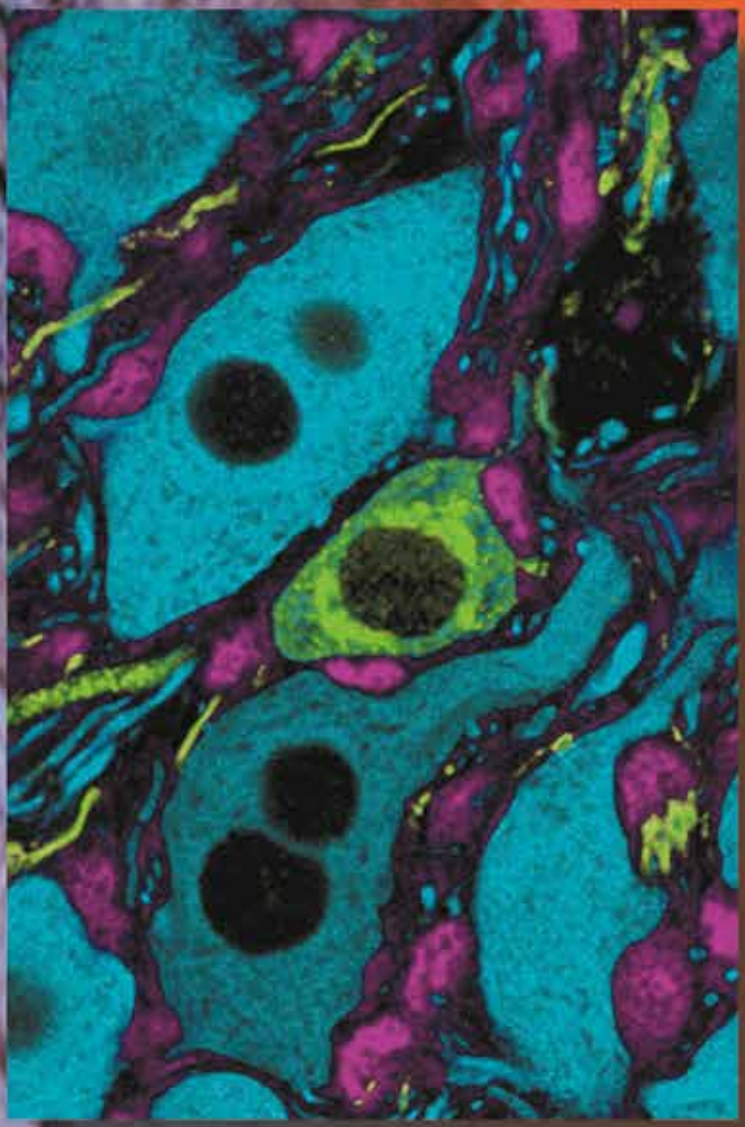
AUSTRALIAN JOURNAL OF
Medical Science
2024



ORIGINAL ARTICLES

CASE-STUDY

QUALITY UPDATE

REGULAR FEATURES

May 2024
Vol. 45
No. 2

UTS Online

Inspire Innovation

Unlock your potential with UTS Online's Master of Medical Science Leadership — a 100% online, tailored postgraduate degree designed for medical professionals.

- First Master's degree of its kind that combines leadership development with innovation in a medical science context
- Ranked top 150 globally for research
- Delivered by experts at the forefront of medical science and leadership
- 100% online

This groundbreaking master's course is your gateway to esteemed career paths such as Director, Manager, Advisor, Head of Laboratory Operations, or Lead Scientist.

- Elevate your leadership in cutting-edge medical fields
- Master effective communication, problem-solving, and adaptability
- Develop skills in analytical thinking, emotional intelligence, and ethical decision-making
- Build a strong network and relationships
- Embrace adaptability for innovation in medical science
- Tackle research, patient care, and healthcare management challenges with new skills

Join us and pioneer the future of medical science, gaining expertise and influence in your field.

