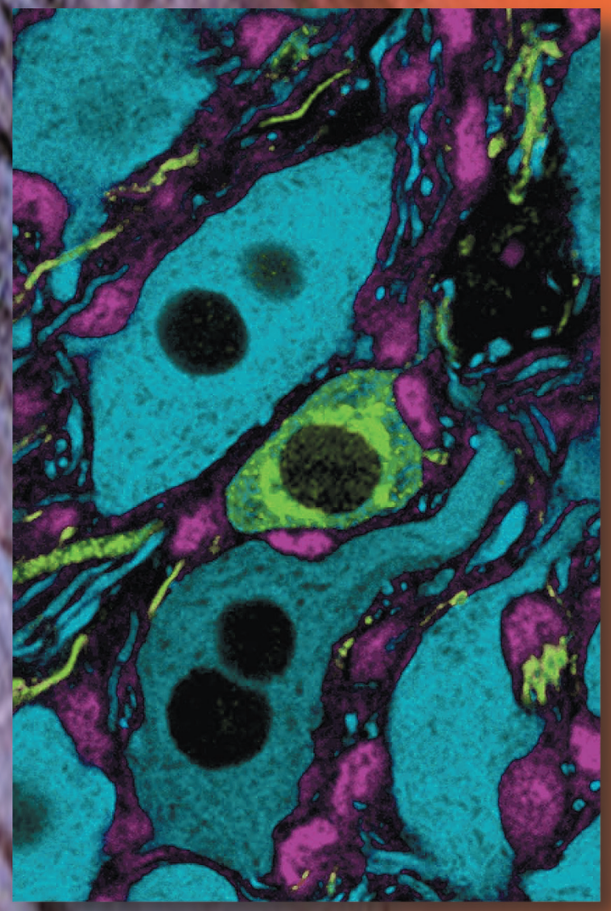
FEBRUARY 2022 VOL. 43 No. 1 Pages 1 - 37 AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

AUSTRALIAN JOURNAL OF
Medical Science
2022



February 2022
Vol. 43
No. 1

https://www.testo.com/en-AU/products/testo-190

Australian Journal of

Medical Science

ADMINISTRATION

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The Australian Journal of Medical Science is the official publication of the Australian Institute of Medical and Clinical Scientists (AIMS).

Circulation 2000 per issue. The Journal is circulated to members in pathology laboratories, universities and research institutes throughout Australia and overseas.

Annual subscription rates are available from AIMS National Office.

Article reprints may be organised on request from AIMS National Office.

Advertising rates are available from AIMS National Office.

Abstraction of the Australian Journal of Medical Science is through the following serial catalogue listings: Australasian Medical Index, Chemical Abstracts, and EMBASE/Excerpta Medica.

The Australian Journal of Medical Science is included on the Australian Research Council ERA 2018 Journal List.

ISSN 1038-1643

Printed by Westminster Eagle Eye Printing, PO Box 161, Paddington Qld 4064.

Design Cover and layout design by Kim Brown, 23 Denman St Exeter SA 5019 kimbo@internode.on.net. Cover photograph courtesy of Prof Ian Gibbins, Flinders Medical Centre, Adelaide.

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ORIGINAL ARTICLE

Immunofluorescence microscopy for the diagnosis of autoimmune blistering skin diseases

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Abstract

Autoimmune blistering skin diseases (AIBDs) are an uncommon and heterogeneous group of skin disorders characterised by painful blistering lesions and autoantibodies directed at skin antigens. These may be investigated by various techniques including fluorescent microscopy. We examined the diagnostic utility of direct (DIF) and indirect (IIF) immunofluorescent microscopy for these disorders. We reviewed samples over a 12- month period that were submitted to our diagnostic laboratory for serum skin antibodies (IIF) and corresponding skin biopsies (DIF). Diagnostic statistics were calculated according the clinicopathological diagnosis established in multidisciplinary meetings. There were 86 patients who had concurrent DIF and IIF requests in the 12-month period. The sensitivities for DIF and IIF were 96.4% and 85.7%, and specificities were 79.0% and 74.7% respectively for any AIBD diagnosis. Fluorescent microscopy is a good screening technique to identify AIBDs with high diagnostic sensitivity.

Keywords: antibody, autoimmune bullous skin diseases, bullous diseases, immunofluorescence, microscopy

Introduction

Autoimmune blistering skin diseases (AIBDs) are disorders characterised by painful blisters on mucosal and non-mucosal skin. As autoimmune disorders, they are often characterised by detection of skin autoantibodies in the diagnostic laboratory. Accurate diagnosis of AIBD rely, in part, on detection of autoantibodies against skin structural proteins. This may be investigated through microscopy via the detection of autoantibodies by indirect immunofluorescence (IIF) microscopy on tissue substrates such as monkey oesophagus, or directly (DIF) on skin biopsies. AIBDs may be broadly divided into pemphigoid skin diseases (characterised by antibody and/or complement at the dermoepidermal junction) and pemphigus diseases and their variants (antibody and/or complement intercellularly between keratinocytes) (Witte *et al* 2018) (Figure 1). The main antigenic targets include BP180 for pemphigoid diseases and desmoglein 1 and 3 for the pemphigus disorders with various techniques, such as enzyme-linked immunosorbent assay (ELISA), available to confirm antibody specificity (van Beek *et al* 2018). We decided to compare the diagnostic value of these differing microscopic techniques in the diagnosis of AIBDs which has not yet been done previously in the literature.

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Materials & methods

All requests for both IIF and DIF on biopsies were identified from our laboratory records within a 12-month period from 2019-2020. Our laboratory services all paediatric and adult patients in the state of South Australia, Australia. IIF was performed on monkey oesophagus substrate (Euroimmun, Lübeck, Germany) using serum diluted in 1:10 as a screening titre and end-point titration performed. “False positives” from sera reacting with red blood cell A and/or B antigens were excluded by introducing a blocking mixture

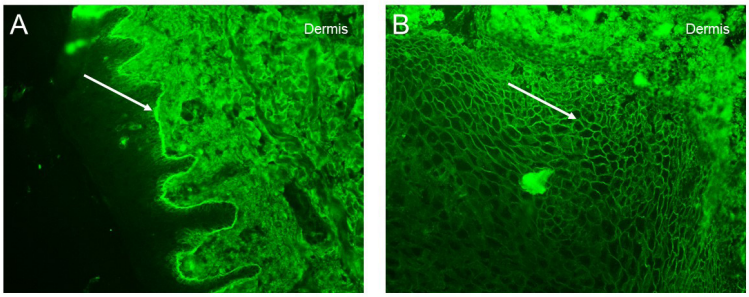

Figure 1. *Detection of antibody on frozen skin sections via direct immunofluorescence microscopy. Epidermal IgG is detected along the dermoepidermal junction in bullous pemphigoid disorders (arrow) (A) and in the intercellular regions of epidermal keratinocytes in bullous pemphigus disorders (arrow) (B). Images are taken at original magnification 100x (A) and 200x (B).*

of A and B antigens prior to sera incubation with substrate (Lee *et al* 2010). Anti-human IgG conjugated to fluorescein isothiocyanate (FITC) was used as the detection antibody. DIF on snap-frozen fresh punch biopsies were cut at 4 µm sections. Antibodies to immunoglobulins (IgG, IgA, IgM), complement (C1q and C3) and fibrinogen, conjugated to FITC, diluted at 1:10 were incubated with the tissue.

Medical records were reviewed for diagnoses. The final diagnoses were determined by integrating pathological findings with clinical presentation in multidisciplinary meetings. Simple descriptive and diagnostic statistics were performed using SPSS statistical software package. Ethics approval to perform this retrospective study was granted by the South Australia Local Health Network Clinical Research Ethics Committee (No. 39.034).

Results

Within a 12-month period, there were 86 requests for concurrent skin antibodies via IIF and DIF methods retrospectively identified. Fifty-four patients were female (62.8%) and the average age ± standard deviation was 71.0 ± 16.7 years. There were 31 AIBDs (36.0%) comprising of 20 pemphigoid, 10 pemphigus and one dermatitis herpetiformis cases.

Using the gold standard clinicopathological diagnosis, the sensitivities for DIF and IIF were 96.4% and 85.7%, and specificities were 79.0% and 74.7% respectively for AIBDs (Table 1). These values are similar to another investigation that found these methods have high sensitivity for AIBDs diagnosis (Buch *et al* 2014). The positive predictive values for DIF and IIF were 69.2% and 55.8%, and negative predictive values were 97.8% and 77.7% respectively. With IIF and DIF taken together, a positive result has excellent sensitivity (96.9%) but moderate specificity (66.7%) for AIBDs.

Overall agreement (support for the same diagnosis) for both methods was 72.7%. Cohen's κ for the presence or not of AIBD was 0.481 indicating only moderate overall agreement between the two methods. When examined according to diagnoses, DIF had good agreement with histology for pemphigoid (Cohen's κ = 0.771) and very good for pemphigus AIBDs (κ = 0.941). For IIF, there was good agreement between IIF and histology for pemphigoid κ = 0.685) and poor for pemphigus AIBDs (κ = 0.132).

Discussion

AAIBDs are a diverse group of skin disorders that have a large range of immunological investigations to support diagnoses and monitor disease activity. IF microscopy is relied on heavily and in this brief investigation, we have evaluated the diagnostic performance and agreement between IIF and DIF techniques for AIBDs. Although there is only moderate level of agreement between these two microscopy techniques when they are considered together there is high sensitivity for a correct AIBDs diagnoses. ELISAs and related assays may help to identify the precise antibody and compared to immunofluorescent microscopy have very high specificities for the diagnosis of pemphigoid and pemphigus skin conditions (Keller *et al* 2016; Kulkollakarn *et al* 2008). Furthermore, these quantitative assays may assist with monitoring disease activity since some levels of antibodies correlate with disease severity.

Autoantibodies may predate the emergence of clinical autoimmune disease (Eriksson *et al* 2011; Lee *et al* 2021) and circulating skin antibodies may also be present before AIBDs (Kridin and Bergman 2018). This is a possible reason for false positive IIF results and reduced specificity of this method for AIBDs. The prozone effect, where there is antibody excess relative to the amount of antigens due to high serum concentrations, has also been observed in IIF (Mathai *et al* 2020). This is caused by suboptimal binding

Table 1. *Diagnostic statistics for autoimmune blistering skin diagnoses*

	Direct immunofluorescence	Indirect immunofluorescence
Sensitivity (%)	96.4	85.7
Specificity (%)	79.0	74.7
Positive predictive value (%)	69.2	55.8
Negative predictive value (%)	97.8	77.7

of the patient's autoantibodies to the substrate antigens and creates false negatives or unusually dim results on visualisation. Nevertheless IIF remains useful in certain cases such as paraneoplastic pemphigus as it has very high specificity (Kelly *et al* 2015).

