4th year University of Otago BMLSc Semester 2, 2020 research abstracts

Modification of the mismatch repair protein MMR protocols at Dunedin Hospital Histopathology Laboratory SCL

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Objectives:
When using the VENTANA BenchMark ULTRA instrument, amplifying the immunohistochemical signal is performed to increase the staining intensity of antibodies for some mismatch repair MMR proteins, namely MLH1 and PMS2. Optimizing amplification-free protocols is beneficial, especially when having a limited supply of the amplification kits. This study aimed to validate amplification-free protocols for MLH1 and PMS2 after modifying one or more variables which include antigen retrieval time (AR time), primary antibody dilution and incubation time.
Methods: The studied colorectal tissue had adenocarcinoma with normal MLH1 and PMS2 protein expression. Eight slides were prepared and tested, with the amplification step performed on one. Both positive and negative controls were included on each slide, and results were interpreted by two qualified pathologists, a scientist, and a student based on a qualitative score between 1 to 5.
Results: Seven amplification-free slides were stained for MLH1, and they showed acceptable results. Two slides with increased primary antibody dilution (1:50 to 1:40), one of which had an increased AR time (64 to 104 min), showed a staining like that of the current protocol slide. One of the seven amplification-free slides stained for PMS2, with a 25-fold increase in antibody dilution (1:50 to 1:25) and an increase in the AR time (48 to 104 min), had excellent staining, and the other six slides showed good and acceptable results.
Conclusion: A ten-fold increase in the MLH1 antibody dilution with or without increasing the AR time was an excellent substitution for the current MLH1 protocol. A 25-fold increase in the PMS2 antibody dilution with a 56 min increase in the AR time showed optimal staining. Using these alternative protocols instead of the current one will reduce costs without influencing the quality of staining.

Validation of the new Elecsys® Total PSA for clinical application

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Objectives: Roche has released a new Elecsys® Total PSA assay this year for prostate-specific antigen measurement with increased biotin tolerance to replace the previous TPSA assay, which has been in use since 2018. The aim of this study was to validate the new assay for clinical use. Validation was based on comparison with the previous TPSA assay to evaluate their degree of agreement.
Methods: Thirty patient samples with completed PSA results retrieved over the course of four weeks were run collectively in duplicate with both assays on the Cobas 6000 e601 unit. Statistical analysis of results was performed using the Bland-Altman plot, Passing-Bablok regression, and the Wilcoxon test.
Results: Visual analysis of the results showed larger variance in data as values increased. Statistical analysis identified a mean difference of 0.42 µg/L between the two assays. Limits of agreement (95%) in the Bland Altman plot were -1.8757 to 1.0313 µg/L and Passing-Bablok regression generated a slope equation of y=0.01177 + 0.9606x, indicating a small proportional bias. A *p*-value of <0.0001 was derived from the Wilcoxon test.
Conclusion: The differences between the assays were deemed statistically significant by the *p*-value, but not clinically significant as they fall within the *Royal College of Pathologists of Australasia* allowable limits of performance for PSA. Thus, the new assay was approved to replace the previous TPSA for running clinical samples. These research findings may be useful for other laboratories and biotin interference studies.

Use of the MAST D68C test for the detection of ESBLs and AmpC β-lactamases

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Objectives: *Enterobacteriaceae* that produce β-lactamases, such as extended spectrum β-lactamases (ESBLs) and AmpC enzymes, are a significant problem for healthcare as they confer resistance to β-lactam antibiotics. ESBL and AmpC detection tests must accurately differentiate between bacteria producing these enzymes and other mechanisms of resistance to β-lactams. The MAST D68C test is a phenotypical test that allows for the detection of ESBL production in *Enterobacteriaceae*, and those also producing AmpC [2]. The purpose of this study was to assess the sensitivity and specificity of the MAST D68C test.
Methods: Forty *Enterobacteriaceae* were tested for resistance using the D68C method and the two currently used methods in the Hawkes' Bay DHB laboratory; the MAST D69C test for AmpC detection and the ESBL double disk synergy test (DDST). A resistance screening method was tested for its sensitivity and the BD Phoenix was used as a confirmation of organism identification (ID) and antimicrobial susceptibility.
Results: The MAST D68C test had a sensitivity and specificity 100% for ESBL-producers, which was greater than the sensitivity and specificity of the ESBL DDST (95% and 98%, respectively). The MAST D68C test had a sensitivity of 50% for AmpC-producers, which was half the sensitivity of the D69C test (100%), and both tests had a specificity of 100%. As no isolates in this study were both ESBL and AmpC producers, we obtained no results for the sensitivity and specificity of their detection with the MAST D68C test.
Conclusion: The D68C test had a superior sensitivity and specificity for ESBL-producers than the DDST and therefore can replace it as the routine ESBL confirmation test. The D68C may be able to replace the D69C for AmpC confirmation, as it does not need to detect chromosomal (inducible) AmpC-producers, which was mostly the reason of the low sensitivity for AmpC-producers. Overall, the MAST D68C test appears to be an acceptable replacement of the two tests currently used for ESBL and AmpC-production.