Scan me for more info

CRICOS 00099F TEQSA PRV12060

Australian Journal of Medical Science

ADMINISTRATION

AIMS National Office

Chief Executive: Ms Sarah Just, BAppSc (MLS), GradCertMgt, MMedSc (Pathology), MAIMS

Design, formatting and management: AIMS National Office
Email: programs@aims.org.au
Website: www.aims.org.au
Telephone: 61 7 3876 2988
Address: PO Box 1911 Milton Qld 4064 Australia

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

Circulation 1500 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.

CONTENTS

May 2024 Vol. 45 No. 2

Original Articles

Quality Update

Case Study

Regular Features

Instructions to authors *are available on the AIMS website: www.aims.org.au*

Application of targeted long-read sequencing on *RHD* gene structural variants

Genghis H. Lopez,[1,2] Mia E. Sarri,[1] Robert L. Flower,[1,3] Catherine A. Hyland,[1,3]

[1] *Research and Development Laboratory, Australian Red Cross Lifeblood, Kelvin Grove, Queensland, Australia*

[2] *School of Health, University of the Sunshine Coast, Sippy Downs, Queensland, Australia*

[3] *School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, Queensland, Australia*

Abstract

Accurate blood typing for blood donors and patients is necessary for safe blood transfusion. Highly immunogenic D antigen, encoded by the *RHD* gene, can cause alloimmunisation in transfusion recipients who lack the D antigen (D-negative). *RHD* variants can produce altered D expression on red blood cells presenting difficulty in determining blood group phenotype. This study aimed to assess the feasibility of Long-Read Sequencing (LRS) technique to characterise *RHD* structural variants (SV). Long-range (LR) PCR amplicons were generated from two samples, *RHD*DV.10* and *RHD*DKG*, known to carry *RHD* gene structural variations. LRS was applied on LR-PCR amplicons and long-read sequences were aligned to *RHD* reference sequences for variant detection. Analysis showed that *RHD*DV.10* and *RHD*DKG* were formed by gene conversion and gene deletion respectively, consistent with known data. Long-read sequencing, as an adjunct to short-read sequencing (massively parallel sequencing), can help resolve complex *RHD* structural variants that produce ambiguous blood group phenotype.

Keywords: ***Long-read sequencing, RH blood group system, RHD structural variants, variant D expression***

Introduction

Background

Accurate blood typing for blood donors and patients is necessary for safe blood transfusion and reduces the risk of alloimmunisation (Cohn and Shaz 2023). The D antigen of the RH blood group system is very immunogenic (Daniels 2013). Patients with a D– or D+ variant phenotype (weak D or partial D) lacking one or more D epitopes, who receive D+ red blood cells (RBCs) can form anti-D. Anti-D is known to cause haemolytic disease of the fetus and newborn and haemolytic transfusion reaction (Quantock *et al* 2017; Sandler *et al* 2017). It is therefore important that D– patients with anti-D do not receive D+ RBCs (Daniels 2013).

The RH system has 56 blood group antigens encoded by *RHD*, *RHCE*, or hybrids of *RHD/RHCE* (ISBT 2023). The two genes are located on chromosome 1p36.11 (Daniels 2013). Each gene has 10 exons which are arranged with their tail ends facing each other (Figure 1) (Wagner and Flegel 2000). *RHD* encodes D (RH1) and *RHCE* encodes C (RH2), E (RH3), c (RH4) and e (RH5) antigens. The *RHD* and *RHCE* genes are 57,295 bp and 57,831 bp long, respectively (Okuda *et al* 2000).