In summary this is the first study to evaluate the agreement statistics between two fluorescent microscopy techniques for AIBDs. Despite only a moderate level of agreement between IIF and DIF for AIBDs diagnoses, the strength of performing these tests together is in the high sensitivity (96.9%) in a correct diagnosis. However, the sensitivity is comparable to DIF alone (96.4%) suggesting that IIF only needs to be considered if using it as a screening test (due to high sensitivity, 85.7%) or if a biopsy/DIF is not going to be performed. Furthermore, the high negative predictive value of DIF makes a negative DIF result a good rule-out test.

Conflicts of interest

The authors declare they have no conflicts of interest

Acknowledgements

Adrian Lee contributed to study design, data collection and drafted the manuscript. Olja Saran and Dimitra Beroukas contributed to study design and data collection. Ming-Wei Lin and Tom Gordon contributed to study design and supervision. All authors have read and revised the manuscript for important intellectual content.

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ORAL PRESENTATIONS

POSTERS

Title: The evaluation of medical tests: lessons learned in the COVID-19 pandemic

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Abstract: In battling the COVID-19 pandemic testing is essential and a wide range of tests has been made available to identify symptomatic and asymptomatic people infected with the SARS-CoV-2 virus, those with COVID-19 disease, and others with antibodies. As in any other medical field these tests should be rigorously evaluated in terms of their analytical and clinical performance, and their consequences. This poses not only logistic challenges but also methodological ones. Problematic for evaluations of the clinical performance of tests for viral RNA has been the absence of an independent reference standard. Many studies lack rigor in terms of the recruitment of study participants. Study reports are often insufficiently informative, which makes it difficult to assess the applicability of study findings. After summarizing current basic principles of test evaluation, we will be discussing a number of key challenges in the evaluation of tests for COVID-19.

Title: Dancing to death

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Purpose of the study/investigation Forensic & Analytical Science Service (FASS) witnessed the tragic impact of drug misadventure at NSW music festivals. Deaths were investigated by Forensic Medicine pathologists who relied upon toxicology results to provide opinion pertaining to cause of death. FASS partnered with the NSW Police Force, NSW Ministry of Health, NSW Poisons Information Centre and NSW hospital-based Clinical Toxicology services to support the NSW Drug Surveillance Strategy with the intent of reducing harm.

Basic procedures This study describes a multi-agency multi-disciplinary response. Drug seizure data was accumulated through expanded surveillance systems in addition to comprehensive expedited toxicology testing.

Results Novel Psychoactive Substances are of interest to the community however toxicology findings indicated methylenedioxymethamphetamine (MDMA) was the most frequent substance above toxic thresholds. The associated drug seizure data identified high dose MDMA tablets, in addition to highlighting the variability in dosage.

Principal conclusion This collaborative research bridged a gap between FASS forensic knowledge and clinical emergency medicine, enabling public drug alerts as a proactive approach to reduce the potential for drug harm.

Title: SARS-CoV-2 neutralizing antibodies: Longevity, breadth, and evasion by emerging viral variants

Fiona Tea[1], Alberto Ospina Stella [2], Anupriya Aggarwa[2], David Ross Darley[3,4], Deepti Pilli[1], Daniele Vitale[5], Vera Merheb[1], Fiona X. Z. Lee[1], Philip Cunningham[6], Gregory J. Walker[7], Christina Fichter[2], David A. Brown[5,7], William D. Rawlinson[7,8,9], Sonia R. Isaacs[7], Vennila Mathivanan[2], Markus Hoffmann[10,11], Stefan Po¨hlman[10,11], Ohan Mazigi[4,12], Daniel Christ[4,12], Dominic E. Dwyer[7,13,14], Rebecca J. Rockett[13,14], Vitali Sintchenko[7,13,14,15], Veronica C. Hoad[16], David O. Irving[16,17], Gregory J.Dore[2,3], Iain B. Gosbell[16,18], Anthony D. Kelleher[2], Gail V. Matthews[2,3], Fabienne Brilot[1,14,19,20], Stuart G. Turville[2]

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The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antibody neutralization response and its evasion by emerging viral variants and variant of concern (VOC) are unknown, but critical to understand reinfection risk and breakthrough infection following vaccination. Antibody immunoreactivity against SARS-CoV-2 antigens and Spike variants, inhibition of Spike-driven virus–cell fusion, and infectious SARS-CoV-2 neutralization were characterized in 807 serial samples from 233 reverse transcription polymerase chain reaction (RT-PCR)–confirmed Coronavirus Disease 2019 (COVID-19) individuals with detailed demographics and followed up to 7 months. A broad and sustained polyantigenic immunoreactivity against SARS-CoV-2 Spike, Membrane, and Nucleocapsid proteins, along with high viral neutralization, was associated with COVID-19 severity. A subgroup of “high responders” maintained high neutralizing responses over time, representing ideal convalescent plasma donors. Antibodies generated against SARS-CoV-2 during the first COVID-19 wave had reduced immunoreactivity and neutralization potency to emerging Spike variants and VOC. Accurate monitoring of SARS-CoV-2 antibody responses would be essential for selection of optimal responders and vaccine monitoring and design.

Title: Optimising DNA recovery of touch DNA from fired cartridge cases

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Purpose of the study/investigation: The recovery of DNA profiles from fired cartridge cases has been notoriously difficult. However, it is of great interest to investigators who are involved in solving gun-related crimes. The aim of the study was to assess the most effective DNA recovery method for sampling touch DNA from fired cartridge cases.
Basic procedures: The study involved three collection methods, swabbing, tape-lifting and a soaking method which were applied to the most common brass cartridges used. The testing was performed on both fired and unfired cartridges that had been handled to leave touch DNA prior to loading and firing.
Results: As expected, lower DNA recovery was observed for the fired compared to unfired cartridges. The swabbing method (which is the current standard operating procedure) recovered significantly less DNA than the tape-lifting and soaking methods. In addition, the tape-lifting technique resulted in more useful profiles than swabbing.
Principal conclusion: This study informs the best sampling strategy to provide the most useful DNA profiling results from fired cartridge cases to assist with the identification of individuals involved in gun crimes.

Title: Extreme weather events and pandemics: managing mosquito-borne disease threats and competing public health priorities

Cameron Webb, Cheryl Toi, Stephen Doggett

Medical Entomology, NSW Health Pathology, Westmead Hospital, Westmead, NSW, 2145

Purpose of the study: Public health responses to the current and emerging threats of mosquito-borne disease rely on an understanding of mosquito ecology with respect to a changing climate. The impact of extreme weather

events on mosquito populations was investigated with additional considerations given to how authorities can adapt their mosquito management programs in light of competing public health priorities.
Basic procedures: In 2020, extreme weather and environmental events impacted Sydney. Bushfire along the Georges River and significant rainfall on the Northern Beaches were investigated to determine their influence on local mosquito populations and activity of arboviruses of human health concern.
Results: Mosquito populations survived, and quickly recovered, from bushfire along the Georges River. Significant rainfall in Northern Beaches resulted in exceptional abundance and diversity of mosquitoes from urban wetlands and bushland habitats. Mosquito-borne pathogens (e.g. Ross River virus, Barmah Forest virus) were detected in both locations.
Principal conclusion: Capacity and resilience is required by local authorities to undertake surveillance in response to extreme weather events while also considering the operational challenges associated with competing public health priorities such as the COVID-19 pandemic.

Title: Mass surveillance of SARS-CoV-2 utilising self-collection swabs and high-throughput laboratory techniques: an Australian case study of asymptomatic Year 12 students at the Qudos Bank Arena

Andrew Sargeant, Christopher Kot, Misha Hashmi, Dr Catherine Pitman, A/Prof Dominic Dwyer, Christopher Bourke, Vicki Pitsiavas, Stephen Parker, Laila Hassan, Hayley Keenan, Therese Atkins

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Purpose of the study/investigation: To determine whether SARS-CoV-2 self-collection and rapid RT-PCR testing could improve result turnaround times, aid in asymptomatic detection and improve testing compliance.
Basic procedures: A total of 15,519 participant performed self-collection of a novel nasal swab (RhinoSwab). They were subsequently tested on a mobile laboratory platform combining a liquid handler (Myra) with a thermocycler (micPCR) and SARS-CoV-2 RT-PCR reagents. Roche Liat was implemented as a confirmation device.
Results: Self-collection averaged less than 5 minutes per collection. RT-PCR focusing on human genes revealed adequate human cell collection of 99.9% of collection. 2,533 Surveyed participants revealed:

- 91% found self-collection easy to perform.
- 1% reported discomfort.
- 75% preferred self-collection.

In total, 15,645 tests were performed, with 6 positive SARS-CoV-2 specimens. On average the time to reporting of a positive result was 3 hours and 25 minutes.
Principal conclusion: Self-collection techniques are preferred, protect healthcare workers and improve result turnaround times.

Title: A national approach to the investigation, identification or exclusion, and management of vaccine induced thrombotic thrombocytopenia (VITT)/thrombosis with thrombocytopenia syndrome (TTS)

Emmanuel J Favaloro[1], Vivien Chen[2,3], Christine Lee[3], Ibrahim Tohidi-Esfahani[3], Lisa Clarke[4],Geoffrey Kershaw[5],Freda Passam[5], Timothy Brighton[6], Dea Donikian[6], Mayuko Kondo[6], Beng Chong[7], Noor Shadood [7], Chris Ward[8], Leonardo Pasalic[1], on behalf of the THANZ VITT Expert Advisory Group[9] and Platelet factor 4 (PF4) VITT ELISA Working Group[9]

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Purpose of the study: COVID-19 a declared pandemic, has high morbidity/mortality prompting rapid development/deployment of many vaccines. A rare but potentially fatal event following immunisation with adenovirus-based vaccines (e.g. AstraZeneca), vaccine induced thrombotic thrombocytopenia (VITT)/thrombosis with thrombocytopenia syndrome (TTS) may arise.
Basic procedure: Several national teams were rapidly formed to enable prompt diagnosis/management of VITT/TTS, as championed by THANZ (https://www.thanz.org.au). Teams comprised the VITT Advisory Expert Group and PF4 VITT ELISA Working Group.
Results: Australian researchers developed assays for VITT one week earlier than the first international publication. An inaugural guidance advisory was posted soon after,

and now updated several times. Nearly 1000 PF4 VITT ELISA tests have been performed in Australia, with over 100 positive cases. Various functional platelet activation tests have also been performed.
Principal conclusion: A co-ordinated national approach to investigation of VITT was achieved rapidly. Early mobilisation of experts in the field permitted rapid nationwide VITT identification/treatment, undoubtedly preventing morbidity and saving many lives.