Establishing the concordance between the ARCHITECT SARS-CoV-2 IgG assay and COVID-19 VIRCLIA IgG Monotest for SARS-CoV-2 IgG immunoassay serology

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Objectives: SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) is the causative agent of the acute respiratory illness coronavirus disease 2019 (COVID-19). SARS-CoV-2 serological testing is an area of growing interest for a variety of applications. The concordance between two SARS-CoV-2 IgG immunoassay kits was investigated by Wellington Southern Community Laboratories (WSCL). These two kits included the COVID-19 VIRCLIA® IgG Monotest from Vircell S.L and the ARCHITECT SARS-CoV-2 IgG assay manufactured by Abbott Laboratories. This was performed by comparing the qualitative results of both assays for patient samples collected within the Wellington region of both cases suspected of COVID-19 infection and samples collected at a timepoint before the emergence of SARS-CoV-2 using WSCL's own serum bank.
Methods: Twenty-three patient samples were collected within a three-month period post-SARS-CoV-2 emergence. The inclusion criteria required patients to either be: 1) individuals who were hospitalised with COVID-19 infection and had positive PCR results; or 2) individuals who had positive PCR results and agreed to provide their sample(s). A further twenty patient samples were collected pre-SARS-CoV-2 emergence. Both sets of patient samples were run using each immunoassay and qualitative results were compared.
Results: From the total forty-three patient samples, eleven samples were determined to be positive by both assays, thirty samples were negative for both assays, and two samples were positive on the VIRCLIA immunoassay whilst negative on the ARCHITECT assay. A weighted kappa of 0.68 (wald 95% CI 0.38-0.98) was calculated.
Conclusion: In conclusion, a substantially strong weight of agreement was made in detecting SARS-CoV-2 IgG antibodies by both assays. There remains many unanswered questions as to how the immune system interacts with SARS-CoV-2 following infection. However, SARS-CoV-2 IgG serology testing can be used for a variety of applications and is best used when samples are collected from individuals at least fourteen days from the onset of COVID-19 symptoms.

The relationship between antibody markers PAX8 and WT1 in endometrial and ovarian serous carcinoma positive cases

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Objectives: Until immunohistochemical (IHC) methods were utilised for their ability to determine tumour type, women with ovarian carcinomas received a diagnosis of carcinoma based only on morphology. Previously specifying the type of ovarian carcinoma did not change the treatment received. With changes in chemotherapy regimens, it has become more important to accurately type ovarian tumours. This report discusses the relationship of antibody markers PAX8 and WT1 performed with IHC methods in ovarian serous cases to provide an indication of the sensitivity and specificity of these markers.
Methods: Patient cases (64) dating back to February 2019 and with a diagnosis of serous carcinoma were retrieved through Lab Solutions. Fifty three of these were harvested to create Tissue Micro Array (TMA) blocks. From these TMA blocks, sectioning, Haematoxylin and Eosin (H&E) staining, immuno-staining with PAX8 and WT1 antibodies and reading of results under the microscope followed.
Results: A total of 32 cases were included in the data analysis. Of these, 23 (~71%) displayed the typical antibody pattern for serous carcinoma of PAX8 positive, WT-1 negative. The remaining 9 cases (~29%) did not follow the pattern and out of these, the WT1 antibody result for 8 (~89%) was negative.
Conclusion: The staining for WT-1 was lower than expected. The literature shows a positive staining in WT-1 of greater than 85% in cases of serous carcinoma of the ovary, compared to 71% in ours. Cases that showed aberrant WT-1 staining should undergo further IHC and morphological typing to determine the tumour type.

Comparison of Immulab and Bio-Rad 3% red cell reagents for ABO reverse grouping/anti-A1 lectin and antibody identification

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Objectives: To evaluate performance of 3% red cell reagents against a variety of clinical samples, and one standalone reagent, to evaluate performance. Bio-Rad DiaCell 3% ABO was compared with Immulab Revercell 3% for ABO reverse grouping (including 'weak' reverse groups) and Anti-A1 lectin. Bio-Rad 3% DiaPanel was compared with Immulab 3% Phenocell panel.
Methods: For a comprehensive reagent comparison, multiple clinical conditions were analysed. Each manufacturer's reagents were tested against 12 samples with known ABO groups; three samples with known weak reverse groups; a cold autoagglutinin disease sample; one sample with known anti-K; three samples with known anti-M; two samples with a known anti-E antibody, one enhanced with polyethylene glycol (PEG); and against anti-A1 lectin.
Results: Revercell 3% versus Diacell 3% - both reagents identified the known ABO blood grouping for the 12 patients tested. However, one sample produced an unexpected reaction against Bio-Rad Diacell 3% red cell reagent which requires further investigation (possible subgroup). Both reagents gave the same result for the 'weak' reverse group samples but Immulab reagent provided stronger agglutination. Both reagents gave the same reaction against anti-A1 lectin. Phenocell 3% versus DiaPanel 3% - both reagents produced a correct positive cold autoagglutinin screen, Phenocell producing stronger reactions. The Phenocell confirmed the presence of anti-M antibodies in all 3 tested plasma samples. However, the DiaPanel failed to identify one. Both reagents identified the anti-K, Anti-E, and anti-E plus PEG. Phenocell produced a stronger reaction with PEG.
Conclusion: Overall, this study has shown that Immulab 3% red cell reagents performed slightly better than corresponding Bio-Rad reagents. This study has limitations, primarily insufficient sample numbers.

