Evaluation of the CerTest VIASURE *Bordetella* real time BDMAX PCR assay

Grace Adams[1] and Trevor Anderson[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: This study compared and evaluated the current in-house multiplex PCR assay to the CerTest VIASURE RT-PCR BDMAX assay for the detection of *Bordetella* in the clinical microbiology laboratory.
Methods: Sixteen confirmed positive archived (frozen) nasopharyngeal samples with varying bacterial loads for *Bordetella pertussis* (n=8) or *Bordetella parapertussis* (n=8), as well as eight confirmed *Bordetella* negatives were sampled. The twenty-four samples that were previously run on the in-house multiplex PCR assay were run on BDMAX using the CerTest VIASURE RT-PCR assay kit as recommended by the manufacture.
Results: The Viasure BDMAX assay detected eleven of the sixteen positive samples that were previously detected on the in-house multiplex PCR assay. The Viasure assay missed two *B. pertussis* and three *B. parapertussis*; all missed samples were weakly detected on the in-house assay. One *B. pertussis* positive on the in-house PCR assay was *B. holmesii* positive by Viasure PCR.
Conclusion: The study demonstrated that both the in-house multiplex PCR assay and the CerTest VIASURE RT-PCR assay for BD MAX are suitable for diagnosis of a *Bordetella* infection. However, the in-house falsely called the *B. holmesii* positive as *B. pertussis* due to cross-reaction in the PCR target. The CerTest VIASURE assay kit, although less sensitive than the in-house assay, offers more simplicity in terms of the assay setup. The BDMAX assay would be appropriate for use in smaller laboratories where staff lack molecular skills and to allow local rapid diagnostics service.

Validation of sexually transmitted infections PCR Assay on the BD MAXTM System

Swaleha Ali[1] and Paul Ogbuigwe[2]
[1]University of Otago, Dunedin and [2]Molecular Biology Laboratory, Te Whatu Ora – Waikato, Hamilton

Objectives: *Chlamydia trachomatis, Trichomonas vaginalis* and *Neisseria gonorrhoeae* are the top three non-viral organisms responsible for a large portion of sexually transmitted infections (STIs), affecting various individuals worldwide. Rapid detection of these infections can reduce the public health burden. The BD MAX System offers a CT/GC/TV assay, designed for rapid detection of these infections. Waikato Hospital currently tests STI specimens using the Abbott STI PCR Assay on the Alinity M Analyzer. Given the significance of this test and high sample volumes, establishing another method for conducting STI PCR was important in case this analyser became non-operational. This study aimed to validate testing of STI samples collected in Alinity M collection tubes on the BD MAX Analyzer.
Methods: Specimens (n=53) were tested on both analysers. BD MAX collection tubes were provided but to reduce time costs and resources, multiple methods were trialled to determine the most economical and efficient approach. Such methods included trialling different patient sample types, buffer media ratios, swapping buffer medias, direct aliquoting of patient sample to the BD MAX buffer tube and swabbing patient samples.
Results: Using swabs provided by BD MAX Multicollection Kit to transfer sufficient patient DNA from the original specimen to the BD MAX Sample Buffer tube proved to be the best method. Valid results were only obtained from specimens tested using this method, as alternative methods either failed to accurately detect the presence of all pathogens (10.8%) or yielded inhibitory results (32.4%).
Conclusion: Result comparison between different methods, using various specimen swabs, revealed that swabbing consistently generated accurate results, firmly establishing the reliability of this method. These findings demonstrate the BD MAX can serve as a backup testing system for STI PCR at Waikato Hospital.

Optimization of PRAME antibody at LabPLUS

Eva Bell[1] and Regie Inigo[2]
[1]University of Otago, Dunedin and [2]LabPLUS, Auckland

Objectives: Preferentially expressed antigen in melanoma (PRAME) has been involved in various studies demonstrating its use as a biomarker in diagnosis of melanoma. Pathologists at LabPLUS believed incorporating a PRAME antibody test into the routine protocols would be of benefit. The aim of this experiment was to optimize PRAME antibody to be used in supporting the diagnosis of melanoma at LabPLUS.
Methods: Cell Marques PRAME (EP461) was used in this optimization, with all protocols being run on the Leica Bond III machine. Known positive controls were included and the bond polymer refine red detection method was used to identify antibody-antigen complexes via a red precipitate. Adaptations were made to the protocol recommended by Cell Marque to identify the optimal PRAME antibody protocol for LabPLUS. Variables trialled in this experiment included primary antibody incubation time, epitope retrieval method and pH.
Results: A total of 16 slides were examined throughout this experiment with heat induced epitope retrieval quickly being discovered as the only epitope retrieval method to produce positive PRAME staining. The testis control demonstrated consistent positive PRAME staining with appropriate protocols, whilst the SOX10 and known cases of melanoma presented with slight difficultly in consistency. Ultimately the optimized PRAME antibody protocol demonstrated great nuclear intensity and compatibility with current protocols at LabPLUS.
Conclusion: Following this experiment, it was concluded that the optimal protocol for PRAME antibody included a 15-minute primary antibody incubation, heat induced epitope retrieval for 60 minutes and a high pH.