Title: Internal audit of two antineuronal immunoblots and the role of indirect immunofluorescence in sample screening

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Purpose of the study/investigation: Anti-neuronal antibodies are associated with a range of clinical phenotypes that are often associated with malignancy. Most of these antibodies can be identified by distinct patterns on indirect immunofluorescence (IIF) using commercial primate neural tissue slides. Antigen specificity can also be determined by immunoassay, though for diagnostic purposes this provides a less specific method. We compared the performance between two line immunoblots(LB), the EUROImmun 12 and Ravo 14 antigen blots and the role of LB and IIF as initial screening methods.
Basic procedures: The two LBs were initially assessed with samples of known positive specificities by IIF. A second phase involved parallel testing of routine pooled negative IIF samples as per institutional standard antineuronal testing algorithm. IIF results and clinical correlation were used to arbitrate on discordant immunoblot results.
Results: Correlation between Ravo and EUROImmun for previously positive results occurred in 12 out of 16 samples (76%) of cases with disagreement in 3 out of 4 (75%) of SOX1 samples and a false positive Recoverin on the EUROImmun blot. Assessment of 318 consecutive routine patient samples did not reveal any cases where a diagnosis was correctly identified by LB and not IIF. There was also no discrepancy between both LB platforms.
Principal conclusion: Subsequent to this audit, Westmead ICPMR has migrated to the 14 antigen RAVO line immunoblot for confirmation of positive IIF or for patients with equivocal IIF results only.

Title: The detection and recovery of foreign biological fluids from the clothing of deceased humans post decomposition

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Purpose of the study/investigation: The study examined the detection and recovery of various foreign (donor) body fluids (mimicking deposition by a perpetrator during a crime) from a deceased individual's clothing following exposure to decomposition fluid.
Basic procedures: Human cadavers were dressed in clothing seeded with foreign blood, saliva and semen and seated in cars at the Australian Facility for Taphonomic Experimental Research for several months. The recovered clothing underwent routine chemical and immunological screening for biological fluid along with DNA testing.
Results: Chemical screening tests for blood and semen gave non-specific and uncharacteristic reactions to both seeded and unseeded areas of the clothing. Immunological confirmatory tests showed more success but were unable to detect the target antigens in heavily stained fabric samples. Microscopy of semen stains failed to detect spermatozoa in several of the seeded areas. DNA analysis of the stained areas either produced degraded DNA profiles or no profile.
Principal conclusion: The study demonstrated that exposure to decomposition fluid has a profoundly detrimental effect on the detection of body fluids and recovery of DNA. The findings from this study provide important information for instructing evidence recovery and DNA profiling strategies from decomposed remains.

Title: Development of ExpressTOX screening methodology for improved detection of novel psychoactive substances

Catherine McDonald

NSW Health Pathology, Forensic & Analytical Science Service, Forensic Toxicology Laboratory, Lidcombe, NSW, Australia

Purpose of the study/investigation: To develop new

methodology within Forensic Toxicology to improve detection of novel psychoactive substances (NPS) and improve efficiencies and turnaround times for public health and post-mortem toxicology analysis.
Basic procedures: Development and validation of a new high resolution mass spectrometry method for analysing 300+ drugs in whole blood. Assessment of analytical techniques and extraction methods included optimising methodology and purchasing reference standards for NPS.
Results: A new fit-for-purpose method allows for flexibility and easy addition of scope, this new workflow has reduced batch extraction time from 6 to 1 hour and analytical instrument time from 30 to 6 hours. New drug detections using this method have included Acetyl-fentanyl, 25C-NBOMe, Etizolam, Flubromazolam and LSD.

Principal conclusion: Development of a method that allows for easy additions and changes according to international and local drug markets. Reduction of sample volume and analytical time allowed drastic improvements on providing real-time intelligence in times emerging public health crises such as drug overdose clusters and unusual emergency room presentations and deaths.

Title: Agreement between measureable residual disease (MRD), by flow cytometric (FC) and molecular methods, in adults with B-lymphoblastic leukaemia (B-ALL) and acute myeloid leukaemia (AML): Interim results from the MRD-FLOW study

Riana van der Linde[1], Prudence Gatt[2], Sandy Smith[3], Jennifer Hsu[3], Marian Fernandez[3], Ming-Wei Lin[3,4,5], Lucinda Berglund[3,4,5], Leo Pasalic[1], Amanda Johnston[1,6], Jennifer Curnow[5,6], Lachlin Vaughan[1,6], Emily Blyth[2,5,6], Elizabeth Tegg[1,5], David A. Brown[2,3,4,5] and Sarah C. Sasson[3,4,5]

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Purpose of the study: To determine the agreement between FC and molecular methods for MRD measurement in adult patients with B-ALL and AML.
Basic procedures: All adult FC-MRD results at ICPMR were studied from 01/01/2021. Presence of a baseline molecular MRD marker was determined. Agreement between tests was determined by Cohen's Kappa statistic.
Results: 18/35 B-ALL patients had a t(9;22) or IGH molecular marker and in these patients there was 82% agreement between methods (Cohen's Kappa 0.55). 13/61 AML patients had a t(8;21) or NPM1 molecular marker, and in these patients there was 55% agreement between methods (Cohen's Kappa 0.13). Of the samples with discrepant results, 4/5 were positive by FC-MRD and negative by molecular-MRD.
Principal conclusion: A sizable proportion of adult leukaemia patients do not have a molecular MRD marker. Agreement between FC and molecular MRD is greater in B-ALL than AML. Planned longitudinal follow-up over 2 years will allow clinical correlation for patients with discrepant results.

Title: The Australian Genetic Reproductive Carrier Screening project – Mackenzie's mission

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Purpose of the study/investigation: Autosomal recessive (AR) and X-linked conditions are individually rare but collectively are common and represent major causes of childhood morbidity and mortality. Most couples only find out about their carrier status after the birth of an affected child. Reproductive genetic carrier screening (RCGS) aims to identify carriers before they have affected children, in order to provide the opportunity for informed decision making. Mackenzie's Mission aims to investigate all aspects of RCGS in order to determine how best to deliver carrier screening to all Australian couples who wish to access it.
Basic procedures: We aim to enrol >8,000 couples from all Australian States and Territories, who will be given the opportunity to be screened for carrier status for ~1,300 genes associated with childhood onset AR and X-linked conditions. The study is investigating uptake of screening, percentage found to be at increased chance, reproductive decision making, psychosocial aspects, health economics, ethics and implementation science issues.
Results: Among the first 4,500 couples screened, 1.9% were found to be at increased chance for one of the screened conditions. 1.8% were found to be carriers for known conditions. Sensitivity for known variants was 85%, with the main reason for not identifying a carrier couple being that one or both carried a variant that could

not be classified as likely pathogenic without knowledge of an affected child (Variants of Uncertain Significance are not reported). Screening conducted on a couples basis is acceptable to participants and greatly reduces analysis time and counselling requirements compared with sequential screening.
Principal conclusion: The study is ongoing but has demonstrated the feasibility and effectiveness of couples-based screening for a very large panel of genes.

Title: Evaluating early evidence kit (EEK) genital wipes in sexual assault investigations

Rebecca Douglas

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Purpose of the study: Evaluation of the efficacy of genital wipes self-collected for early evidence in the preservation of forensically significant material, compared with results obtained from sexual assault investigation kits (SAIKs) collected at a later time.
Basic procedure: Results from genital wipes and SAIK samples collected for the same patient were compared based on the evidence recovered such as the presence/absence of semen, whether DNA was detected, and which DNA profiling system was used. The time elapsed between an alleged sexual assault and collection of the genital wipe and SAIK samples was also compared.
Results: The loss of evidence prior to the collection of SAIK samples was demonstrated as genital wipes (collected at the same time or earlier than the SAIK) added information to sexual assault investigations in approximately 30% of cases.
Conclusion: This study demonstrated the value in using genital wipes in sexual assault cases and therefore the use of EEKs was recommended and was subsequently implemented state-wide.

Title: Human cytomegalovirus dysregulates cellular dual-specificity tyrosine phosphorylation-regulated kinases and sonic hedgehog proteins in astrocytes and cerebral organoids: a mechanism for virus-induced fetal injury

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Purpose of the study: Cytomegalovirus (CMV) infection is the leading non-genetic cause of congenital malformation in developed countries. CMV infection causes significant neurological injury in fetuses. We provide first evidence in primary human astrocytes (NHA) and cerebral organoids that CMV dysregulates neurodevelopmental proteins and pathways including dual-specificity tyrosine phosphorylation-regulated kinases (DYRK) and sonic hedgehog (SHH).
Basic procedure: Cerebral organoids were generated from human induced pluripotent stem cells. DYRK and SHH expression were investigated *in-vitro* in CMV-infected NHAs and cerebral organoids using co-immunoprecipitation, western blot, immunofluorescence, and bulk RNA-sequencing.
Results: CMV-infected NHAs showed altered protein expression of DYRK1A, DYRK1B, Gli2, Rb, ULK3 and Sh. Immunofluorescence demonstrated accumulation and re-localisation of DYRK and SHH proteins differentially in NHAs and organoids. Bulk RNA-sequencing of infected cerebral organoids revealed CMV induced aberrant expression of DYRK, SHH, and crucial developmental pathways.
Conclusion: CMV differentially dysregulates cellular DYRKs and SHH proteins in primary human astrocytes and cerebral organoids which may have important implications for congenital CMV-induced neural malformation.

Title: Electron microscopy: Does an 80-year-old analytical technology still have a place in 21st century pathology?

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Purpose of the study: There is a perception in pathology that electron microscopy (EM), while valuable in applications such as renal biopsy diagnosis, is an ageing

analytical tool of the 20th century. The reality is quite different with the last two decades seeing revolutionary improvements in resolution, usability and automation.
Basic procedures: A demonstration of EM technological advances not yet adopted by pathology.
Results and conclusion: Cryogenic specimen preparation has produced enormous improvement in resolution. Single particle analysis resolves individual macromolecules. Nanoparticle immunocytochemistry enables unprecedented resolution of tissue biomarkers. Wide-area imaging and multibeam SEM are key to high-throughput diagnostics and 3-D electron microscopy for clinical imaging correlation. Technological advances justify EM remaining a valued tool in pathology for (i) improved patient care through precision diagnosis (ii) increased multidisciplinary collaboration (iii) improved laboratory efficiency (iv) phenotypic characterisation for analytics such as mass spectrometry, genomics and immunocytochemistry.