A comparison of methods used for direct antiglobulin testing

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Objectives: Direct antiglobulin testing is performed for a number of reasons, including investigation of auto-antibodies, as part of new-born blood typing to check for haemolytic disease of the new-born, as part of a transfusion reaction investigation, and as part of the accreditation of blood donors. The objective of this project was to compare the tube-, manual card-, and automated card-based methods used to perform direct antiglobulin testing.
Methods: Three methods were used for the direct antiglobulin test comparison, with each sample being tested using each method. The first method was a tube-based technique, performed using Immulab Epiclone AHG Poly reagent and Lorne Laboratories Anti-Human IgG. The other two methods employed a card-based technique using Grifols DG Gel DC Scan cards. The first method was performed manually, while the second method was performed using an automated Grifols Erytra analyser.
Results: Comparing the manual and automated card methods, 90.62% of samples showed negatives results for both methods, or positive results within one grading of each other. Comparing the tube method against each of the manual and automated card methods, 68.75% of samples showed negative results for the both the tube and either card method or had positive results within one equivalent grading of each other. Of the remaining 31.25% of samples, either one method showed a positive result but was negative with the alternative method, or the results from each method had a difference of greater than one equivalent grading.
Conclusion: The manual and automated card-based methods showed comparable results, whereas the tube-based method displayed more discrepancy in results compared to the card-based methods. This is likely due to the more subjective nature of grading the results from the tube-based method as opposed to the card-based methods.

A method comparison between the Abbott Architect and Roche Cobas e602 for the analysis of cyclosporine

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Objectives: Cyclosporine is an immunosuppressant used for solid organ transplants to prevent rejection and increase organ lifespan. Due to variable rates of metabolism as treatment progresses, monitoring of patients is required to avoid numerous toxic side effects. The Architect is currently used for testing cyclosporine and has been a challenging assay to complete. Before committing to any new assay, the Biochemistry department wanted to evaluate the Roche Cobas e602 assay (competitive assay) against the existing Abbott Architect method (chemiluminescent microparticle immunoassay (CMIA) technology).
Methods: Whole blood samples (46) requiring cyclosporine testing were analysed using both systems. Both use a haemolytic pre-treatment process, but their methods differ. Quality Control (QC) material was obtained from Bio-Rad and two RCPA samples provided external quality control.
Results: Aspects that were considered were: price difference between reagents and calibration material; pre-treatment process; precision data run on quality control material; whether data collected is comparable between methods (helps to avoid retesting monitored patients).
Conclusion: Passing-Bablok and Student's t-test showed the differences between the two methods were statistically significant but not clinically significant. The price of Roche reagents, while more expensive is likely to be balanced out by the greater degree of precision demonstrated on the Cobas as it will require fewer reruns to ensure QC is acceptable. The potential for operator error is greater in Abbott method and this is reflected in the poorer precision data. Given the above points, the Roche method is an acceptable alternative testing method for cyclosporine.

A method evaluation of the tacrolimus assay on the Abbott Alinity and the Roche Cobas 8000

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Objectives: Tacrolimus is an immunosuppressive agent primarily used in patients receiving transplants. Southern Community Laboratories, Dunedin currently performs the tacrolimus assay on the Abbott Alinity. This method evaluation aimed to compare the Tacrolimus assay currently in use at Southern Community Laboratories, Dunedin with another available assay on the Roche Cobas.
Methods: The current method on the Alinity uses a chemiluminescent microparticle immunoassay, while the tacrolimus assay on the Cobas uses a competition principle. Both assays require sample pre-treatment before being tested on their respective analysers. Forty-four patient samples were tested on both analysers plus two RCPA samples.
Results: The raw data from the testing showed only minor variation between the results for the two methods of analysis. The quality control results from the Cobas showed a greater level of precision than the quality control results from the Alinity, with a slightly smaller standard deviation in the results from the Cobas. The Passing-Bablok graph had a regression line with a slight downwards bias. Student's t-Test and the Altman graph were used to estimate the differences between the results on the Alinity and the Cobas.
Conclusion: The higher level of precision of quality control results on the Cobas indicated that the analyser would need fewer calibrations as opposed to the Alinity. The downwards bias of the Passing-Bablok graph indicated that the results from the Alinity were higher than those from the Cobas. The differences established between results using Student's t-Test and the Altman graph showed no clinical significance. This means that the analysers could theoretically be used interchangeably.