Usefulness of the Sysmex XN 2000 Body-Fluid HF-BF Panel to detect early malignancy in pleural fluid and ascites fluid

Laura Bungard[1], YiiSen Wee[2] and Paul Spek[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: The microscopic examination and cellular analysis of body fluids (BFs) is critical for accurately diagnosing many diseases. BF cell counts have previously been performed manually, but automation allows for faster and more accurate analysis. The BF mode on the haematology analyser, Sysmex XN-2000, differentiates cells into polymorphonuclear and mononuclear white blood cells (WBC) and high-fluorescent cells (HFC). The aim of this study was to evaluate the performance of HFC in the detection of malignant cells in pleural and ascites fluid, and to establish a cut-off value to aid in the early diagnosis of malignancy.
Methods: A total of 200 BF samples, including 42 malignant, from preceding years were reviewed to establish a cut-off value to investigate. Samples received during the permitted time frame were analysed on the Sysmex XN-2000, and if greater than the established cut-off of 4.2/100 WBC, were sent to cytology for

manual microscopy.
Results: In pleural and ascites fluids, malignant cells were not detected by cytological microscopic examination in all samples that were received over the data collection period.
Conclusion: In conclusion, the BF mode on the Sysmex XN could be an alternative method for BF cell counts, with the HF-BF parameter acting as a screening tool to determine whether samples require further investigation by microscopy but has its limitations. Therefore, in cases where the concentration of HF-BF is greater than the cut-off, or there is clinical suspicion of malignancy, additional microscopic review will be required.

Evaluation of Fortress and Bio-Rad TPHA kits as confirmatory assays for Treponema pallidum antibodies

Abbey Burgess[1] and Bronwyn Findon[2]
[1]University of Otago, Dunedin and [2]Waikato Hospital Laboratory, Hamilton

Objectives: Identification of *Treponema pallidum* antibodies are an essential requirement for the diagnosis of syphilis infection. The objective of this study was to assess the performance of two *Treponema pallidum* haemagglutination assays in order to validate a new assay to replace the discontinued Serodia kit, currently in use for the confirmation of syphilis.
Methods: Serum samples (n=40) previously tested at the Waikato Hospital were selected for repeat testing to evaluate the performance of the Bio-Rad and Fortress haemagglutination kits. Previously positive sera (n=30) were selected, including samples from Waikato Hospital, Hamilton Sexual Health, surrounding rural laboratories and the Royal College of Pathologists of Australasia. Negative samples (n=10) were selected based on a negative *Treponema pallidum* immunoassay result. Serodia, Bio-Rad and Fortress procedures were performed on each sample and results were compared.
Results: The Bio-Rad kit demonstrated 100% sensitivity and specificity, detecting the 30 true positives and 10 true negative samples. One hundred percent sensitivity and 90% specificity were achieved with the Fortress kit, detecting 30 true positives, but only 9 of the 10 true negatives. Of the 30 positive results identified using the Bio-Rad kit, three exhibited consistent equivocal findings in contrast to the Fortress kit, where five of the 30 showed repeat equivocal results. Both the Bio-Rad and Fortress haemagglutination methods describe a repeatable equivocal as a positive result.
Conclusion: Both Bio-Rad and Fortress haemagglutination assays provided results concordant with the current Serodia kit. Higher specificity, user friendliness and reagent quality infer Bio-Rad is the best replacement for the current Serodia kit.

Investigating CD61 as a novel marker for identifying vascular invasion in follicular thyroid cancer

Megan Casey[1] and Michael Lau[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Background: Follicular thyroid cancer is a commonly diagnosed cancer with vascular invasion being the predominant route of metastasis, hence vascular invasion is seen as a key marker for diagnosing malignancy. Currently the only way to diagnose vascular invasion in follicular thyroid cancer is through a haematoxylin and eosin (H&E) stain. The study aimed to determine if CD61 could be a relevant marker in helping to confirm vascular invasion in a patient with thyroid cancer.
Methods: Seven most recently diagnosed cases of follicular thyroid cancer and one adrenal cancer case were chosen based on the uncertainty of vascular invasion. Tissue samples were stained with H&E stain using a Leica autostainer XL, and a CD61 monoclonal mouse antibody using the Ventana Benchmark Ultra machine. The resulting stains were viewed and interpreted by a pathologist to determine if the CD61 marker provided a clear view of vascular invasion.
Results: CD61 was successful in demonstrating vascular invasion in the known positive cases of follicular thyroid cancer, while not showing any vascular invasion in cases that were diagnosed as non-invasive from a H&E stain. In the non-invasive cancers, there was still staining of the platelets within the vessels in the capsule, but no breach of the capsule wall into the vasculature was evident.
Conclusion: Implementing CD61 as a novel marker to identify vascular invasion in thyroid cancer could be beneficial in confirming the initial diagnosis made from a H&E stain. Further investigation using more known positive vascular invasion cases and a larger sample size is needed before it can be fully implemented into the thyroid cancer immunohistochemistry panel.
Acknowledgement: The author thanks Spencer Walker for supervision.

Verification of the Aptima BV assay and Aptima CV/TV assay

Katelyn Carter[1], Jenna Paterson[2], Gayleen Parslow[2], James Ussher[1,2] and Juliet Elvy[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Abnormal vaginal discharge is a common reason for women to visit the doctor. The majority of cases are Bacterial Vaginosis, characterised by a change in flora from lactobacilli to anaerobic pathogens including *Gardnerella vaginalis* and *Atopobium vaginae*. Abnormal discharge can also be caused by vulvovaginal candidiasis and *Trichomonas vaginalis*. The aim of this study was to compare two molecular real-time transcription-mediated-amplification assays by Hologic, Aptima BV and Aptima CV/TV, to gold standard methods in women with relevant symptoms.
Methods: A total of 287 specimens with both Aptima and Amies swabs were analysed. Gold standard methods were defined as Nugent scoring for bacterial vaginosis, SAB culture for candidiasis and Aptima *T. vaginalis* assay for *T. vaginalis*. Nugent scoring was performed on Gram stains from Amies swabs with a score ≤ 3 negative, 4 – 6 indeterminant, and >7 positive. Amies swabs were streaked onto sabouraud dextrose agar (SAB) to observe Candida growth. Aptima swabs were run on Aptima BV, CV/TV, and T. vaginalis assays.
Results: The Aptima BV assay performed with specificity and sensitivity of 97.4%, with 97.4% concordance against Nugent scoring. 45 specimens with indeterminant Nugent scores were excluded from these calculations and require further investigation. The Aptima CV/TV assay for candidiasis performed with a specificity of 91.3% and a sensitivity of 96.2% with 92.7% concordance against SAB growth, and 100% specificity, sensitivity, and concordance for T. vaginalis.
Conclusion: The Aptima assays performed excellently compared to current methods. Although cost per assay is more expensive than current reference methods, time saved performing manual gram stain readings is valuable and promotes a shift to molecular testing.