Title: State-wide surveillance and whole genome sequencing confirm circulation of enterovirus D68 (EV-D68) causing respiratory illness in NSW

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Purpose of the study: Enteroviruses (EV) commonly cause fever, respiratory illness, and hand foot and mouth disease. However, some individuals develop severe neurological syndromes including meningitis, encephalitis and acute flaccid myelitis (AFM). Outbreaks of different subtypes occur, but the incidence of enterovirus D68 (EV-D68) in NSW is unknown. We developed a state-wide surveillance program to determine the incidence of enteroviruses, with an initial focus on EV-D68.
Basic procedure: Samples positive for EV were collected by collaborating laboratories over 16 months and sent to the Virology Research Laboratory NSW Health Pathology SEALS Randwick for whole genome and next generation sequencing.
Results: Over the surveillance period, samples from 332 patients were collected. The EV-D68 was amplified from 31/332 (9.3%) samples/patients (27 children and 4 adults). Patients with EV-D68 presented with respiratory symptoms including exacerbation of asthma, bronchiolitis and two had AFM.
Conclusion: Our surveillance demonstrates that EV-D68 circulates in NSW and can cause severe disease. These data strengthen the importance of having an active EV surveillance network in NSW and nationally.

Title: Implementation of artificial intelligence capability to an existing automated scanning microscope for sperm detection.

Hiu Tan Lau

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Purpose of the study/investigation: In the examination of sexual assault cases, it is important to identify whether

semen is present. However, microscope examination for sperm detection is very laborious and time consuming. This study involved investigating whether new artificial intelligence (AI) components added to an existing automated scanning microscope could improve the accuracy and efficiency of sperm detection.

Basic procedures: FASS incorporated AI functionality into an existing automated scanning microscope. A comprehensive in-house validation study was carried out on the upgraded system by comparing sperm counts obtained from manual scans with automated scans on sperm slides with varying levels of thickness.

Results: The study demonstrated the upgraded system is sensitive and effective in detecting sperm with reproducible results. With the AI enhancement, manual involvement to obtain a result from a microscope slide decreased by 50%, with significant impact on capacity for higher throughput slide examination in shorter timeframes.

Principal conclusion: FASS was the first Australian forensic laboratory to implement this automated scanning system with AI technology which has provided greater efficiency in sperm detection for criminal casework.

Title: Driver mutation status of cutaneous scalp melanoma

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Purpose of the study/investigation: Primary scalp melanomas (PSM) are associated with worse outcomes compared to non-scalp counterparts of cutaneous head and neck melanoma (CHNM). Driver mutations vary with age, primary site and degree of cumulative sun damage (CSD). We sought to identify driver mutations and CSD in PSM, compared to non-scalp CHNM, and determine whether differences in driver mutations account for adverse outcomes.

Basic procedures: Patients with stage III/IV CHNM with molecular testing performed (2000-2021) were identified from our institution database.

Results: Of CHNM, 166/395 (42%) arose on the scalp. High CSD correlated with higher rates of BRAF V600K mutations (vs V600E, p=0.0006). A similar driver mutation profile was seen in scalp and non-scalp melanomas (p=0.1486). Posterior PSM showed low CSD and high rates of BRAF mutation, and more frequently metastasised to the brain than anterior PSM (p=0.0019).

Principal conclusion: CSD is associated with differences in driver mutations in PSM. Posterior PSM and BRAF driver mutation are associated with adverse outcomes in these patients.

Title: Enzyme immunoassays for SARS-CoV-2 serology: correlation with neutralising antibodies, and detection on dried blood spots collected from patients

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Purpose of the study: This study aimed to 1) determine the correlation between enzyme immunoassays (EIA) and neutralising antibody titres, and 2) evaluate DBS for the detection of SARS-CoV-2 antibodies on commercially available EIAs.

Basic procedures: We correlated neutralising antibody titres of sera (n=300) with signal cut-off ratios of six scalable EIAs in parallel. Evaluation of DBS for detection of SARS-CoV-2 antibodies on three commercially available EIAs was performed by analysing paired DBS and serum samples from 54 subjects.

Results: The spike-based Euroimmun Anti-SARS-CoV-2 ELISA had the best quantitative relationship with neutralising antibody titre. On this assay testing of DBS samples was highly sensitive and specific, and quantitative results strongly correlated with those of paired serum.

Conclusion: Some EIAs can effectively be used as a surrogate test (for neutralisation) in determining protection following SARS-CoV-2 infection or vaccination. DBS derived blood is a viable alternative to plasma or serum for use in EIAs, and has particular utility as a non-

invasive collection tool for COVID-19 serological testing of infants and children.

Title: Autoimmune autonomic ganglionopathy: redefining the 'Gold-Standard' autoantibody immunoassay for a rare but underdiagnosed disorder

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Purpose of the study/investigation: Autoimmune autonomic ganglionopathy (AAG) is an uncommon immune-mediated neurological disease that results in failure of autonomic function and is associated with autoantibodies directed against the ganglionic acetylcholine receptor (gnACHR). Until now, testing for these antibodies have not been available in Australia. We sought to try and create an assay that could be implemented in a diagnostic laboratory without the need for complex or specialised transfected cell lines.
Basic procedures: We have constructed a live cell flow cytometric immunoassay to detect the function of autoantibodies to the gnACHR, and performed extensive validation of staining protocols, and compare the results of 190 serum samples from unaffected individuals with blinded positive controls that were internationally sourced.
Results: At the defined positive decision limit, we demonstrate perfect qualitative agreement (AUROC = 1) and excellent quantitative correlation with the former standard (radioimmunoprecipitation assay). Furthermore, unlike existing assays, we found no false positive results in a variety of disease controls.
Principal conclusion: We were successful in creating a new functional assay for the detection of gnACHR antibodies.

Title: Characterising the population of all viruses in clinical specimens using comprehensive virome capture sequencing and profiling the global repertoire of antiviral antibodies

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Purpose of the study: Current diagnostic methods characterise only a small number of viruses within a clinical specimen. Although highly sensitive, they are suboptimal for characterising co-infections and viruses of yet unknown significance, and the surveillance of emerging pathogens.
Basic procedure: Virome capture sequencing and antiviral antibody profiling (VirScan) were applied to characterise all human viruses in samples (stool, respiratory and plasma) collected from multiple study populations, including confirmed SARS-CoV-2 adult cases in NSW and children at risk of developing type 1 diabetes within the Viruses In the Genetically at Risk (VIGR) cohort.
Results: Of 92 SARS-CoV-2-positive cases, 8% were co-infected with rhinovirus (6%) or influenzavirus (2%). VirScan of 50 VIGR plasma samples (25 cases with pre-diabetes and 25 controls) identified 326 viral peptides differentially abundant between cases and controls, most belonging to enteroviruses.
Conclusion: Both approaches provide highly sensitive, unbiased and comprehensive summary of total viral content in any given clinical specimen without *a priori* knowledge of viruses in the sample.

Title: Keeping up with the fentanyls

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Purpose of the study: Fentanyl analogues are emerging on International and Australian illicit drug markets,

detected unsuspectingly with other illicit drugs. Due to the low dose toxicity of these analytes, severe strain is placed on health systems. Identification and detection of these drugs in clinical and coronial settings is crucial.
Basic procedures: Fentanyl analogue standards were obtained and characterised using Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry and added to existing libraries for identification in biological samples. Where standards were not available, peer reviewed libraries and theoretical fragmentation patterns were used to indicate the presence of these analytes.
Results: Several fentanyl analogues have been identified in NSW patients using these methods, including Etodesnitazene, Carfentanyl, Acetylfentanyl, and 4-ANPP, with over 30 fentanyl analogues added to the reference library.
Conclusion: Continual enhancement of screening techniques and expansion of reference libraries has allowed for greater detection of fentanyl analogues in clinical and coronial samples, resulting in public health alerts across NSW.

POSTERS

Title: Pathologist-initiated "reflex" BRAF mutation testing in metastatic melanoma at a tertiary referral centre

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Purpose of the study/investigation: Testing for *BRAF* mutations in advanced melanoma is pivotal in identifying patients for systemic therapy. BRAF V600E immunohistochemistry (IHC) results can streamline decisions during oncology consultations. This study aimed to evaluate a local protocol for pathologist-initiated "reflex" BRAF IHC in all diagnoses of stage III/IV melanoma when status is unknown.
Basic procedures: All stage III/IV melanomas diagnosed within the department during quarter one (Jan-Mar) over three consecutive years (2019-2021) were identified, and data obtained from corresponding pathology request forms and pathology reports.
Results: Pathologists established the *BRAF* mutation status in 352/408 cases (87%). When a *BRAF* mutation status was previously known (202/408), this was rarely communicated by clinicians via the pathology request form (1%, 3/202). Pathologists performed IHC in 74% (153/206) of cases with an unknown *BRAF* mutation status, and testing was omitted in 53 cases (26%), often in small-volume (including cytology) specimens.
Principal conclusion: Incorporating BRAF IHC testing within routine diagnostic protocols of advanced melanoma was successful in most cases.

Title: Familial relationship study to determine thresholds for familial searching of forensic DNA profiles

Carole Field

Forensic Biology/DNA Unit, Forensic & Analytical Science Service, NSW Health Pathology, Sydney, Australia

Purpose of the study/investigation: Close relatives share DNA which can be used to identify familial relationships in a database. Children can be identified through specific DNA sharing patterns. Full siblings will share DNA, however, some siblings do not share enough DNA making the identification of potential siblings in a familial search problematic. A familial study was carried out to evaluate known family relationships to determine the degree of DNA sharing.
Basic procedures: The study included full sibling and parent/child relationships. The degree of DNA sharing was determined using two DNA typing kits. Likelihood ratios (LRs) which indicate the statistical support for the specified relationships were calculated for both DNA typing kits.
Results: The LR range was determined for parent/child and full sibling relationships. LR thresholds for familial searching were determined for the two DNA typing kits underpinning the current procedure for familial searching on the DNA database.
Principal conclusion: LR thresholds were determined for familial searching on the DNA database for both criminal investigations and identification of human remains optimising the balance of resources and outcomes.