DxH800 research parameters, low haemoglobin density, microcytic anaemia factor, and red cell size factor for identification of iron deficiency anaemia

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Objectives: The DxH800 has a range of research parameters, which are not currently used clinically. This study assessed the use of low haemoglobin density (LHD%), microcytic anaemia factor (MAF), and red cell size factor (RSF) in the diagnosis of patients presenting with iron deficiency anaemia (IDA) and from patients without iron deficiency anaemia (normal).

Methods: Normal samples were identified from analysis of full blood count (FBC). Abnormal ferritin, transferrin saturation, and CRP results were used to identify patients with iron deficiency anaemia. This was to exclude conditions which may affect iron stores, including anaemia of chronic disease. If low ferritin and transferrin saturation plus normal CRP were seen in samples, a FBC and reticulocyte count was run, which in turn included the three parameters of interest.
Results: The patient data consisted of 33 IDA samples, and 110 normal samples. A statistically significant difference was seen between the normal and IDA samples using LHD%, MAF, and RSF. There was no statistically significant difference in RSF between male and females, for both normal and IDA samples. However, a p value of < 0.001 was obtained for MAF between male and females in the normal sample. In addition, a p value of 0.0309 was seen for LHD% between male and females in the normal sample. This trend was also not seen in the IDA sample.
Conclusion: The significance of these parameters in differentiating IDA and normal illustrates its possible usefulness in a clinical scenario. With further investigation of these parameters, their use clinically would potentially aid in diagnosis. Previous studies have illustrated the use of these parameters in differentiating thalassaemia's from IDA. Analysis of these parameters in other forms of anaemia would illustrate its overall usefulness clinically.

Validation of the updated Aptima Combo 2 assay for detection of diagnostic-escape *Chlamydia trachomatis* variants

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Objectives: The identification of numerous diagnostic-escape *Chlamydia trachomatis* variants (C1515T; C1514T; G1523A; C1522T single nucleotide polymorphisms) has necessitated an update to the Aptima Combo 2 (AC2) assay (Hologic; San Diego, CA); a duplex nucleic acid amplification test used for the detection of *C. trachomatis* (23S rRNA) and *Neisseria gonorrhoeae* (16S rRNA). These variants possess mutations in the probe targeted sequence, enabling detection evasion. Subsequently, a secondary probe was introduced, targeting a differing region within the 23S rRNA. Updated kit validation is necessary to demonstrate novel variant detection ability, without compromising other diagnostic and analytical expectations.
Methods: The proposed verification study of the updated assay was executed by testing specimens of known outcome on the updated kit on the Hologic Panther (San Diego, CA) and comparing the outcome to the original AC2 result, thus assessing the concordance, specificity, and sensitivity across various clinical expectations. This included testing Panel G verification specimens spiked with four of the most prevalent novel variants of *C. trachomatis*, confirming their detection ability. Investigation of discrepant results (n=15) was performed through secondary AC2 testing and confirmation on the AusDiagnostics Urogenital Infections and Resistance Panel.
Results: All Panel G verification specimens (n= 10) returned positive for *C. trachomatis* with the updated AC2. Overall, results demonstrated excellent sensitivity and specificity for both *C. trachomatis* (98.5%; 98.6%) and *N. gonorrhoeae* (98%; 99.4%), with the concordance (Cohens kappa coefficient) between results being 0.947 (95% CI: 0.900-0.993), and 0.970 (95% CI: 0.938-1.00), for each respective target.
Conclusion: The study validates the updated AC2 assay, which is of adequate accuracy and can successfully detect recently emerged *C. trachomatis* diagnostic-escape variants. This will benefit reducing undetected transmission and the health implications pertaining to untreated infections, whilst additionally conferring protection against future diagnostic-escape variants.