Stability of vitamin B12

Sathu Chandrasiri[1], Heather Hughes[2] and Teresa Wong[2]
[1]University of Otago, Dunedin and [2]Canterbury Southern Community Laboratories, Christchurch

Objectives: At Canterbury Southern Community Laboratories (CSCL), the current levy to add tests for vitamin B12 is 24 hours. This is based on a published study by the World Health Organization in 2002. However, the laboratory receives many test-adds for vitamin B12 beyond 24 hours. This study aimed to

evaluate the stability of vitamin B12, to see whether the results were significant enough to extend the allowable time to add tests. Methods: Twelve patient samples were collected using gold top tubes, then centrifuged to separate the serum. Each sample was aliquoted into four sample cups labelled day 0, 1, 2, and 3. These samples were then frozen periodically over three days. They were all thawed and tested on the same day to eliminate kit variations on the Cobas.
Results: The allowable limit of performance according to the Royal College of Pathologists of Australasia Quality Assurance Programme (RCPAQCP), is + or – 15% of results >120pmol/L, the upper limit. Based on the data collected from days 1, 2 and 3, and comparing each sample to its respective result from day 0, it was evident that all results were within 15% of the allowable limit.
Conclusion: Based on this, we can say that the stability of vitamin B12 is within the allowable limit of performance according to the Royal College of Pathologists of Australasia Quality Assurance Program, for the upper limit. However, further testing with samples of a lower limit <120pmol/L and an increased number of samples is required to make any changes to the allowable test-add time at CSCL.

Application of the GeneXpert in a 3-step algorithm for indeterminate *Clostridioides (Clostridium) difficile* diagnostic results

Ysaure Chemaslé[1], Tina Lusher[2] and Rebecca Lucas-Roxburgh[2]
[1]University of Otago, Dunedin and [2]Med Lab Central, Palmerston North

Objectives: The aim of this project was to implement a three-step testing algorithm for patient samples with indeterminate (GDH positive/Toxin negative) *Clostridioides difficile* results using the GeneXpert Dx System, ensuring higher diagnostic sensitivity and accurate results for clinicians, thereby providing better patient outcomes.
Methods: Samples were collected between March 20th and April 6th 2023. A total of 17 samples had previously been tested using the current GDH/Toxin testing algorithm, these samples comprised four GDH positive/Toxin positive, three GHD negative, and 10 indeterminates. An additional three samples previously tested using an alternative molecular method (Aus Diagnostics faecal PCR) were also included to allow comparison across molecular platforms.
Results: All previously confirmed positive and negative samples yielded the same respective results on the GeneXpert, verifying its function. Two of 10 indeterminate (GDH positive/Toxin negative) samples were PCR positive on the GeneXpert with Ct values ranging from 25.2-28.9; one of these positive samples also had a Binary Toxin Ct value of 24.3. One sample negative for both GDH and Toxins was positive by PCR on the GeneXpert with a Ct value of 34.4.
Conclusion: Introducing the GeneXpert Dx System in a three-step algorithm for confirmatory diagnosis of indeterminate GDH positive/Toxin negative results increases the sensitivity and ensures positive infections are detected giving clinicians more accurate results. In turn, this reduces transmission and upholds infection and prevention controls, thereby improving patient outcomes.

Investigation of the cut-off value for dilute thrombin clotting time

Jessie Chen[1], Aileen Wemyss[2], Nalita Bali[2] and Laura Chen[2]
[1]University of Otago, Dunedin and [2]Middlemore Hospital Laboratory, Auckland

Objectives: The study aimed to establish the cut-off value for dilute thrombin clotting time (DTCT) by performing DTCT and Dabigatran assays on patients on Dabigatran, where performing a Dabigatran assay would be unnecessary as the Dabigatran levels would be below detectable levels.
Methods: Thirty-four samples were collected from patients receiving Dabigatran therapy over a 5-week period. The plasma was aliquoted, and frozen at -35 degrees Celsius after routine testing was completed. Testing was carried out in two batches. Both DTCT and Dabigatran assays were performed on the Diagnostica Stago STA R® Max 2 analyser. A retrospective study was performed using the same reagents and methods due to the relatively few samples collected in this study.
Results: For DTCT results greater than 80 seconds, 10 patients had a Dabigatran assay result which ranged from 42 ng/mL to 413 ng/mL. For DTCT results within the range of 40 to 68 seconds, 10 patients had a Dabigatran level between 11 ng/mL to 82 ng/mL. There were 12 patients in the range of 30 to 38 seconds for DTCT with a Dabigatran level between 3 ng/mL to 25 ng/mL. One patient had a Dabigatran level of 9 ng/mL below DTCT of 30 seconds.
Conclusion: Our study demonstrated that performing a Dabigatran assay on patients who had a DTCT of 30 seconds or less would be unnecessary as Dabigatran levels would be below detectable levels. The retrospective study, which was performed at Middlemore Hospital Laboratory, confirmed the cut-off value for DTCT to be 30 seconds. Due to the relatively few samples collected, further testing is required before implementing a change in the cut-off value for DTCT.