The *RHD* gene is highly polymorphic (Daniels 2013). Variations in the *RHD* can produce a range of quantitative and/or qualitative changes in D expression termed D variants. These D variants on RBCs can be classified as 1) weak D – quantitative change in D antigen expression, or negative to weak agglutination reaction in standard immediate spin test but are moderately or strongly reactive in the anti-human globulin phase, 2) partial D – lacking one or more D epitopes, and 3) Del – an extremely low expressing D antigen that is not detected by standard serological techniques but by adsorption and elution technique (Daniels 2013; Sandler *et al* 2017; Wagner 2023). There are over 300 *RHD* variants that give rise to these altered D phenotypes (ISBT 2023). Generally, these *RHD* variants can be categorised as 1) single nucleotide variant (SNV), 2) insertions and deletions (Indels), and 3) structural variants (SV). SVs are large genomic alterations involving over 50 nucleotide bases (Cameron *et al* 2019). Over 60 *RHD* SV formed by gene conversion, gene deletion and gene duplication have been reported (Wagner and Flegel 2023). Accurate genotyping of *RHD* variants is

Address correspondence to:
Genghis H. Lopez, PhD
Australian Red Cross Lifeblood
44 Musk Avenue, Kelvin Grove, Queensland, Australia
Email: glopez@redcrossblood.org.au

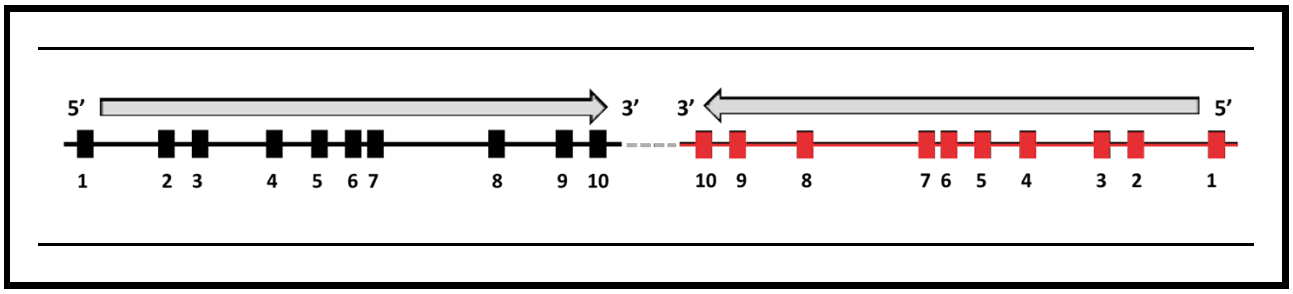


Figure 1. *Schematic representation of RHD and RHCE genes in the RH locus.*

RHD and RHCE genes are arranged in opposite orientation with their 3'-ends facing each other and are separated by approximately 30,000 bp (Okuda et al 2000, Wagner and Flegel 2000). Not shown in the diagram are the two 9 kb Rh boxes flanking the RHD, and the TMEM50A gene which is positioned between RHD and RHCE.

important to achieve correct phenotype prediction. Readers are referred to these review articles for further information on blood group genotyping (Cone Sullivan *et al* 2022; Westhoff 2019).

Majority of *RHD* variants are due to SNVs occurring in the exon region, however, SNVs located in the intron region disrupting RNA splicing have also been reported (ISBT 2023;Wagner 2023). Recently, a novel *RHD* c.1–110A>C SNV disrupting the GATA1 motif at the proximal promoter region was identified producing a Del phenotype (McGowan *et al* 2024b).

Known blood group gene SNVs, including *RHD*, can be detected by SNP genotyping methods such as sequence-specific primer PCR assay, real-time PCR, and SNP-microarray (Veldhuisen *et al* 2009). However, the more advanced massively parallel sequencing (MPS) technology that generates DNA sequence reads up to 300 bp, known as short-read sequencing (SRS), can easily detect known and novel SNVs and indels (Cone Sullivan *et al* 2022; Lane *et al* 2016; Schoeman *et al* 2017). Characterising *RHD* SVs using short-read sequencing can be challenging (Khandelwal *et al* 2023). Short-read sequences for *RHD*