Verification of ELISA commercial kits in detecting intrinsic factor IgG antibodies

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Objectives: Anti-intrinsic factor (IF) IgG antibodies lead to the malabsorption of vitamin B12, strongly associated with autoimmune-pernicious anaemia. Accuracies in detecting IF antibodies are significant for patient management. This study aims to compare the verification of the current commercial ELISA kit (Generic Assay (GA)) against other ELISA kits (Genesis Diagnostics (GD) and Euroimmun (EI)) in detecting IF IgG antibodies.
Methods: Seventy-four serum samples of IF IgG antibodies diluted into 1/101 factor. Diluted samples (100uL), calibrators, and QCs were added prior to incubation. Plates were washed and incubated with 100uL of the conjugate. After the second washing step, plates were incubated with 100uL of TMB substrate, followed by the addition of 100uL stop solution. The optical density of plates was measured to calculate the concentration of IF antibodies. Wilkson signed-rank test statistically analysed the variances of results among the kits. QC procedure completed via testing two of the in-house and manufactured QC samples in every assay.
Results: All three test kits demonstrated different variances among positive, negative, and borderline-positive samples ($p < 0.05$; CI >95%). Positive samples indicated significant variances (p-values of GA and GD=0.0319; GA and EI=0.0244; GD and EI=0.0049). Negative samples in GA against GD and EI indicated significant variances ($p<0.0001$; p=0.0061). GD against EI showed no variances between their results ($p>0.999$). Borderline samples displayed variances of GD against EI (p=0.0273). Borderline-positive p-values of GA against GD and EI were unacceptable due to insufficient samples tested.
Conclusion: Significant variances of GA results determined against other kits. The normal ranges and cut-off values of the IF IgG antibody concentration through GA should be adjusted for accurate analysis.

Establishing Sysmex XN cell population data reference intervals for healthy individuals

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Objectives: Middlemore Emergency Department (ED) clinicians have requested the haematology laboratory investigate whether the cell population data (CPD) obtained from the laboratory's XN Series cell counter, comprising of one XN10 analyser and two XN20 analysers, will provide useful and significant information for the early detection of sepsis in ED patients. The aim of this study was to determine precision and establish an initial reference interval for selected parameters of the CPD for healthy individuals, to use as a comparison for suspected sepsis patients.

Methods: To determine precision for each CPD on each XN analyser module, two K2EDTA samples were collected from a healthy staff member and pooled. A complete blood count (CBC) was repeatedly sampled on each analyser module 10 times for the pooled sample. The coefficient of variation (CV) was calculated for each CPD for each analyser to determine precision. To establish the reference interval, a K2EDTA sample was collected from 20 healthy volunteers. A CBC was analysed for each sample on each analyser within 2 hours of collection. The 95% confidence intervals were calculated using the data mean and standard deviation (SD), assuming there was a normal Gaussian distribution. Intra-analyser comparisons, Anderson-Darling test, Extreme studentized Deviation test, and reference intervals were calculated.
Results: Monocyte (MO-X, MO-WX, MO-WY), lymphocyte (LY-X, LY-Y, LY-WX, LY-WY), and neutrophil (NE-SSC, NE-SFL, NE-WY, NE-FSC) results from each XN analyser were aligned within 2SD and followed a Gaussian distribution with no outliers. MO-Y on the XN10 module did not pass the Anderson-Darling test. MO-Z and MO-WZ results from the three analysers were outside 2SD.
Conclusion: The monocyte, lymphocyte and neutrophil reference intervals established can be used for comparison with septic patient results. However, comparison and standardisation of the analysers are required before using the MO-Z, MO-WZ and MO-Y reference intervals.

Accuracy verification for the validation of Lorne anti-Fy^b antiserum use in the Grifols system

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Objectives: The New Zealand Blood Service currently utilises the Lorne anti-Fy^b antiserum for Fy^b phenotyping blood donors and recipients. This study aimed to determine whether this antiserum can accurately detect both the presence and absence of the Fy^b antigen using the Grifols DG Gel Coombs cards.
Methods: The accuracy verification protocol used for this study recruited 37 samples with varying levels of Fy^b expression. The Lorne anti-Fy^b antiserum was used to Fy^b phenotype these samples by tube and card indirect antiglobulin testing. A card-mediated direct antiglobulin test (DAT) was also run on each donor and patient sample as those which produced a positive DAT, and/or a card indirect antiglobulin test (IAT) grade <2, were excluded from analysis. Linear regression and the correlation coefficient were used to compare the efficacy of this antiserum for both IAT methods.
Results: Three clusters of results were evident on the generated scatterplot. Upon investigation, 83% of Fy$^{(a-/b+)}$ and 33% of Fy$^{(a+/b+)}$ samples were found to produce tube and card readings of 8 and 3 respectively, while 17% of Fy$^{(a-/b+)}$ and 42% of Fy$^{(a+/b+)}$ samples generated grades of 5 and 3. Alternatively, 100% of Fy^b-negative samples presented grades of 0 for both IAT techniques. The correlation coefficient obtained (R=0.939) indicated a strong correlation between the tube and card IAT results.
Conclusion: This study verified that the Lorne anti-Fy^b antiserum was able to accurately detect the presence of Fy^b using the Grifols system despite the variation in antigen expression pertaining to the dosage phenomenon. It also showed the antiserum has high antigen specificity as 100% of the Fy^b-negative samples produced negative results for both methods. Despite these results advocating the suitability of the Lorne anti-Fy^b antiserum for use with the Grifols system, further testing of its precision is required to complete the validation.