Case Study: Immune acquired anti-D antibody in pregnancy

Yi Xuan Chong[1] and Talalelei Teu[2]
[1]University of Otago, Dunedin and [2]Middlemore Hospital Blood Bank, Auckland

RhD negative individuals who are exposed to the D antigen may be sensitised and start producing anti-D antibodies. This can be through blood transfusions or fetomaternal bleeds during pregnancy where the fetus has RhD positive blood. Production of anti-D in pregnancy is dangerous for the fetus as it can cause haemolytic disease of the fetus and new-born (HDFN). A pregnant woman who was previously admitted to the hospital for her first pregnancy returned three years later with her second pregnancy. She was 37 weeks pregnant and was admitted in preparation for the birth of the second baby. The patient had a history of immune anti-D and the routine antibody screen confirmed that she had immune acquired anti-D antibodies, rather than the usual probable passive anti-D that is the result of routine antenatal anti-D prophylaxis (RAADP). The baby was slightly jaundiced but healthy, and further testing and an elution determined that maternally acquired anti-D was bound on the surface of the baby's red cells. True immune acquired anti-D is uncommon because any situation where there is a risk of fetal cells in the maternal circulation indicates that a dose of anti-D is offered to the mother. Therefore, the likely explanation for the anti-D is that the patient had a miscarriage or bleed between pregnancies that she did not know about. Due to a gap in the patient's medical records, it is difficult to determine the exact cause of sensitisation that produced the anti-D.

Validation of the BioFire FilmArray Torch Gastrointestinal (GI) panel

Tiffany Gunn[1] and Tony Barnett[2]
[1]University of Otago, Dunedin and [2]MedLab South, Nelson

Objectives: This verification was done to test the accuracy, precision, sensitivity and specificity of the BioFire FilmArray Torch Gastrointestinal (GI) Panel for the identification of gastrointestinal pathogens in inpatient faecal samples received during the weekend.
Methods: This was achieved by first testing a negative faecal sample to establish a base solution in which to spike with positive

synthetic genetic material, and to ensure no contamination was present within the instrument or reagents. Then, two synthetic positive control samples were run, as well as a positive faecal sample, and a 1 in 4 dilution of the positive faecal sample. Each panel was set up and run by a different laboratory staff member to test for potential user to user variation.
Results: The validation results showed adequacy, with all positive control samples outputting results as expected. Only one result was unexpected, which was the initial attempt at a negative control sample. However, this unexpected result has led to the validation of the panel being able to differentiate *E. coli* strain O157 from other pathogenic strains of *E. coli*, which was an aspect not verified by any of the positive controls. The failed negative control also additionally uncovered an unexplained false positive to or low-level detection of Norovirus, which was unable to be confirmed by another molecular method
Conclusion: The verification showed that the BioFire FilmArray Torch GI Panel is able to be used efficiently with inpatient faecal samples, has the ability to identify all pathogens listed in the panel, can identify pathogens at 25% of the ideal concentration, and has no risk of user-to-user variation.

Kleihauer testing requests in Canterbury Health Laboratories; audit of testing and alignment with ANZSBT guidelines

Margaret Lilley[1,2] and Catherine Neal[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: The Kleihauer test quantifies the fetomaternal haemorrhage in RhD-negative mothers to determine the dosage of RhD immunoglobulin (anti-D). It is important to give anti-D to RhD-negative mothers to prevent alloimmunisation that could cause haemolytic disease of the fetus and newborn in current/ future pregnancies. This study aimed to evaluate Canterbury Health Laboratories standard operating procedures against the Australian and New Zealand Society of Blood Transfusion guidelines.
Methods: Data from past Kleihauer tests performed at Canterbury Health Laboratories across two different quarters (November 2021 to January 2023 and November 2022 to January 2023) was used to assess if Canterbury Health Laboratories standard operating procedures are in line with the Australian and New Zealand Society of Blood Transfusion guidelines. Data was collected using the Requesting Form Identifier and Eclair.
Results: On average, 33.65% of the Kleihauer tests performed at Canterbury Health Laboratories across the two quarters were not recommended based on the Australian and New Zealand Society of Blood Transfusion guidelines. The majority of the not recommended Kleihauer tests performed at Canterbury Health Laboratories were for antepartum haemorrhage and abdominal pain in RhD-positive mothers.
Conclusion: A third of the Kleihauer tests performed at Canterbury Health Laboratories each quarter are not recommended by the Australian and New Zealand Society of Blood Transfusion guidelines. This should prompt Canterbury Health Laboratories to review and update their Kleihauer test standard operating procedure. A clinician will need to oversee and give input to ensure the proposed standard operating procedure is appropriate before being put into practise.

Prostate-specific antigen staining in poorly differentiated prostate adenocarcinomas

Celeste Linley
University of Otago, Dunedin

Objectives: NKX3.1 is a prostate specific gene located on chromosome 8p. NKX 3.1 has been shown to be positive in most primary prostatic adenocarcinomas as well as being highly sensitive and specific for high grade prostate adenocarcinomas. The aim of this study was to optimise NKX3.1 antibody within our laboratory and compare its performance to Prostate-specific antigen (PSA) staining in poorly differentiated/ high grade prostate cancer (adenocarcinomas).
Methods: Tissue samples (n=4) from four cases of poorly differentiated/ high grade prostate adenocarcinoma were selected and PSA staining was rerun as well as NKX 3.1 staining. This IHC staining was run on a Ventana Benchmark Ultra using an Optiview detection kit. PSA staining was carried out using a protocol optimised within our laboratory. For NKX 3.1 staining, the NordiQC recommend protocol was used.
Results: In two of the cases, a majority of cells stained positively for PSA and NKX 3.1. For these cases similar staining patterns were seen, although it did appear that NKX 3.1 was staining slightly more cells than PSA. The other two cases had weak or patchy PSA staining but a majority of cells still stained positively for NKX 3.1.
Conclusion: These results suggest that the NKX 3.1 staining is useful for identifying cells of prostate origin even when PSA expression has been decreased or lost. Due to this added sensitivity, NKX 3.1 could be beneficial with PSA for identifying high grade/ poorly differentiated prostate adenocarcinoma.
Acknowledgement: The author thanks staff at Medlab Central, Palmerston North for their supervision and support of this project.