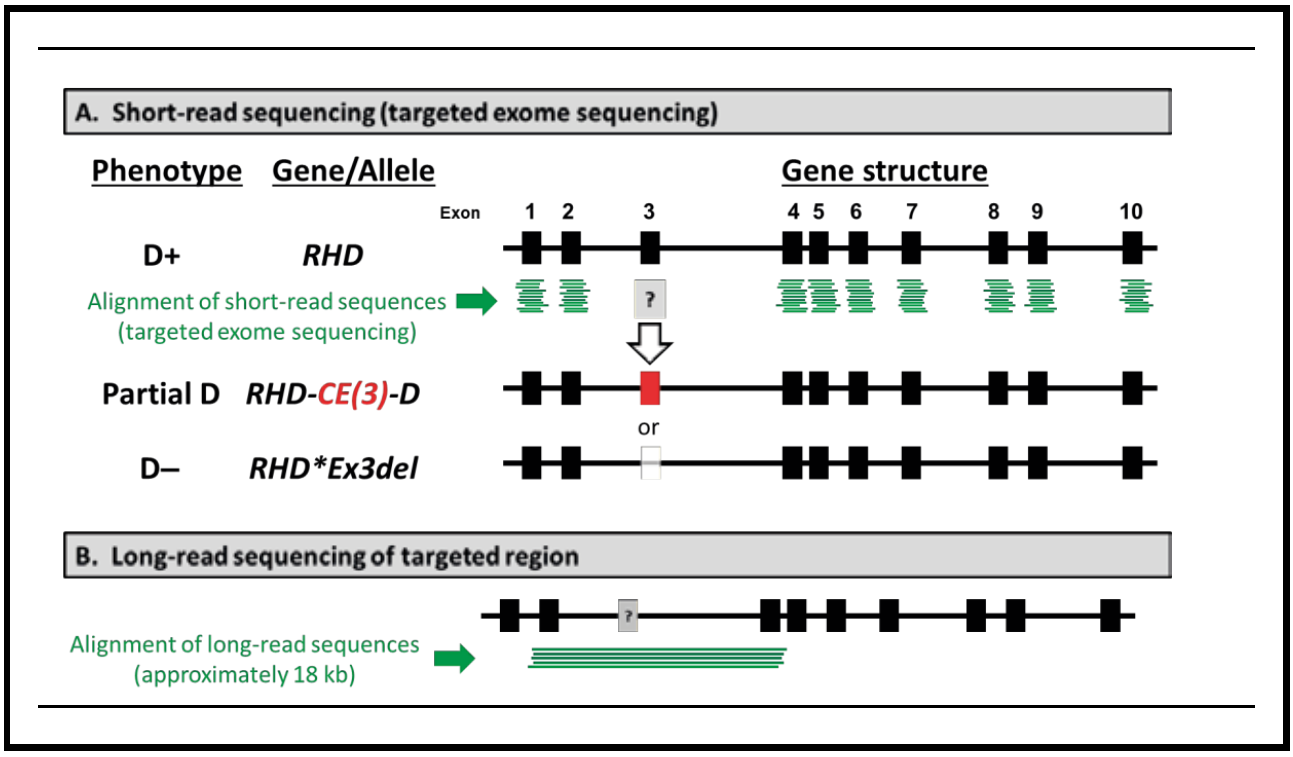


Figure 2. *Alignment of short-read sequences and long-read sequences when presented with RHD structural variants involving RHD exon 3.*

*The RHD-CE(3)-D and the RHD*Ex3del alleles were identified in a Caucasian and an African individual, respectively (Beckers et al 1996, Hyland et al 2017). Question mark (?) = in 2A, indicates absence of mapped sequence reads; in 2B, indicates the region spanned by long-read sequences.*

and *RHCE* are prone to mis-mapping because the two genes share 93.8% sequence homology (Daniels 2013; Schoeman *et al* 2017). Some *RHD* exon sequence reads have been shown to mis-map or misalign to *RHCE* and vice-versa making it difficult to give an accurate prediction of the gene structure. An example of this is shown in Figure 2A as the absence of mapped reads in *RHD* exon 3 can have two possible structures; 1) an *RHD-CE(3)-D* hybrid gene, the *RHD* exon 3 replaced by *RHCE* exon 3, or 2) *RHD*Ex3del* - a structural variant with an exon 3 deletion (Beckers *et al* 1996; Hyland *et al* 2017).