An investigation on the use of fresh frozen plasma with 3-factor prothrombin complex concentrates for warfarin reversal

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Objectives: Our aim was to investigate the use of fresh frozen plasma (FFP) in conjunction with the 3-factor prothrombin complex concentrate (3F-PCC, Prothrombinex VF) for warfarin reversal. 3F-PCC contains the clotting factors II, IX, and X, but very little or no F VII. FFP, which contains all the clotting factors, is used as a source of FVII, but the importance of FVII during warfarin reversal is questionable. If FFP use can be avoided, costs, and potentially adverse effects in patients, can be reduced.
Methods eTraceline and Eclair were used to gather data on Christchurch District Health Board patients receiving both Prothrombinex and FFP for warfarin reversal during 2019. Patients with other indications were not included. Information was extrapolated to the whole of New Zealand, assuming the percentage of patients receiving both would be similar across the country. Using this information, estimated usage of FFP and the savings by reducing this usage were calculated.
Results: It is estimated that in New Zealand about 78 patients per year receive FFP with Prothrombinex (for warfarin reversal) unnecessarily. This is about 0.6% of total FFP issues per year, or NZ$18,744.23 worth of FFP.
Conclusion: The small percentage (0.58%) of the total FFP issues in this context is of no great financial significance, as the rate of FFP issues changed by 5-10% per year from 2014-2018, after the 15% drop in demand in 2013. However, reduction in these FFP issues could potentially improve patient outcome through reducing risk of transfusion reactions.

Verification of two HbA1c point-of-care analysers

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Objectives: A Cobas b101, an HbA1c point-of-care (POC) device, was recently purchased for the Central Otago diabetic nurse specialist. A verification study of this new Cobas b101 was required and in conjunction with this, a verification study of the Abbott Afinion 2 was also performed. The verification study included both analytical performance analysis and an ease-of-use assessment.
Methods: The analytical performance of both point of care analysers was established by running forty-one K2EDTA venous samples once on all three devices; Cobas b101, Afinion 2, and Bio-Rad D-100 (reference method). Imprecision was estimated by calculating the measurement of uncertainty (MOU) from twelve paired samples and repeatedly tested quality control samples. Lastly, an ease-of-use assessment was performed by five health-worker participants.
Results: Results were reported in mmol/mol, with both PoC devices having strong correlations when compared to the D-100. Results: b101 R^2 = 0.9824; Afinion 2 R^2 = 0.9864. The MOU calculated from QC results was higher for the Cobas b101. Results: b101 = QC1 = ±4.72; QC2 = ±6.65; Afinion 2 = QC1 = ±2.17; QC2 = ±2.76. The ease-of-use assessment found a preference for the Afinion 2, primarily due to difficulties faced whilst filling the b101 test cartridges.

Conclusion: The verification study showed good correlation between both POC devices and the reference method. The higher MOU estimated from QC results on the Cobas b101 needs further investigation. Limitations of this study include: use of venous samples (in practice finger prick specimens are used); lack of data points above the clinical reference value (>50mmol/mol); and not investigating the effect of haemoglobin variants on HbA1c measurements.

Overview of Anti-DFS70 antibody identification in Southern Community Laboratories Dunedin: Accuracy, methodologies, and an exclusion criteria for systemic autoimmune rheumatic disease

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Objectives: The dense fine speckled pattern on antinuclear antibody indirect immunofluorescence pattern is often caused by the anti-DFS70 antibody. Anti-DFS70 antibodies occur in higher frequencies in healthy individuals compared to patients with systemic autoimmune rheumatic disease. The aim of this research project was to: a) analyse the accuracy in detecting anti-DFS70 antibodies using the screening method of antinuclear antibody indirect immunofluorescence testing; b) compare with a different methodology for detecting anti-DFS70 antibodies (chemiluminescence) and; c) discuss the use of anti-DFS70 antibody as an exclusion criteria for systemic autoimmune rheumatic diseases.
Methods: Patient samples with dense fine speckled pattern detected through indirect immunofluorescence antinuclear antibody testing using the Immuno Concepts Hep 2000 test system were confirmed for anti-DFS70 antibody using a chemiluminescent immunoassay by Wellington Southern Community Laboratories on a Bioflash instrument.
Results: The Dunedin Immunology department was able to correctly identify 80% of the patient samples with positive anti-DFS70 antibodies.
Conclusion: The accuracy of the results in identifying anti-DFS70 antibodies using the initial antinuclear antibody testing were similar to other studies. It has been found that, regardless of the choice of method for confirmation of anti-DFS70 antibody, the initial finding of the dense fine speckled pattern using antinuclear antibody testing must be confirmed using chemiluminescence or line immunoassay. Studies have found that testing for anti-DFS70 antibodies as an exclusion criterion for systemic autoimmune rheumatic disease may provide benefits in decreasing costs through reducing follow up testing and reducing referrals to a rheumatologist. However, it is still important to identify the presence of other antibodies such as anti-dsDNA antibodies and extractable nuclear antigen panels.