Title: Sepsis management using T2 magnetic resonance technology panels

Andrew Sargeant, Christopher Kot, Misha Hashmi, A/

Prof Sharon Chen, A/Prof Hemalatha Varadhan, Dr Amith Shetty, Prof Jonathan Iredell, A/Prof Rajesh Malik, Dr Maria Martino, Dr John-Paul Smiles, Dr Aditee Parab, Tom Olma, Jane Drury, Simon Holmes, Catriona Halliday, Justin Ellem and Dr Bente Talseth-Palmer

WSLHD (Westmead Hospital), HNELHD (John Hunter Hospital) and/or NSW Health Pathology, New South Wales

Purpose of the study/investigation: Bloodstream infections (BSIs) are a common cause of death in hospitals. Delayed administration of antimicrobial therapy may contribute to adverse outcomes in patients with BSIs. Blood cultures are considered the gold standard for diagnosis of bacteraemia, however, results can take time. The T2Dx uses molecular PCR and nuclear magnetic resonance for fast result turnaround.
Basic procedures: Emergency Department (ED) patients who met qSOFA criteria and/or hyperlactataemia were deemed appropriate for the study, having 2 EDTAs collected concurrently as blood culture bottles. Patients admitted into Intensive Care Units (ICUs) met pre-defined criteria for suspected fungaemia, in particular candidaemia, having 1 EDTA collected concurrently as mycolytic blood culture bottles.
Results: We have collected:

- 115 samples in ED with 43 positive on T2Bacteria panel (8 additional samples detected growth on the T2 system).
- 30 samples in ICU with only 1 positive for *C. parapsilosis*.

Principal conclusion: The T2Dx offers an advantage in detection of BSIs, aiding in targeted antibiotic therapy.

Title: Evaluating the long- term effects of a data- driven approach to reduce variation in emergency department pathology investigations: study protocol for evaluation of the NSW Health Pathology Atlas of variation

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NSW Health Pathology and Macquarie University

Purpose of the study/investigation: The objectives of this study are to evaluate whether a data- driven approach (ie, using Atlas Analytical Model to engage with LHDs) is associated with: (1) a reduction in test order variation; (2) improvements in patient outcomes through a reduction in ED LOS and a reduction in hospital admission rates and (3) cost benefits (total pathology costs per ED encounter).
Basic procedures: The objectives of the study will be assessed using an interrupted time series (ITS), a type of quasiexperimental design, using both retrospective and prospective data. It is considered the best approach to evaluate the effect of an intervention at the population level when a randomised trial is not feasible. One of the strengths of ITS design is that it evaluates the effect of the intervention accounting for underlying preintervention trends in the outcome variables.
Results: We will disseminate the findings of the project to diagnostic informatics, health service/policymakers and broader audiences through conference presentations and peer- reviewed journal publications. We will follow the REporting of studies Conducted using Observational Routinely- collected health Data statement when writing publications. We will also engage directly with key stakeholders including the NSW Ministry of Health, NSWHP and LHDs within NSW to disseminate the project findings and to inform policies related to pathology testing in EDs.
Principal conclusion: TBA

Title: Whole-genome sequencing analysis of invasive *Streptococcus pyogenes* isolates from patients in Hunter New England Health District, New South Wales

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Purpose of the study/investigation: *Streptococcus pyogenes* (*S. pyogenes*) is a Gram-positive cocci bacteria and is a significant cause of invasive infections. Three-year moving case average suggests invasive *S. pyogenes* (iGAS) cases are increasing in HNELHD from average of 32.0 cases/year for 2008-2010 to 57.3 cases/year for 2017-2019. The purpose of this study was to perform epidemiological typing of iGAS isolates by whole genome sequencing (WGS), attempt in-silico *emm* typing and identify resistance determinants. We sequenced 78 *S. pyogenes* specimens collected between 2009 and 2021 from patients located within HNELHD, Australia.
Basic procedures: *S. pyogenes* strains were frozen after isolation and antimicrobial resistance (AMR) testing, which was performed and reported in accordance with EUCAST. Isolates sub-cultured on to blood agar were identified using a Bruker-MALDI Biotyper. Genomic DNA was extracted using ZymoBIOMICS DNA Microprep Kits. Illumina DNA Prep kits were used for library preparation

and 2×300bp sequencing was performed on an Illumina MiSeq. A bioinformatics pipeline was established using Galaxy. Published databases were used to assess AMR genes.

Results: At least 31 different *emm*-types were identified from the 78 iGAS isolates assessed by our study. *emm*-typing data revealed the most common *emm*-types found were *emm*1, 28, 89, 3.1, 4 and 12. A range of antibiotic resistant genes were identified from results obtained from the databases namely Resfinder, NCBI and CARD. 5/78 isolates were found to be phenotypically resistant to macrolides and tetracycline (ECOFF (EUCAST) method), however, tetracycline resistance genes were found in six isolates.

Principal conclusion: To the best of our knowledge this is the first genomic survey of iGAS cases in Northern NSW, Australia. The prevalence of AMR genes in iGAS cases is currently low in our healthcare district, occurring in approximately 8% of cases. Resistance to tetracycline and macrolides appears to be the most common form of AMR. We note association of certain *emm* type clusters with year of isolation and whether these represent clonal expansion is uncertain. Further epidemiological studies may be indicated

Title: A state-wide approach to the evaluation, verification and implementation of ACL TOP instruments to facilitate standardised/harmonised testing of coagulation tests and haemostasis assays across NSW Health Pathology

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Purpose of the study: To verify and implement a single platform of hemostasis instrumentation, the ACL TOP 50 Family, comprising 350, 550, and 750 instruments, and associated tests, across the entire network of NSW Health Pathology laboratories (n=60).

Basic procedure: Comparative evaluations of three instrument classes using a large battery of test samples for routine coagulation tests and specialised haemostasis assays using HemosIL and reference comparator reagents/instruments to satisfy ISO15189 national accreditation standards. Verification of manufacturer normal reference ranges (NRRs), generation of harmonised APTT heparin therapeutic range (TRR), and harmonisation of all applicable methods and documentation.

Results: The three instrument types were verified as a single instrument class, permitting standardisation of methods/NRRs/TRR across all instruments (n= 75) deployed in all (n=60) laboratories. Harmonisation of documentation is in process.

Principal conclusion: Co-ordinated state-wide approach permitted method harmonisation for routine coagulation and specialised haemostasis assays.

Title: Verification of biogeographical ancestry and phenotype prediction for forensic investigations

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Purpose of the study/investigation: This study was conducted to verify a massively parallel sequencing (MPS) method for the prediction of biogeographical ancestry (BGA) and externally visible characteristics (EVCs; hair and eye colour). This capability enables FASS to provide additional intelligence to investigators about the ancestry and physical appearance of a person associated with a crime sample, which can be valuable when other investigative leads have been exhausted.

Basic procedures: The study followed the validated Genetic Ancestry Laboratory (GAL) methodology utilising the Precision ID Ancestry and Ion Ampliseq™ DNA Phenotyping panels. Automated library and template preparation was undertaken on an Ion Chef™ with sequencing on an Ion GeneStudio™ S5 Plus. The study assessed genotype and prediction concordance of results from unknown donors and prediction accuracy using known donors.

Results: Results were highly concordant with those generated by the GAL, and BGA and EVC predictions

were also consistent with self-reported information from known donors.
Principal conclusion: FASS was the first forensic laboratory in Australia to implement this MPS capability. Further research is underway to optimise the methodology and improve interpretation and reporting guidelines.

Title: Digital adaptability in the COVID-19 world

Jason Tran, Brynley Munro, Catherine M[c]Donald

NSW Health Pathology, Forensic & Analytical Science Service, Forensic Toxicology Laboratory, Lidcombe, New South Wales

Purpose of the study/investigation: The impact of COVID-19 has forced the world to adapt and survive. Previous forensic toxicology laboratory (FTL) methods, procedures and casefiles were heavily paper based and on-site restricted. To protect the staff and allow them to safely work from home (WFH) and continue to provide essential services paperless workflows were developed.
Basic procedures: Technology such as Acrobat, remote access and increased network capabilities were introduced. All documents, results and casefiles, along with FTL procedures were converted to a digital format including virtual access to instrumentation.
Results: FTL has been able to continue to provide essential services whilst improving efficiencies in workflow and also maintain the safety of staff. Workforce is 50% WFH, ongoing monetary savings, reduction of physical storage requirements and improved productivity were demonstrated.
Principal conclusion: FTL has shown resilience during the COVID-19 pandemic by evolving the way it serves and protects the community. New efficiencies were discovered, carbon footprint lowered and FTL has safeguarded itself for the future.

Title: Markers of neuroinflammation and autoimmunity in patients with psychiatric disease

Jocelyn X Jiang[1,2], Nicole Fewings[3], Prudence Gatt[1,3], Suat Dervish[8], Matthew Silsby[2,4], Alessandro F Fois[2,4], Stephen Duma[2,4], Sushil Bandodkar[2,5], Sudarshini Ramanathan[2,9,0], Andrew Bleasel[2,4], Bryne John[7], Anthony Harris, Ming-Wei Lin[1,2,3] and David Brown[1,2,3]

[1]Department of Immunopathology, NSW Health Pathology-ICMPR, Westmead Hospital, Westmead New South Wales
[2]Sydney Medical School, University of Sydney, Sydney New South Wales
[3]Centre for Immunology and Allergy Research, The Westmead Institute for Medical Research, Westmead New South Wales
[4]Department of Neurology, Westmead Hospital, Westmead, New South Wales
[5]Department of Clinical Biochemistry, The Children's Hospital at Westmead, Westmead, New South Wales
[6]Department of Psychiatry, Westmead Hospital, Westmead, New South Wales
[7]Department of Anaesthetics, Westmead Hospital Westmead, New South Wales
[8]Westmead Research Hub, The Westmead Institute for Medical Research, Westmead, New South Wales
[9]Department of Neurology, Concord Hospital, Concord, New South Wales
[10]Neuroimmunology Group, Kids Neuroscience Centre, Children's Hospital at Westmead, New South Wales

Background: Increasingly, the immune system is recognized in the pathophysiology of psychiatric disease. We measured serum autoantibodies and CSF markers of neuroinflammation in patients with atypical psychiatric disease.
Methods: Patients were referred to a research immunology clinic by their psychiatrist. A panel of serum autoimmune investigations and cerebral spinal fluid (CSF) investigations were performed.
Results: Thirty-five patients were enrolled in the study: twenty-nine females and six males. Eighteen non-inflammatory controls were included. Most patients had mixed mood and psychotic phenomenon. Nineteen patients had presence of a low titre ANAs without associated ENA. One patient had a 1:2560 speckled ANA and associated anti-SSa/Ro antibodies. Two patients had CSF pleocytosis (>5 monocytes) and three had CSF restricted oligoclonal bands. CSF cytokines GMCSF and interferon gamma were associated with psychiatric disease when compared to non-inflammatory controls.
Conclusion: Further study of a larger cohort of patients is needed to confirm these results.