This limitation of SRS can be overcome by long-read sequencing (LRS) (Mantere *et al* 2019). LRS, which can produce sequence reads of more than 15 kb, has the potential to span the length of the altered region (Figure 2B) making this technique suitable to investigate structural variants (Khandelwal *et al* 2023; Thun *et al* 2023). The aim of this study is to apply targeted LRS on two known *RHD* variant samples and assess its performance to characterise structural variation in the *RHD* gene.

Materials and Methods

This study has approval from the Australian Red Cross Lifeblood Human Research Ethics Committee Application Number 2010#07.

Variant *RHD* samples and *RHD*-positive DNA control

Two blood donor samples from previous studies with rare *RHD* structural variations, *RHD*DV.10* and *RHD*DKG*, were selected for this study (Figure 3) (Lopez *et al* 2016; Lopez *et al* 2018). The *RHD*DV.10* is an *RHD-RHCE-RHD* hybrid allele. This variant is formed by gene conversion where *RHD* exons 5 and 6 were replaced by homologous sequences from the *RHCE* gene. This allele gives rise to a partial D phenotype, lacking D epitopes 1.2 and 5.1, and expresses low-frequency antigen D^{W} (RH23). The *RHD*DKG* allele has a 1,012 bp deletion that extends from intron 8 to intron 9, including exon 9. This variant encodes a Del partial D phenotype. Both the *RHD*DV.10* and *RHD*DKG* were hemizygous.

The *RHD*-positive DNA control used in this study is homozygous for *RHD*. Red blood cells for *RHD*-positive and *RHD*-negative DNA controls express the R1R1 and rr phenotype, respectively.

Long-range polymerase chain reaction (LR-PCR)

Whole blood samples were collected in EDTA tubes. Genomic DNA (gDNA) was extracted using the DNA Blood 350 Kit on the EZ1 Advanced (QIAGEN) instrument, a semi-automated machine using a magnetic bead-based DNA isolation method as per the manufacturer's instructions. DNA samples were kept frozen at –30 °C until testing.

Two long-range PCR (LR-PCR) assays were used to amplify specific regions in the *RHD* gene (Lopez *et al* 2016; Lopez *et al* 2018). For *RHD* exon 3-7 LR-PCR assay, D-specific forward primer D-3-383, (5'-TGTCGGTGCTGATCTCAGTGGA-3') located in Exon 3, and reverse primer re621 (5'-CATCCCCCTTTGGTGGCC-3') located in Intron 6 were used to amplify a 16.055 kb product. For *RHD* exons 7-10 LR-PCR assay, D-specific forward primer D-7-1048 (5'-TGCCGGCTCCGACGGTATC-3') located in Exon 7, and reverse primer D-10-1358 (5'-CAGTGCCTGCGCGAACATTG-3') located in the 3'-UTR amplified a 22.554-kb product. Primers were added into TaKaRa PrimeSTAR GXL DNA polymerase (Scientifix Pty Ltd, Clayton, VIC) kit to produce long-range amplicons.