Comparison of BACTEC blood culture and conventional routine culture for sterile body Fluids other than blood

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Objectives: Diagnosis of infection in sterile body fluids (SBF) is complicated. Infection of SBF is associated with high mortality and morbidity. In this study we evaluated aspirates, chronic ambulatory peritoneal dialysis (CAPD) fluid, ascites fluids and pleural fluid. The study was designed to evaluate positive diagnostic yield of BACTEC blood culturing system and conventional routine culture for SBF other than blood.
Methods: Data was collected retrospectively. Patient specimens were received at Canterbury Health Laboratory, Christchurch. Specimens which were included in the study were synovial fluids, CAPD, ascites, and pleural fluid. Laboratory protocol from UK Standards for Microbiology Investigation was followed.
Results: In total there were 109 specimens included in the study. Sixteen (14.7%) were positive using the BACTEC blood culture system and 10 (9.12%) were positive on conventional routine culture. Differences in diagnostic yield for synovial fluids, CAPD, ascites, and pleural fluids were 7.55%, 5.56%, 4.35%, and 0% respectively. BACTEC had higher diagnostic yield compared to conventional culture in SBF. The specificity (0.95) of ascites fluid was lower using the BACTEC system compared to culture (1.0).
Conclusion: In conclusion, diagnostic yield using the BACTEC blood culture system was higher than routine culture for aspirates and CAPD. Data for pleural fluids was too limited to compare both methods. To improve specificity, ascites fluids with low WBC counts should be excluded from the BACTEC system. The increase in yield of positive culture using the BACTEC system is probably due to increased sample volume, resins which can neutralise the antibiotics and growth supplements in BACTEC bottles. Combining both methods for aspirates, CAPD and pleural fluids is considered optimal

Developing a reportable immature platelet fraction reference range

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Objectives: The immature platelet fraction (IPF) parameter is a non-reportable parameter on Sysmex analyers with PLT-F capabilities. It is an exciting parameter with many possible clinical applications. The objectives of this study were to verify a published adult reference range for use at Waikato Hospital and to publish the relevant clinical information on immature platelet fractions in the Waikato Hospital Test guide.
Methods: Donor samples were run on the PLT-F channel of Sysmex XN analysers, and non-parametric methods, as described in CLSI EP28A3C guidance document, were used to determine a 95% confidence interval.
Results: The reference range published by Johnson and Baker in 2018 (*International Journal of Laboratory Hematology*, December issue) was used. The range in the present study was 0.0 – 13.5 x10^9/L for absolute immature platelets. The range reported by Johnson and Baker was 1.6 – 12.6 x 10^9 /L. Use of the published range at Waikato Hospital, as yet, still requires review and approval by the haematologist. The clinical information summarising the utility and precautions regarding absolute immature platelet count is yet unpublished, however it has been approved by the Routine Technical Specialist. Publication of IPF for clinical use at Waikato Hospital awaits Hematologist range approval and requires registration testing.
Conclusion: The immature platelet fraction is a useful measure of the causes of abnormal platelet count. Further study should be conducted to determine its relationship better to age.

The effects of time delay and sample storage temperatures on EDTA-anticoagulated whole blood samples sent for complete blood count and white blood cell differential performed on Sysmex XN-9000 analyser

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Objectives: Complete blood count and white blood cell differential are common tests requested to assess patient's health, therefore the accuracy of the produced results is essential. The aim of this study was to assess the effects of time delay and storage temperature on the stability of haematological parameters in EDTA-anticoagulated whole blood samples when tested on a Sysmex XN-9000 analyser.
Methods: Over a period of one week, blood samples collected from five healthy volunteers were tested for complete blood count, reticulocyte, and white blood cell differential counts at different time intervals and storage temperatures (room temperature and 4°C).
Results: Maximum analytical variation and limits were set based on the Analytical Performance Specifications Guidelines and were used for assessment of sample stability. The samples stored at 4°C revealed the stability of all haematological parameters for up to 48 hours, producing minimal variations between the results. In contrast, some complete blood count parameters were less stable at room temperature, namely MCV and MCHC, which produced unacceptable variations at 48 hours post-collection. Additionally, RDW-CV and haematocrit showed non-significant upward trends after 2 hours of storage at room temperature.
Conclusion: The results showed stability of reticulocyte and white cell differential counts over time regardless of the storage temperature. However, some complete blood count parameters produced unreliable results at room temperature. Thus, it is recommended to store samples at 4°C if a delay in sample processing is anticipated. The stability of haematological parameters varies between different analysers, therefore, the conclusions drawn from this study are only comparable with studies performed on Sysmex XN-9000.