Title: CoSMoSS: monitoring drug seizures from music festivals in New South Wales

Kelsey E Seyfang, [1], Daniel Moawad, [1], Una Cullinan, Daniel Barry, Christine Harvey[2], Matthew O'Reilly[3]
[1]NSW Health Pathology Forensic and Analytical Science Service, Lidcombe, New South Wales
[2]NSW Ministry of Health, New South Wales
[3]NSW Police Force, New South Wales

Purpose of the study/investigation: The State Coroner's

Court of NSW reported in 2019 on the deaths of six young people at music festivals in NSW. These deaths caused concern in the community and generated significant media attention. Although MDMA toxicity was confirmed as a causal factor in each death, concern remained as to whether toxic adulterants are present in substances circulating in NSW.

Basic procedures: NSW Police Force, NSW Ministry of Health (MoH) and NSW Health Pathology Forensic & Analytical Science Service (FASS) have convened the Combined Surveillance and Monitoring of Seized Samples (CoSMoSS) project, facilitating collaboration and information exchange between the agencies. The principal aim of the project is to better monitor 'street-level' drug seizures by Police. The project's pilot phase focused on seizures of MDMA at music festivals. The second phase has monitored street-level seizures from different regions of NSW.

Results: The illicit drugs analysis unit at FASS report: weight, purity, dose per capsule/tablet, secondary drugs, cutting agents and any manufacturing by-products. Samples suspected to contain heroin are screened for fentanyl type substances. Methylamphetamine, MDA, MDMA, Heroin, Cocaine, ketamine, pharmaceuticals and isobutyl nitrite have been observed among other drugs.

Principal conclusion: Information is triaged by the CoSMoSS representatives from each agency, and by additional clinical and expert advisors to the MoH to ensure appropriate public health response. Responses to date have included the publication of drug warnings by NSW Health. These have had a wide impact through social media, television, radio and print media.

The information gained from this project has allowed the IDAU to add new service capabilities and make focussed decisions about ongoing service development priorities.

Title: Targeting sub-therapeutic concentrations of medications in an air crash investigation

Lily Molinari, Catherine McDonald

NSW Health Pathology, Forensic & Analytical Science Service, Forensic Toxicology Laboratory, Lidcombe, NSW, Australia

Purpose of the study/investigation: To identify sub-therapeutic concentrations of low-dose medications in post-mortem samples from the pilot in an air crash investigation and to determine the relevance of the medication to the cause of the crash.

Basic procedures: Extraction techniques were adapted from existing in-house methods to optimise sensitivity for the compounds of interest. Certified Reference Standards were used to create calibration ranges capturing sub-therapeutic concentrations to determine medication compliance. The medications were over-the-counter and prescription.

Results: Appropriate calibration ranges were achieved in order to determine that the medication, if present, would be detected in the sample. This data enabled the medication to be excluded as a cause of the crash. In combination with carbon monoxide testing performed, these results allowed investigators to identify the cause of the crash and the opportunity to implement additional safety protocols.

Principal conclusion: The existing in-house methods of our laboratory can be readily adapted to target non-routine drugs as required to support investigations of public health and safety interest.

Title: Application of 3D printing in post mortem reconstruction – a pilot study

Bilton, LM [1], Harvey, SB [2], I'Ons, B [3], Green, H [4]

[1]Forensic Medicine Wollongong, Forensic & Analytical Science Service, New South Wales
[2]Medical Physics, Illawarra Shoalhaven Medical Imaging Service, New South Wales
[3]School of Science and Health, Western Sydney University, New South Wales

Purpose of the study: This research examines current techniques of reconstruction, particularly of the face and skull after post-mortem examination, medical and forensic reconstruction, and investigates protocols and devices to advance reconstruction techniques.

Basic procedures: The project investigates whether additively manufactured (3D printed) models and supporting devices of the craniofacial region and cervical spine are a viable alternative to current reconstruction techniques, in addition to potentially improving outcomes for families, particularly in trauma and paediatric cases.

Results: Initial results of this pilot study indicate that 3D printed models can be generated relatively quickly, and that certain plastics are better suited to this application than others. Next steps will involve testing the methods of connecting 3D printed components to existing bone structures and trialling these implants.

Principal conclusion: While preliminary results are promising, more work is required to determine the usefulness in reconstruction as well as timeliness, and importantly any improvement to family outcomes.

Title: Increased discrimination power using the Precision ID mtDNA Whole Genome Panel

Lisa Filippi[1], Felicity Poulsen[1], Catherine Hitchcock[1]

Forensic Biology/DNA Unit, Forensic & AnalyticalScience Service, NSW Health Pathology, Sydney, New South Wales

Purpose of the study/investigation: FASS currently conducts mitochondrial DNA (mtDNA) analysis utilising Sanger sequencing of the control region. However, the discriminatory power of this method is limited when common mtDNA profiles are identified. Analysis of the whole mtDNA genome can potentially increase the power of discrimination as many variants are located outside the control region.
Basic procedures: This study describes part of the validation of the Precision ID mtDNA Whole Genome Panel using massively parallel sequencing involving the comparison of the profiles generated using the two sequencing methods. Automated library and template preparation were undertaken on an Ion Chef™ and an Ion GeneStudio™ S5 Plus Sequencer.
Results: Whole genome sequencing was concordant with Sanger sequencing for the control region but 60-85% of variants recorded were outside the control region allowing profiles that were previously identical to be differentiated.
Principal conclusion: Implementation of whole genome mtDNA sequencing provides an increased discrimination power that will assist with identification of unidentified human remains and donors of crime samples.

Title: DNA profiling and confirmation of blood from minute blood samples

Louise Wallis[1], Catherine Hitchcock[2], Dennis McNevin[3], Jennifer Raymond[1]

[1]Forensic Evidence & Technical Services Command, NSW Police Force
[2]Forensic Biology/DNA Unit, Forensic & Analytical Science Service, NSW Health Pathology, Sydney, Australia
[3]Centre for Forensic Science, School of Mathematical & Physical Sciences, Faculty of Science, University of Technology Sydney, Australia

Purpose of the study: When blood stains at crime scenes are too small to undertake testing to confirm the presence of blood and perform DNA profiling, preference is given to DNA profiling. This study's aim was to develop a method in which both confirmatory blood testing and DNA profiling could be performed.
Basic procedure: The ABACard® Hematrace® kit is the confirmatory blood testing kit used. Previous testing revealed various parts of the kit could be used to generate a DNA profile including the membrane, buffer and swab, with the swab providing the best results. Minute blood stains of varying sizes, also different substrates were tested to determine the smallest blood stain size that generated a useful DNA profile.
Results and conclusion: The results demonstrated the swab used for the Hematrace® testing can be submitted for DNA analysis and generate a useful DNA profile with stains 0.6–0.7 mm or larger.
This enables an update of the standard operating procedures incorporating both confirmatory blood testing and DNA analysis for appropriately sized stains.

Title: An illicit drug pre-precursor newly encountered in NSW

Matthew Paul

Forensic & Analytical Science Service, NSW Health Pathology, Sydney New South Wales

Purpose of the study/investigation: Methylamphetamine is a routinely seized illicit drug and has been encountered to be manufactured in NSW. The common precursors to manufacture methylamphetamine are listed in the NSW Drug Misuse and Trafficking Regulation and recently this list was expanded.
One newly listed precursor is methyl *alpha*-phenylacetoacetate, commonly abbreviated to MAPA. MAPA is a precursor for 1-phenyl-2-propanone (P2P), which is one precursor that can be used in the manufacture of methylamphetamine.
When police prosecute offenders, the courts need to understand the potential yield that seized precursors can achieve as well as an understanding of the success of the methods undertaken.
To date, there is no published data for the yield of P2P from MAPA nor an understanding of the best methods to carry out this conversion.
Basic procedures: Small amounts of **MAPA** were treated with different acids and solvents under varying conditions to measure the amounts of **P2P** produced.

Title: Assessment of the Y-quantification threshold for routine Yfiler® Plus amplification

Rebecca Douglas

Forensic Biology/DNA Unit, Forensic & Analytical Science

Service, NSW Health Pathology, Sydney, Australia

Purpose of the study: Re-evaluated the Y-quantification threshold for Yfiler® Plus amplification of casework samples to streamline workflow.
Basic procedure: The Y-quantification data and Y-STR profile information for >1000 casework samples was assessed. Counts of Y-STR loci with labelled peaks in each profile were recorded across 3 categories selected to represent the minimum comparable profile (≥5 loci), a comparable but not uploadable profile (≥10 loci) and an uploadable profile (≥20 loci).
Results: Approximately 55% of samples had a Y-quantification value less than 0.0015 ng/ul and at this threshold it is expected that profiles will contain peak information at ≥5 loci in >50% of amplifications and ≥10 loci in approximately 25% of amplifications. ≥20 loci recovery reached >10% success at 0.0022 ng/ul.
Conclusion: Raising the Y-quantification threshold from Y: 0.0004 to Y: 0.0015 ng/ul was suitable for routine casework samples. The reduced number of samples proceeding to amplification translates to savings in consumables and analyst's time spent processing samples and reporting results.