The PCR conditions were as follows: 30 cycles of 10 seconds at 98°C (denaturation step) followed by 10 minutes at 68 °C, for LR-PCR *RHD* 3-7, or 70 °C, for LR-PCR *RHD* 7-10 (annealing step). Following the LR-PCR, amplicons were electrophoresed in a 0.5% agarose gel added with DNA intercalating dye (GelRed, Biotium), at 20V for 18 hours. Molecular weight marker (GeneRuler High Range DNA ladder, SM1351, ThermoFisher Scientific) was used to estimate the size of the PCR products. Agarose gels were photographed using an imaging equipment (Gel Doc XR+, Bio-Rad, South Granville, NSW).

Long-read sequencing on a PacBio Sequel II system

PCR products from four samples, including *RHD*-positive DNA controls, were sent to the Australian Genome

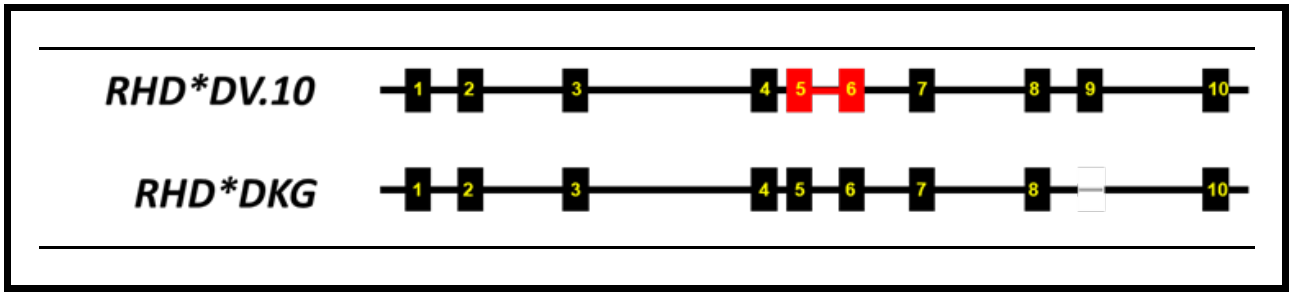


Figure 3. *Molecular structure of RHD variants investigated in this study.*

*The RHD*DV.10 is also named as RHD*05.10 while RHD*DKG is also known as RHD*DEL48 or RHD*Ex9del (ISBT 2023).*