Optimization of RNA scope for identification of TP53 isoform Δ133p53β in macular degeneration

Kyle McLean, Harrison Dolan, Francesc March de Ribot, and Tania Slatter
University of Otago, Dunedin

Objectives: The primary cause of blindness in patients over 65 years is age-related macular degeneration (AMD) which affects over 196 million people worldwide. AMD pathogenesis involves several complex retina changes not fully understood, involving abnormal blood vessel growth, fibrosis, and inflammation. P53 isoforms are associated with an increase in inflammation and VEGF production. The basis of this study was to optimise an RNAscope method to determine if the TP53 isoform, Δ133p53β, was increased in a model of AMD.
Methods: A cell line model of macular degeneration was used with serum-starved retinal epithelial cells (RPE). Different preparations of RPE cells (serum-starved and non-serum starved) were made. Slides were stained using the RNAscope 2.5 HD Detection Reagent – RED method. The probes used were UBC (positive control), DapB (negative control) and Tp53 (towards Δ133p53β). The protocol was optimised through alterations in the standard procedure, which included additional time for protease and AMP5 hybridisation steps. Slides were visualised using digital pathology, positive cells counted, and the percentage of positive cells compared between serum starved and non-serum starved cells.
Results: The method was optimised for RPE cells, as evidenced by strong positive staining in the UBC control and negative staining with the DapB probe. Δ133p53β staining was evident in the AMD model of serum-starved cells and not in the control.
Conclusion: The RNAscope method in relation to RPE cells was successful. Further work is required to determine if Δ133p53β was increased in the model of AMD and if it contributes to inflammation.

Comparison of a one-point calibration system with a six-point calibration system for the Opiate assay performed on the Roche Cobas c502 analyser

Abi McCullagh[1], Sian Horan[2] and Christian Christian[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Drugs of abuse testing is performed on the Roche Cobas c502 analyser. The opiate assay is currently programmed

to perform a six-point calibration and the c502 analyser reports results as a numerical value (semi-quantitative). The purpose of this project was to assess the implications of a one-point calibration system (rendering the assay qualitative) and investigate how it affects patient results when compared to the current system and confirmation testing.

Methods: Patient samples (n=30) were re-tested on the c502 analyser using the six-point calibration system and the new one-point system. All samples were sent to the liquid chromatography-tandem mass spectrometry laboratory for confirmation testing. Statistical analysis was used to assess the performance of the one-point calibration system in comparison to the six-point calibration using the confirmation results from the liquid chromatography-tandem mass spectrometry laboratory.

Results: The sensitivity of the one-point method increased to 100% (six-point method had a sensitivity of 86.6%). The specificity remained unchanged (86.6%). The assay precision was comparable to both methods; the coefficient of variation changed by only 1% for both positive and negative quality control material tested daily over a period of ten days.

Conclusion: Patient results will not be compromised if the one-point method is implemented. All positive samples are sent to the liquid chromatography-tandem mass spectrometry laboratory for confirmation testing so, if there are false positives, these will be detected and corrected. A one-point calibration will also save time in terms of preparation and money.

Comparison of combination disc tests with different concentrations for the detection of extended-spectrum β-lactamases

Rei Miyamoto[1], Catherine Gordon[2] and Bronwyn Davison[2]
[1]University of Otago, Dunedin and [2]Canterbury SCL, Christchurch

Objectives: To compare the performance of confirmatory combination disc tests using cefotaxime 5µg and ceftazidime 10µg discs with and without clavulanic acid 10µg, with the cefotaxime 30µg and ceftazidime 30µg discs with and without clavulanic acid 10µg, in extended-spectrum β-lactamase (ESBL) detection at Canterbury SCL.

Methods: ESBL-positive Enterobacterales (n=34) and ESBL-negative Enterobacterales (n=31) were used to carry out the combination disc tests. For each isolate, a bacterial suspension was inoculated across two Mueller Hinton Agar plates and incubated according to the MAST combination disc kit manual. Each set consisted of a plate with MAST® cefotaxime 5µg and ceftazidime 10µgdiscs with and without clavulanic acid 10µg, and a plate with MAST® cefotaxime 30µg and ceftazidime 30µg discs with and without clavulanic acid 10µg. The inhibition zone sizes were measured and recorded in Microsoft Excel, then analysed using RStudio version 4.3.0.

Results: For both combination disc sets, the sensitivity was 100%. The specificities of the new and current discs were 90% and 93%, respectively. They both displayed good repeatability and there were no statistically significant differences. The ranges of zone differences between the ESBL-positive and negative isolates in both combination discs were sufficiently distinct to be used confidently in the laboratory. ESBLs could not be consistently detected with these combination discs alone in isolates with AmpC β-lactamase or carbapenemase coproduction.

Conclusion: The overall performance of the cefotaxime 5µg and ceftazidime 10µg discs with and without clavulanic acid 10µg were similar to the discs with 3µg of cefotaxime and 30µg of ceftazidime. This allows standardised detection and other susceptibility testing using the same antimicrobial concentrations.

Stability of ionised calcium measured in vacutainer tubes compared to blood gas syringes, and the potential differences in measured whole blood versus plasma

Nasrin Neyyan[1], Sian Horan[2], and Christian Christian[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Ionised calcium (iCa) reflects true calcium homeostasis, and thus measurements for the iCa are increasingly requested in patient care. Smaller laboratories are often unable to do an immediate analysis of the iCa due to the method's unavailability, analytical performance, and stringent sample collection and handling. The study aimed to compare the measurement of iCa in heparinised whole blood syringes to vacutainer tubes, and to compare the iCa concentration of whole blood to plasma. The study can help to determine whether samples can be sent from remote laboratories to Dunedin for analysis of iCa.