Title: Use of multidimensional flow cytometry (MD-FC) to improve measurable residual disease (MRD) monitoring of acute myeloid leukaemia (AML)

Riana van der Linde[1,4], Sandy Smith[3], David A Brown[2,3,4], Sarah C Sasson[2,3,4], Elizabeth Tegg[1,3,4]

[1]Department of Laboratory Haematology, ICPMR, Westmead hospital, NSW Health Pathology, [2]Department of Clinical Immunology, Westmead hospital, New South Wales
[3]Flow cytometry Unit, ICPMR, NSW Health Pathology, New South Wales
[4]Sydney Medical School, Faculty of Medicine and Health, University of Sydney, New South Wales

Purpose of the study: Is MRD analysis by MD-FC better than 2D-FC?
Basic procedure: We compared 2D- and MD-FC analysis (Kaluza software) in 80 consecutive MRD samples and validated methods in a further 36 cases, examining results with Cohen's Kappa and McNemar analysis.
Results: With a reporting limit of 0.01% of CD45 positive cells, 84/116 were concordant (k=45%, p=0.21). At a cut-off of 0.1% concordance was 99/116 (k=60%, p=0.14). MRD <0.1% by either test was present in 31/116 with fifteen having +MD-FC/-2D-FC and two of these were molecular positive. Molecular markers were available in 36/116 cases. Of these, seven had detectable molecular MRD below 0.011% and no FC-MRD detected and eight had. positive MD-FC (0.03% to 0.39%), but negative molecular MRD. Two had +2D-FC but were negative for MD-FC and molecular methods.
Conclusion: There is agreement between MD- and 2D-FC-MRD, but MD-FC appears to perform better at MRD<0.1%

Title: The ExpressTOX Quant: development and validation of new analytical method for quantitation of basic drugs in blood

Sergei Bidny, Catherine McDonald, Jason Tran,

NSW Health Pathology, Forensic & Analytical Science Service, Forensic Toxicology Laboratory, Lidcombe, New South Wales

Purpose of the study/investigation: Until now in Forensic Toxicology laboratory three different methods were used for quantitation of Amphetamines, Benzodiazepines and Narcotic analgesics. The goal of this project was to combine three methods into one method and develop and validate the new sensitive and specific analytical method for identification and quantitation of 75 analytes belonging to different classes of drugs.
Basic procedures: Instrumental analysis was performed on a Sciex Exion LC Ultra-High Pressure Liquid Chromatograph coupled to a Sciex Triple Quad 4500 Mass Spectrometer. For each analyte, two MRM transitions were established with optimised cone voltages and collision energies to provide information on quantitation and additional transitions and ion ratios for confirmation.
Results: Calibration curves were linear over the concentration range 0.005-3 mg/L and limits of detection were between 0.0005-0.001 mg/L.
Principal conclusion: The new method has seen efficiency gains in analysis time, sample volume, staff training and saves resources, reagents and solvents.

Title: The rise and fall of invasive serotype 3 pneumococcal disease in Australian children

Rebecca J Rockett[1,2], Shona Chandra[2,3], Shahin Oftadeh[3,4], Vitali Sintchenko[1,2,3]

[1]Sydney Medical School, The University of Sydney, Sydney, New South Wales
[2]Centre for Infectious Diseases and Microbiology-Public Health, Westmead Hospital, Westmead, New South Wales
[3]Institute of Clinical Pathology and Medical Research, NSW Health Pathology, Westmead, New South Wales

[4]NSW Pneumococcal Reference Laboratory, Institute of Clinical Pathology and Medical Research, NSW Health Pathology, Westmead, New South Wales

Purpose of the study/investigation: Invasive pneumococcal disease (IPD) is caused when *Streptococcus pneumoniae* penetrates normally sterile sites. Prior to the introduction of pneumococcal conjugate vaccines (PCV7 & PCV13), IPD was associated with high rates of morbidity and mortality in children and the elderly. This study aimed to understand the evolution and genomic characteristics of IPD caused by the virulent serotype 3, which continues to persist despite high vaccine coverage.
Basic procedures: Historical serotype 3 IPD isolates from 1999-2020 (*n*=134) were subject to serotyping, whole genome sequencing and bioinformatics analysis.
Results and **principal conclusion:** The genomes produced were predominately ST180 (113/134) with the detection of an increasing number of divergent and more virulent clade II and III isolates. Internationally studies have also reported the increasing divergence of ST180 IPD genomes which is postulated to be driving the increased incidence of serotype 3 IPD in Australia.

Title: Evaluation of sampling techniques for large scale prohibited drug seizures

Simone Burge

Purpose of the study: In recent years, there has been an increase in the number and complexity of prohibited drug cases seized in NSW. There are physical demands and work health and safety implications for staff involved in sampling these cases, along with the time required for the current extensive sampling process.
Basic procedures: A comparative study was conducted to evaluate three sampling techniques: homogenisation; incremental sampling from five points within the item; and single point sampling. The objective is to ascertain any differences in purity between each of the sampling techniques. The seizures selected for this study included large quantities of compressed/crystalline substances, and powder which contained methylamphetamine, MDMA, cocaine, or heroin.
Results: The results of this study will allow for review of current drug sampling procedures to determine whether a more efficient and less labour-intensive sampling technique is viable.
Conclusion: This study has the potential to reduce the need for laborious tasks currently undertaken by staff and reduce the WH&S risks, in addition to increasing laboratory efficiency by minimising sampling time.

Title: Phenotypic and genotypic characterization of BLNAR and BLPACR strains of *Haemophilus influenzae*: A rare finding of presence of ESBL and highly resistant *ftsI* mutations in Australian isolates

Syeda Shaher Bano Naqvi[1], Hemalatha Varadhan[1], Nouri Ben Zakour[2], Jo Barfield[3], Fiona Oehme[1], Rodney Givney[1]

[1]Department of Microbiology, John Hunter Laboratory, NSW Health Pathology, NSW, Australia
[2]Westmead Institute for Medical Research, New South Wales
[3]Hunter Medical Research Institute, New South Wales

Purpose of the study/investigation: To characterize the dual mechanism of β-lactam resistance in *H. influenzae* isolates.
Basic procedures: One hundred isolates resistant to both ampicillin and amoxicillin-clavulanate on disk-diffusion were collected from 2017-2019 and phenotypically classified as pBLNAR and pBLPACR based on nitrocefin-hydrolysis. Disk-diffusion results were verified with gold-standard BMD using EUCAST and also by CLSI disk-diffusion standards to look for concordance. Whole-genome-sequencing performed on 44 isolates to study *ftsI* and *bla* genes.
Results: Among the pBLNAR strains, 25.5% isolates had ampicillin MIC of 1mg/L at the borderline of susceptible range, and 4.6% had a low MIC of 0.5mg/L, making them Low-BLNAR strains. Conversely, 100% pBLPACR strains had high MIC (8mg/L). Both BLNAR and BLPACR, had a similar pattern of amoxicillin-clavulanate MIC, higher if *ftsI* gene mutations were of Ubukata group III (D350 N, S357N, S385T and L389F). Three different ESBLs were also detected, not previously reported in *H.influenzae*.
Principal conclusion: EUCAST disk-diffusion showed better correlation with genotype than CLSI. Detecting ESBLs in *H.influenzae* may have critical management implications.

Title: Use of next generation sequencing technologies for respiratory virus detection from clinical samples: review of recent advances and barriers to its potential in clinical use

Xinye Wang[1], Sacha Stelzer-Braid[1,2], Matthew Scotch[1, 4], William D. Rawlinson[1,2]

[1] School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, New South Wales
[2] Virology Research Laboratory, Prince of Wales Hospital, University of New South Wales, Sydney, New South Wales
[3]College of Health Solutions, Arizona State University,

Phoenix, AZ, USA
[4] The Kirby Institute, Sydney, New South Wales

Purpose of the study/investigation: Next generation sequencing (NGS) technology was developed in 2009 and since then has become cheaper and easier to use and thus more applicable to modern clinical laboratories. We aimed to review the application of next generation sequencing (NGS) technologies for detecting respiratory viruses from clinical samples. Furthermore, the barriers of NGS use in clinical laboratories have been discussed.

Basic procedures: Five different databases were searched for studies published from 01 January 2010 to 01 February 2021, restricted to articles in English. The titles and abstracts of articles were manually searched based on defined inclusion and exclusion criteria.

Results: Fifty-two studies matching the inclusion criteria were included in the final review. Included studies demonstrated that NGS technologies could be successfully used for the detection of influenza A/B virus (9, 17%), SARS-CoV-2 (21, 40%), parainfluenza virus (3, 6%), respiratory syncytial virus (1, 2%), human metapneumovirus (2, 4%), or a viral panel including other respiratory viruses (16, 31%). Second-generation sequencing platforms were performed in the majority of studies (41, 79%), particularly Illumina (39, 75%).

Principal conclusion: Next generation sequencing technologies were shown to contribute significantly to novel virus detection, virus subtyping, mutation identification, variants monitoring, and clusters of transmission of infections identification in included studies. Although there are many challenges associated with NGS use in clinical laboratories (e.g., the cost of performing NGS and complex bioinformatic analysis), NGS could be seen as a promising tool in future diagnostic practice to improve understanding of respiratory viruses and provide a more accurate diagnosis.

RESEARCH ENGAGEMENT GRANT SCHEME

AIMS is delighted to announce a new round of the exciting program of bursary and grant funding opportunities known as the AIMS Research Engagement Grant Scheme.

The scheme aspires to recognise, support and engage with research-oriented medical scientists through annual bursary and grant opportunities to encourage pathology-related research and CPD.

AIMS will be accepting applications for 2022 until 30 March 2022.

https://www.aims.org.au/cpd/research-engagement-grant-scheme

FUNDING OPPORTUNITIES	ELIGIBILITY	VALUE (**AUD**)
Scientific Communication Bursary	Graduate or final year student enrolled in an AIMS accredited program	2 x $500
Honours Research Bursary	Candidates enrolled at a university preferably offering an AIMS accredited program	3 x $1,000
MPhil or Master by Research Seeding Grant	Candidates enrolled at a university preferably offering an AIMS accredited program	2 x $2,500 or 1 x $5,000
Doctor of Philosophy (PhD) Research Seeding Grant	Candidates enrolled at a university preferably offering an AIMS accredited program	2 x $5,000 or 1 x $10,000
AIMS Fellowship (Stage 4) Bursary	Candidates enrolling in or undertaking Stage 4 of the AIMS Fellowship	3 x $2,000
Clinical or Research Laboratory Grant	Medical scientists with an acceptable* science degree	2 x $5,000 or 1 x $10,000
Education or Leadership & Management Research Grant	Medical scientists with an acceptable* science degree	2 x $5,000 or 1 x $10,000

Australian Professional Acknowledgement of Continuing Education (APACE)

3 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly.
24 credits maximum per accreditation period claim.

Journal-based CPD No. 82
Page 1 of 1

Questions relating to the article *'Immunofluorescence microscopy for the diagnosis of autoimmune blistering skin diseases'* at page 2 of this issue.