Comparison of three IAT techniques for erythrocyte alloantibody identification - two microcolumn assays and one tube-based

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Objectives: Indirect antiglobulin tests (IATs) are utilised within blood banks for the detection and identification of clinically significant antibodies; a critical element of pretransfusion testing as transfusion of incompatible alloantibodies can lead to adverse reactions in patients. Conventionally tube-based methods have been used, although the development of technology has seen the introduction of microcolumn card assays. This study was done to compare the efficacy of three IAT techniques: a card IAT using 0.8% suspension of reagent cells, another employing a 3% cell panel (typical utilised for tube methods), and finally a standard 3% cell tube IAT.
Methods: In this study, 12 samples known to contain at least one alloantibody were tested using the three different IAT techniques for antibody identification – 0.8% cell card, 3% cell card, and 3% cell tube. Grifols DG Gel microcolumn cards and Grifols Perfect Panel reagent cells (0.8% and 3% suspensions) were utilised.
Results: The comparison between the two microcolumn card techniques found them to have similar results and the lowest degree of variability. Contrasting results between card and tube technology demonstrated increased variability, with the highest degree of inconsistency being between the 3% cell card method and the tube-based technique. Two cases of weak Kpa antibody were not detected by the card IAT using 0.8% cells, while the tube IAT was able to identify one of these cases and the card IAT using 3% cells successfully recognised both.
Conclusion: Microcolumn card techniques were found to be more sensitive for antibody identification than the traditional tube approach overall, although the 0.8% cell microcolumn assay failed to identify two cases of weak anti-Kpa.

Method comparison between two C-reactive protein assay kits using patient samples

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Objectives: The objective of this method comparison was to determine comparability between the old soon-to-be phased out of production Roche CRPL3 assay kit and the new to-be-introduced Roche CRP4 assay kit, in preparation for the switch from CRPL3 to CRP4 in a diagnostic laboratory. If comparability cannot be demonstrated, reference ranges and quality control targets may need to be evaluated, and referrers notified prior to usage of the CRP4 kit.
Methods: Patient samples previously tested for CRP concentrations were selected to provide a range of samples spanning the measurable range of the CRP kits (0-350mg/L). Each sample was run on each automated analyser line using both kits. The results were transcribed to a spreadsheet and analysed using statistical software – Bland-Altman, Passing-Bablok, and linear regression graphs were generated. In addition, the coefficient of variance of the CRP4 assay was generated using data from daily runs of quality control material.
Results: Passing-Bablok and linear regression analysis showed that results produced by CRPL3 and CRP4 kits were comparable. The linear regression equation of Line 1 was CRP4 = 2.399 + 0.9616 CRPL3, and Line 2 was CRP4 = 3.279 + 0.9382 CRPL3. The coefficient of variance of Line 1 for 44 runs of two controls were 2.0% and 2.6%, and Line 2 for 38 runs of two controls were 1.4% and 1.8%.
Conclusion: The produced statistical graphs showed adequate comparability between CRPL3 and CRP4. In addition, the coefficient of variance of the CRP4 assay was acceptable. As a result, no target values required adjustment, and the switch from CRPL3 to CRP4 kits in the automated analysis for CRP concentrations was approved. The CRP4 assay went live on 25 August 2020.

Methodology and clinical interpretation of blood gas analysis

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Objectives: Blood gas analysis (BGA) plays an integral role in laboratory and point-of-care testing for assessing acid-base balance and respiratory physiology. While arterial BGA is the gold standard test for evaluating oxygenation and acid-base disturbances, venous blood gas is being increasingly used as an alternative; both of which provide useful information that aid

clinical decision-making and patient monitoring. Despite BGA's diagnostic value, result accuracy is dependent on several pre-analytical factors, and its usefulness relies heavily on correct result interpretation. The aim of this project was to describe the methodology of BGA and discuss how results can be interpreted clinically using data obtained at Waikato Hospital Laboratory.

Methods: BGA was performed using the Radiometer ABL90 FLEX PLUS analyser. Sample collection involves anaerobic collection of whole blood (arterial/venous) into plastic syringes with lyophilised heparin. Sample quality was checked before measurement and analysis was performed within 30 minutes of collection to ensure accurate results.

Results: Fifty BGA (27 arterial & 23 venous) were performed, of which 2 cases were chosen for interpretation, with 2 additional abnormal sets of results obtained from result log records to cover various disease states, including diabetes, pneumonia, heart failure, and kidney injury.

Conclusion: Interpretation can be difficult, particularly in mixed acid-base disorders; but a stepwise approach can be taken. The overall acid-base status can be determined by reviewing changes in pH, pCO_2 and HCO_3^-, followed by pO_2, O_2 saturation and pCO_2 for evaluating oxygenation and ventilation status. Base excess, anion gap and other derived values also aid in assessing severity and deducing underlying causes. Most importantly, results must be interpreted alongside clinical details and history which may provide clues to the aetiology of acid-base derangements.

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