Methods: The study was conducted on 18 subjects from Southern Community Laboratories, Dunedin, on both blood gas syringes and lithium heparinised tubes to compare the stability of iCa. The measurement was done on heparinised whole blood syringes, whole blood, and plasma (immediate, 24 hours and 48 hours), and the plasma samples were further divided into two Hitachi cups and stored at 40C and –200C. Measurements were done using a direct Ion Selective Electrode (ISE) blood gas analyser ABL900. The level of statistical significance was set at a P-value of <0.05 and clinical significance was determined by comparing the difference to assay performance specification of ionised calcium (4%) criteria established by the Royal College of Pathology of Australasia.

Results: The study showed a statistically and clinically significant difference in all the sample distributions compared to the whole blood gas syringe for iCa.

Conclusion: The results suggest using a heparinised whole blood syringe for analysis of iCa. It is not recommended to send samples from remote areas for analysis of iCa.

Secretor status and susceptibility to IgG ABO antibody-associated haemolysis

Annabel Parker[1] and Krishna Badami[2]
[1]University of Otago, Dunedin and [2]New Zealand Blood Service, Christchurch

Objectives: Intravenous immunoglobulin (IVIg) is used to treat a variety of disorders. Haemolysis is a complication of IVIg, especially in non-O (A, B, AB) group individuals. This is due to the presence of IgG anti-A, anti-B, and anti-AB in IVIg. Haemolysin-positive plasma poses a similar risk. This in-vitro study aimed to investigate secretor substance as a possible mitigation of haemolysis due to IgG ABO antibodies.

Methods: IVIg or haemolysin plasma (containing high-titre IgG anti-A), fresh group AB serum as a complement source, and serum with or without secretor substance was tested for haemolysis against group AB red cells. Appropriate controls were used. As Lewis phenotype is correlated to secretor status, samples were phenotyped to determine the presence of secretor substances. Haemolysis was detected using an adapted haemolysin screening method and semi-quantified using cell button width. Direct antiglobulin testing (DAT) was performed to investigate extravascular haemolysis.

Results: Haemolysin plasma induced haemolysis in 8/8 samples containing non-secretor serum and 12/16 secretor serum samples. The relative risk of haemolysis in samples containing non-secretor serum was 1.33 times higher than in samples containing secretor serum. However, IVIg did not induce haemolysis, regardless of the secretor status of additional serum. The DAT results with IVIg were positive using both group AB and O RBC.

Conclusion: In-vitro haemolysin testing showed that secretor substance potentially conveys a lower risk for IVIg-, or haemolysin plasma-related haemolysis. Results using IVIg were inconclusive. Explanations for the IVIg results may be relatively low titres of IgG ABO antibodies and, for the DAT result, the presence of anti-D.

Investigation of INNOVANCE® D-dimer QC reagent stability past expiration date

Anjali Raju[1]
[1]University of Otago, Dunedin

Objectives: This experimental study aimed to examine whether the stability of the INNOVANCE® D-dimer quality control reagents can be extended to an extra seven days past the expiry date as originally stated by the manufacturer.
Methods: A vial of D-dimer QC material was reconstituted according to manufacturer's directions and used to perform a quality control assay on the Sysmex CS 2500 automated coagulation analyser. The QC assay was performed twice daily from days 1-7 (the stability period specified by manufacturer). The same QC vial was then assayed for an extended 7 days, with the same procedure of performing two QC assays per day from day 8-14. This method was used to analyse seven vials each of D-dimer QC1 and QC2. The results for each vial from days 1-7 were compared to days 8-14 to evaluate whether there was a significant difference in the assay values in the days after the QC set expired.
Results: The mean D-dimer concentration for all 7 vials of INNOVANCE® D-dimer QC1 and QC2 measured on the days after expiration met the limit of allowable performance set by the Royal College of Pathologists of Australasia. However, further statistical analysis showed increased variability of the data, suggesting there were procedural errors in the experimental phase of this study.
Conclusion: This study demonstrated QC results obtained from assays using expired QC reagents for D-dimers were within acceptable limits. However, due to the variability and experimental errors, the results were not reliable. Further testing is required to validate the results.
Acknowledgement: The author thanks staff at Canterbury Southern Community Laboratories for their and supervision and support of this project.

Shortened erythrocyte sedimentation rate evaluation in community patients

Jacinta Richardson[1] and Nicole Keegan[2]
[1]University of Otago, Dunedin and [2]Taranaki Pathology Services, New Plymouth

Objectives The erythrocyte sedimentation rate (ESR) test is most commonly used for monitoring and diagnosis of inflammatory conditions. The aim of the study was to identify whether a final erythrocyte sedimentation rate (ESR) result can be estimated based on values from earlier set times.
Methods: Demographics and clinical details were collected from patients who had ESR requests sent to Taranaki Pathology services between the 5th of April and the 24th of May 2023. Once the ESRs for these patients were set up, the results were recorded at 15, 30, 45, and 60 minutes. These results were then graphed to see if there was a relationship between the final result, and the values collected at earlier times.
Results: The mean age +/- standard deviation (SD) of the patients was 64.3 +/- 17.4. Thirty-six percent of the patients were male and 64% were female. The mean ESR value at 15 minutes was 1.2 (SD+/-1.8), the 30-minute mean was 5.2 (SD+/- 5.7), the 45-minute mean was 11.4 (SD+/- 11.3), and a 60-minute mean was 19.9 (SD +/-17.3). A statistically significant correlation was found between 15 and 60 minutes (r=0.731), and 30 and 60 minutes (r=0.943). However, the most statistically significant correlation was between 45 and 60 minutes (r=0.979). In order to estimate the 60-minute (y) value based on the 45-minute (x) value the following equation can be used: y=1.5x + 2.8. The 45-minute estimation was the most reliable regardless of age, gender, and if the end result was normal or not.
Conclusion: Sixty-minute ESR values can be predicted based on 30-minute and 45-minute estimations. This finding may be helpful in cases where an ESR result is time critical.