1.	Autoimmune blistering skin diseases (AIBDs) are disorders characterised by painful blisters on mucosal and non-mucosal skin.	True/False
2.	The main antigenic targets include BP180 for pemphigoid diseases and desmoglein 1 and 3 for the pemphigus disorders with various techniques, such as enzyme-linked immunosorbent assay (ELISA), available to confirm antibody specificity.	True/False
3.	IIF was performed on monkey oesophagus substrate (Euroimmun, Lübeck, Germany) using serum diluted in 10:10 as a screening titre and end-point titration performed.	True/False
4.	"False positives" from sera reacting with red blood cell A and/or B antigens were excluded by introducing a blocking mixture of A and B antigens prior to sera incubation with substrate.	True/False
5.	Anti-human IgG conjugated to fluorescein isothiocyanate (FITC) was used as the detection antibody.	True/False
6.	Antibodies to immunoglobulins (IgG, IgA, IgM), complement (C1q and C3) and fibrinogen, conjugated to FITC, diluted at 10:10 were incubated with the tissue.	True/False
7.	Simple descriptive and diagnostic statistics were performed using SPSS statistical software package.	True/False
8.	There were 31 AIBDs (56.0%) comprising of 20 pemphigoid, 10 pemphigus and one dermatitis herpetiformis cases.	True/False
9.	Cohen's κ for the presence or not of AIBD was 0.681 indicating only moderate overall agreement between the two methods.	True/False
10.	For IIF, there was good agreement between IIF and histology for pemphigoid (κ = 0.885) and poor for pemphigus AIBDs (κ = 0.332).	True/False

Name: _____

Email: _____

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Journal-based CPD No. 83
Page 1 of 1

Questions relating to the oral presentations from the NSW Health Pathology Research Forum 2021 at page 5 of this issue.

1.	Novel Psychoactive Substances are of interest to the community however toxicology findings indicated methylenedioxymethamphetamine (MDMA) was the most frequent substance above toxic thresholds.	True/False
2.	The testing was performed on both fired and unfired cartridges that had been handled to leave touch DNA prior to loading and firing.	True/False
3.	A total of 17,719 participant performed self-collection of a novel nasal swab (RhinoSwab).	True/False
4.	Most of these antibodies can be identified by distinct patterns on indirect immunofluorescence (IIF) using commercial primate neural tissue slides.	True/False
5.	A co-ordinated national approach to investigation of VITT was achieved rapidly.	True/False
6.	To determine the agreement between FC and molecular methods for MRD measurement in adult patients with B-ALL and AML.	True/False
7.	Nanoparticle immunocytochemistry enables unprecedented resolution of tissue biomarkers.	True/False
8.	Over the surveillance period, samples from 332 patients were collected. The EV-D68 was amplified from 31/332 (13.3%) samples/patients (29 children and 8 adults).	True/False
9.	FASS incorporated AI functionality into an existing automated scanning microscope.	True/False
10.	CSD is not associated with differences in driver mutations in PSM. .	True/False

Name: _____

Email: _____

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Changes to APACE due to COVID-19 pandemic UPDATE

An APACE Certificate is usually awarded on attaining 100 CEU credits within a two year period.

As webinars and online conferences, meetings and workshops are all interactive, it was considered that this is the same as attending in person, therefore the same number of points will be awarded for attendance either virtually or face-to-face. This should enable more members to attend as no travelling time, costs and in some cases the online attendance with be without cost to the attendee.

Therefore, the extended time frame due to Covid-19 will no longer apply.

https://www.aims.org.au/apace

BOOKS FOR REVIEW

Following is a list of books available for review by resource consultants and members of the Institute with particular expertise in the field. The reviewer is invited to retain the complimentary copy of the book once the review is received.

As per our agreement with the book publishing companies, complimentary books are submitted to the Institute provided that all reviews are published in the Australian Journal of Medical Science. These reviews must be of a high quality as buying decisions and the reputation of the book and author are important considerations.

Books not requested will be allocated at discretion of the Editors for the Australian Journal of Medical Science. Reviews should be 300 to 700 words depending on the volume of the book. Time limit for return of review is **six** weeks.

Please send your request to: Australian Institute of Medical Scientists PO Box 1911 Milton Qld 4064
Tel: (07) 3876 2988 Fax: (07) 3876 2999 Email: programs@aims.org.au

1. **Bifidobacteria: Genomics & Molecular Aspects** edited by B. Mayo, & D. Van Sinderen. Caister Academic Press. xii + 260 pages.

2. **Medicine and Sport Science Volume 55: Cytokines, Growth Mediators & Physical Activity in Children during Puberty** edited by J. Jurimae, A.P. Hills & T. Jurimae. Karger. viii+178 pages.

3. **Digestive Diseases The Keys to IBD 2010: Treatment, Diagnosis & Pathophysiology.** Edited by G. Rogler & W. Sandborn. Karger. 188 pages.

4. **Else Kröner-Fresenuis Symposia Volume 1: Molecular Mechanisms of Adult Stem Cell Aging** edited by K.L. Rudolph. Karger. xii+108 pages**.**

5. **Endocrine Development Volume 24: Hormone Resistance and Hypersensitivity** edited by M. Maghnie, S. Loche, M. Cappa, L. Ghizzoni & R. Lorini. Karger. viii + 160 pages.

6. **Frontiers of Hormone Research Volume 41: Endocrine Tumor Syndromes and Their Genetics** edited by C.A Stratakis. Karger. xii + 187 pages.

7. **Frontiers of Hormone Research Volume 39: Kallmann Syndrome & Hypogonadotropic Hypogonadism** edited by R. Quinton. Karger. x+174 pages.

8. **Generic: The Unbranding of Modern Medicine** by Jeremy A. Greene. John Hopkins University Press. 368 pages.

9. **Human Pathogenic Fungi: Molecular Biology and Pathogenic Mechanisms** edited by Derek J. Sullivan & Gary P. Moran, Caister Academic Press. x + 342 pages.

10. **Internal Medicine: A Doctor's Stories** by Terrence Holt. Black Inc. 273 pages.

11. **Intolerant Bodies: A Short History of Autoimmunity** by Warwick Anderson and & Ian R. Mackay. John Hopkins University Press. 250 pages

12. **Lyme disease and relapsing fever spirochetes** edited by Justin D. Radolf and D. Scott Samuels. Caiser Academic Press. 760 pages

13. **More Than Hot: A Short History of Fever** by Christopher Hamlin. John Hopkins University Press. 400 pages.

14. **Pediatric and Adolescent Medicine Volume 19: Metabolic Syndrome and Obsesity in Childhood and Adolescence** edited by W. Kiess, M. Wabitsch, C. Maffeis, A.M. Sharma. Karger. x + 202 pages.

15. **Phage Therapy - Current Research and Applications** edited by Jan Borysowski, Ryszard Miedzybrodzki & Andrzej Gorski. Caister Academic Press. 368 pages.

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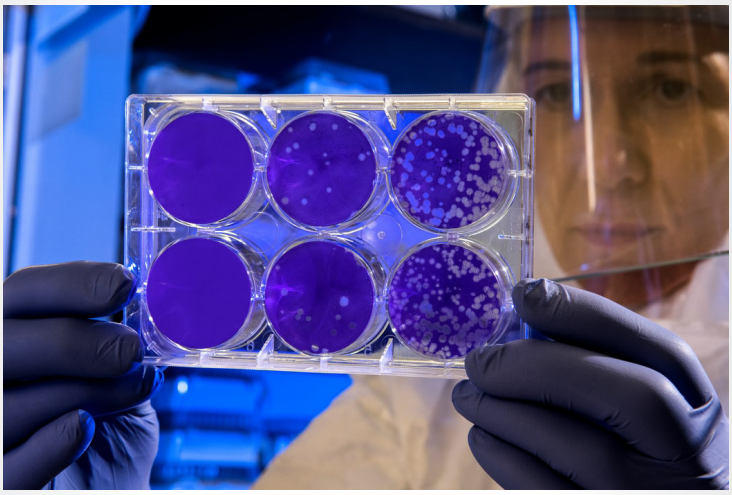

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AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

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The following instructions are based on the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", also known as the Declaration of Vancouver, and on the *Australian Government Style manual: for authors, editors and printers*, 6th edition, 2002. URLs were correct on September 29th, 2008.

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The *Australian Journal of Medical Science (AJMS)* will consider for publication any paper relevant to the field of Medical Science. Disciplines include blood banking, clinical biochemistry, haematology, histopathology, immunology, microbiology and molecular biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

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The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

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Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

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Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

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Personal Author(s) of a book:

Osler AG 1976. *Complement: mechanisms and functions*. Englewood Cliffs: Prentice-Hall.

Editor, Compiler, Chairman as Author:

Rhodes AJ, Van Rooyen CE, comps. 1968. *Textbook of virology: for students and practitioners of medicine and the other health sciences*. 5th ed. Baltimore: Williams and Wilkins.

Chapter in Book:

Weinstein L, Swartz MM 1974. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 457-472.

Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

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Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence:

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In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

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Use only standard abbreviations (see list of commonly used abbreviations).

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Report measurements in the units in which the measurements were made. In most countries the International System of Units (SI) is standard.

Commonly used abbreviations

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g	gram
g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

O'Connor M, Woodford FP. *Writing scientific papers in English: an ELSE-Ciba Foundation guide for authors.* Amsterdam, Oxford, New York: Elsevier-Excerpta Medica, 1975.

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

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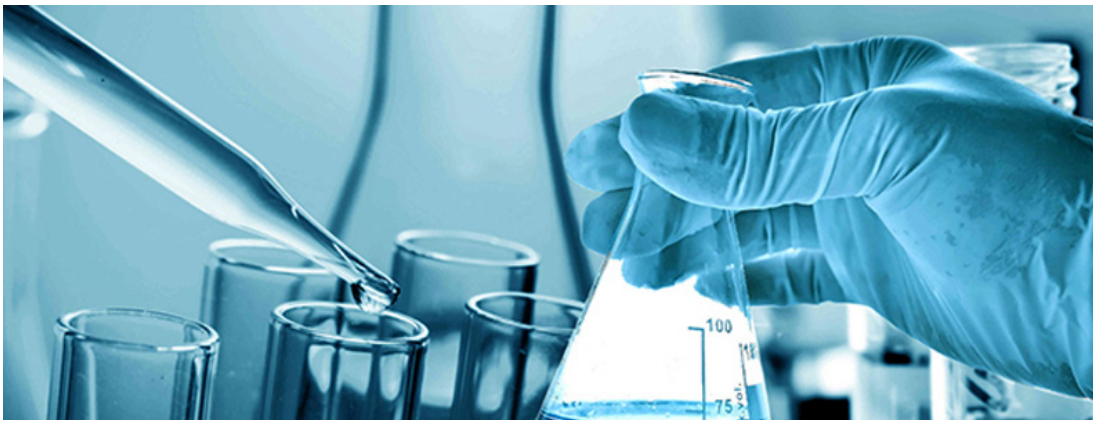

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