the chaotropic ion method, and reason for replacement of the current New Zealand Blood Service testing method.

Comparison of the effect of C reactive protein on activated partial thrombin time using three different reagents

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Objectives: C reactive protein (CRP) is an acute phase protein made by the liver in response to inflammation. CRP has a known affinity for phospholipids. It is this affinity for phospholipids that can prolong activated partial thromboplastin time (APTT) times in a calcium dependant manner. The aim of the study was to compare the effect of elevated CRP has on APTT results using three commonly used reagents.
Methods: Sodium citrated blood samples from 24 patients with elevated levels of CRP and 10 controls (normal CRP) were double centrifuged and platelet-poor plasma was frozen for analysis. Rapidly thawed samples were then tested on a Sysmex CS-2500 automated coagulation analyser using three different APTT reagents in series, Actin FS, TriniCLOT aPTT HS, and Actin FSL. The hypothesis for the study was that there is a significant difference between the reagents APTT times due to the interference of an elevated CRP level (>5mg/L).
Results: When patients with normal and elevated CRP were compared, there was a neglible difference in APTT times for the Actin FS and FSL reagents. However, for TriniCLOT aPTT HS, there was a significant difference ($P < 0.05$). The overall sample size was insufficient for statistical validation.
Conclusion: In this study, elevated CRP affect APTT times when TriniCLOT aPTT HS was used. In view of the small sample size, further investigation is required to confirm this result.

Early antenatal predictors of HDFN-affected fetuses and newborns in pregnant women with anti-D.

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Objectives: Rhesus D incompatibility between mother and fetus is the most common cause of haemolytic disease of the fetus and newborn (HDFN). Managing HDFN can be expensive and invasive. However, there has been little research on determining early antenatal indicators of HDFN-affected fetuses and newborns due to rhesus D incompatibility. The aim of this project was to examine possible early predictive antenatal factors of fetuses and newborns significantly affected by HDFN.
Methods: Antenatal records were accessed from New Zealand Blood Service, Christchurch hospital for pregnant women known to have antenatal immune anti-D between 2008 and 2018. Between the affected pregnancies and unaffected pregnancies, antenatal factors obtained from the mothers and the fetuses or newborns were compared.
Results: Among all the antenatal factors analysed in the 98 pregnancies, fetal R2r phenotype was more frequent in the 23 affected pregnancies than the 75 unaffected pregnancies. Initial anti-D titres were generally higher in the affected pregnancies than the unaffected pregnancies.
Conclusion: It was interesting to find that fetal rhesus phenotype and initial anti-D titre were likely early indicators of severe HDFN. To confirm our findings, this study needs to be repeated on a larger and more complete dataset.