Stemcell EasySep positive cell selection kits for isolation of T cells and granulocytes for chimerism analysis

Sneha Sathish[1], Michelle Burns[2] and Geraldine Duncan[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: Chimerism analysis is performed separately on myeloid and lymphoid cells to determine the success of a stem cell transplant. EasySep cell selection kits offer a more rapid method to isolate cells from smaller volumes of blood than the current method. This study reports the first trials of the EasySep CD3 and CD66b+ kits for isolating T cells (lymphoid) and granulocytes (myeloid) respectively.
Methods: EasySep selection kits isolate cell populations using antibody complexes and magnetic particles. Purity was determined by flow cytometry and DNA was extracted using a Qiagen kit. The DNA was used as a template for PCR using the Identifiler kit. Initially, cells were isolated from blood with each kit, and cell numbers/purity were checked, DNA extracted and chimerism PCR performed. Chimerism results (% donor) for T cells and granulocytes from two patients were compared using cells prepared by the current method or the EasySep kits.
Results: Cells were isolated from 2 ml of blood and sufficient DNA obtained for the chimerism PCR. Over several experiments flow cytometry showed that cell populations were >99% pure T cells or granulocytes. The kits also successfully isolated pure cell populations from blood two days after collection which is important for non-Christchurch patients. Percent donor results for T cells and granulocytes from two patients were similar for cells isolated by the current method or EasySep kits.
Conclusion: Both kits isolated >99% pure T-cell and granulocyte populations. The EasySep CD3 and CD66b+ kits are easy to use, rapid (30 minutes hands-on), require small blood volumes, and are relatively inexpensive. Further parallel testing will now be conducted for patients with chimerism analysis requests.

Validation of the N435 and XN25 VITEK 2 AST cards

Johnny Slade[1], Koen Van Der Werff[2], Rachel Roth[2]
[1]University of Otago, Dunedin and [2]Wellington Southern Community Laboratories, Wellington

Objectives: This project aimed to validate the performance of two new Vitek 2 cards (N435 and XN25) for antimicrobial susceptibility testing (AST). This project aimed to determine whether the new cards are viable options for routine AST based on the reproducibility of expected results.
Methods: Isolates (n=37) from various gram-negative species with a range of known resistance phenotypes were used. The N435 card validation compared the resistance determination to the current standard N311 AST card, as both cards contain a similar selection of antimicrobials at different dilutions. The validation of the XN25 was done using manual disc diffusion and measuring zones of inhibition to compare to the resistance determinations from the card.
Results: Of the 11 drugs analysed in this project, five yielded identical results to their reference standard. The results from the cefepime and ciprofloxacin of the N435 card, and the ceftolozane/tazobactam of the XN25 card showed an increased number of resistances detected relative to their reference method. We hypothesise that this is a result of increased accuracy due to dilutional changes closer to the calling ranges, but it may affect the clinical outcomes and treatment availability in some cases if these are false positives.
Conclusion: Future investigations using the gold standard microbroth dilution and E-test strips and utilising more bacterial species and resistance phenotypes are required to finalise the

validation of any discrepant results before these cards become used routinely in the WSCL microbiology laboratory.

The stability of gentamicin over time and effects on EDTA-mediated platelet clump dissociation

Judea Smith[1] and Linda Williams[2]
[1]University of Otago, Dunedin and [2]Canterbury Southern Community Laboratories, Christchurch

Objectives: Gentamicin is an antibiotic used to dissociate platelets in platelet clumped samples. This study investigated the effectiveness of gentamicin over time and the ability to extend the stability of open gentamicin over its 24-hour limit.
Methods: The EDTA tubes and corresponding citrate tubes (if available) of 14 patients with known platelet clumping were tested. Mixed patient sample (400uL) was pipetted into a tube and mixed with 200uL of gentamicin. After 15 minutes at room temperature, the sample was remixed and manually sampled through an XN20 unit. The diluted platelet count was obtained by multiplying by 1.5 to correct for dilution. The diluted WBC, RBC and HGB values were also multiplied by 1.5 and compared to the undiluted values. If the diluted values were within 5% of the undiluted values, the platelet count was accepted. A blood film was prepared and examined for successful platelet dissociation. This method was repeated hourly for 6 hours using one gentamicin vial and daily for 5 days (120 hours) using one gentamicin vial and repeated using a new gentamicin vial as a control.
Results: Our analysis showed significant increases in platelet counts when gentamicin was added to EDTA tubes over 6 hours and added to EDTA and citrate tubes over 5 days, with similar patterns in results between both open gentamicin and new gentamicin. However, blood films of EDTA samples with gentamicin from the same vial, revealed platelet clumps were present at hour 0 and hour 24 then began to appear again by hour 120.
Conclusion: This study demonstrated that the effectiveness of gentamicin can be extended beyond the current 24 hour limit.

Verification of the Cobas 4800 HBV viral loads on the Cobas 6800

Brandon Su[1] and Megan Burton[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Wellington

Objectives: The performance of the Cobas 6800 Hepatitis B virus quantitative DNA assay was evaluated and compared to that of the Cobas 4800. This was to verify the use of the Cobas 6800 for the quantification of hepatitis B viral loads at Southern Community Laboratories (SCL), Wellington.
Methods: The specificity, sensitivity, and agreement were assessed by comparing the results of hepatitis B viral loads from historic samples evaluated by the Cobas 4800 to the results obtained when tested by the Cobas 6800. Precision was assessed through a serial dilution of a sample with a high viral load tested in triplicate to assess the variability in the results at three titres.
Results: Cobas 6800 hepatitis B DNA results were linear across the tested range of 1.0 log IU/ml to 8.4 log IU/ml when compared to results from the Cobas 4800. There was excellent agreement between results from the two systems, with a mean difference of -0.01 log IU/ml. Among the 116 samples tested, all samples except for two were correctly identified as detected or not detected for hepatitis B. Both incorrectly identified samples were false negatives at the limit of detection for the assay. At the investigated titres, there was great precision within and between runs, with the coefficient of variation for inter and intra-run variation all below 5% for their respective titres.
Conclusion: The Cobas 6800 hepatitis B assay displayed a high level of agreement and precision when compared with the Cobas 4800. Thus, the Cobas 6800 is suitable for testing hepatitis B viral loads at SCL, Wellington.

Comparison of two different analysers for the screening of syphilis

Mosie S'ua[1] and Helen Vanderloo[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: To compare and contrast the screening test for syphilis (detection of the bacterium, *Treponema pallidum*) between two different analysers supplied by different manufacturers.
Methods: The Alinity i-series (supplied by Abbott) is the currently used analyser at SCL Dunedin and reference point for this research. Patient samples (n=116) that were previously screened on the Alinity were re-run on the Liaison XL, supplied by DiaSorin, to see if the two analysers gave concordant results.
Results: Of 116 samples, a total of 17 discrepant outcomes were identified between the two analysers. Specifically, these results displayed a low positive signal on the Alinity analyser, indicated by values equal to or exceeding 1.0 S/CO. However, when compared to the Liaison XL analyser, the majority of these discrepant results (15 out of 17) exhibited negative signals, falling below the threshold of 1.0 S/CO. Notably, the remaining 2 discrepant results obtained from the Liaison XL analyser yielded equivocal signals, ranging between 0.9 and 1.0 S/CO.
Conclusion: The Alinity analyser seemed to be more sensitive than the Liaison XL, picking up a few false positives from time to time. However, it is preferred that the method of screening has a high sensitivity. The Liaison XL analyser was, for the most part, concordant with the Alinity for the vast majority of patient samples and was actually more accurate when it came to the 17 discrepant results obtained. It seems the Liaison would be a good alternative to the Alinity if a backup screening test for syphilis is ever needed, albeit a larger sample size would need to be tested to see the true measure of agreement between these two analysers.

Determining the optimal input DNA concentration range for AmpFLSTR® Identifiler™ assay

Harry Ward-Hayes[1] and Amanda Dixon-McIver[2]
[1]University of Otago, Dunedin and [2]Igenz, Auckland

Objectives: Forty randomly selected buccal swabs were retrospectively analysed to determine an optimal DNA concentration range for the AmpFLSTR Identifiler assay to generate complete DNA profiles.
Methods: DNA profiles obtained from the samples previously run using the AmpFLSTR Identifiler assay, were categorised as Passed (obtained a complete profile), Rerun (required a rerun with adjusted test parameters to obtaining a complete profile) or Fail (where no or an incomplete profile was obtained). DNA concentration was quantified on the QUBIT and quality was assessed on the Nanodrop to determine an optimal range which would reduce the number of re-runs and fails. Other factors such as sex, age and collection location were also examined to determine if they affect the DNA profile outcome.
Results: The existing protocol using a standard 1 in 15 dilution had a 62.5% pass rate, along with 20% Rerun rate and 17.5% Fail rate. DNA concentration ranged from 0.263 ng/uL to 6.54 ng/uL with no clear correlation between DNA concentration and the ability to yield a complete DNA profile. Quality was assessed using absorbance ratios 260/280 and 260/230, and values between 1.08 and 1.88 and 0.25 to 1.07 were obtained, respectively. Again, with no correlation to pass rates. There was also no correlation to pass rate between age, sex or collection location found.
Conclusion: This investigation found there was no optimal DNA

concentration range which could be implemented to reduce the number of failed samples and in turn to reduce cost and time to generate a complete profile and obtain a result for the client. Collection location, age and sex did not appear to have an impact on pass/failure rate.

The impact of various pre-analytical centrifugation settings on ABO/D typing

Chloe Williams[1] and Amy Christie[2]
[1]University of Otago and [2]New Zealand Blood Services, Dunedin

Objectives: The current pre-analytical centrifugation setting at Dunedin Blood Bank is 1000 RCF for a duration of 5 min. This research investigates the impact different centrifuge settings have on the analytical quality of samples for card ABO/D typing. By conducting this research, the aim is to further reduce the centrifuge duration. Reducing the duration could potentially decrease the laboratory turnaround time.
Methods: Using the original centrifuge setting as a control, four other centrifuge settings were chosen based on guidance from the manufacturer's literature of the tubes used. The four other chosen settings were as follows: 1300 RCF for 10 min; 2000 RCF for 3 min; 3000 RCF for 3 min; and 3500 RCF for 3 min. These were evaluated using five samples from three healthy individuals. The analytical quality of both manual and automated card ABO/D typing was assessed.
Results: None of the five chosen centrifuge settings compromised the analytical quality of manual or automated card ABO/D typing among the three individuals. Each manual ABO/D result was compared to results obtained from the control centrifuge conditions. Furthermore, the automated system successfully tested and accepted samples spun at each centrifuge setting.
Conclusion: The chosen centrifuge settings delivered identical results in manual and automated card ABO/D typing for each healthy individual. This suggests the possibility of reducing the duration to 3 min for a broader population of healthy individuals. However, implementing a different centrifuge setting requires further investigation involving a larger and more diverse sample population, including both patients and healthy individuals. Additionally, future research should encompass a comprehensive range of blood bank tests beyond just ABO/D typing.