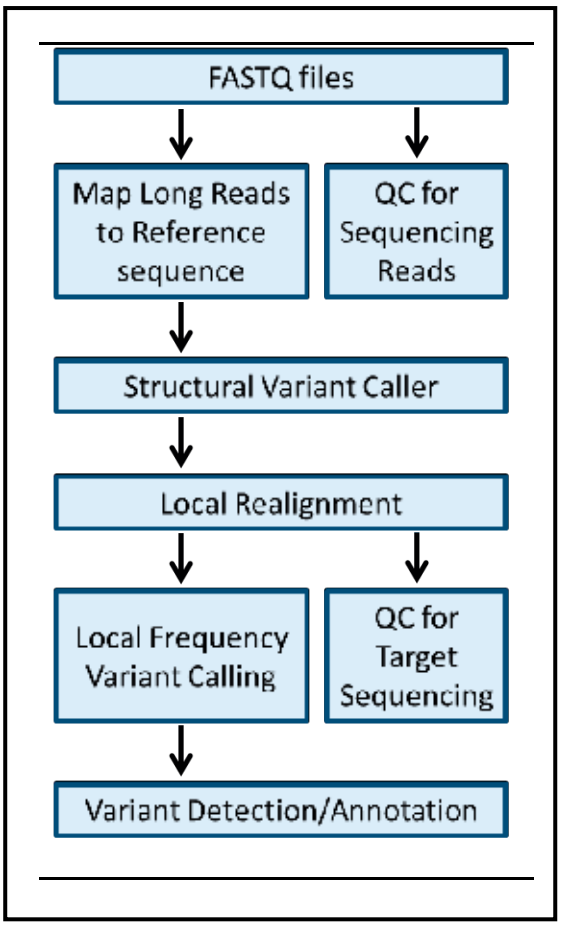


Figure 4. *Overview of bioinformatics analysis workflow.*

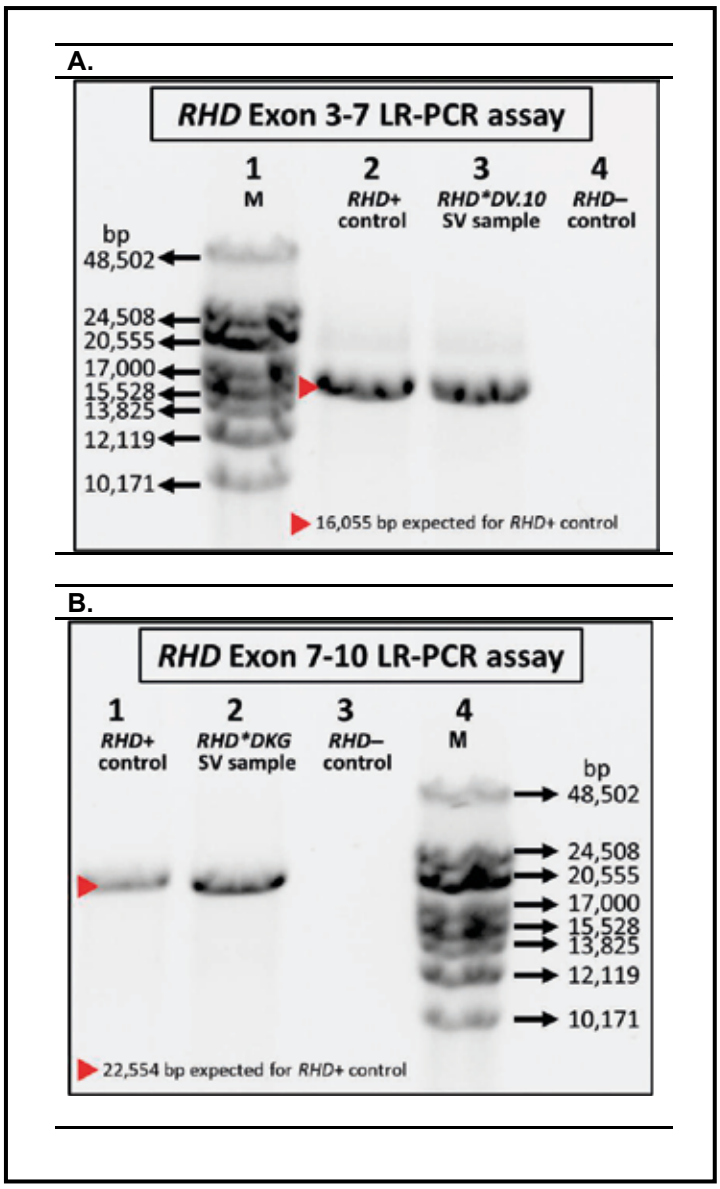


Figure 5. *Agarose gel electrophoresis of long-range PCR amplicons.*

LR-PCR = long-range polymerase chain reaction; SV = structural variant; M = molecular weight marker; bp = base pair. Red triangles indicate expected bands for RHD+ controls.

Research Facility, University of Queensland (St Lucia, QLD) for LRS using a PacBio Sequel II SMRT Cell 8M following the HiFi amplicon sequencing workflow. Sequencing mode circular consensus sequencing was used to generate HiFi reads as output (Q20+ quality).

Data analysis

FASTQ files were imported into CLC Genomics Workbench software version 23.0.5 (QIAGEN, Clayton, VIC) for analysis. Trimmed reads were aligned to *RHD* reference sequence NG_007494.1 (genomic) and variant detection for structural variants and single nucleotide changes was performed. CLC software was used to identify variants (exon numbers and amino acid changes). Key steps in the bioinformatics analysis workflow are presented in Figure 4. *RHD**DV.10 and *RHD**DKG exons and its flanking introns were checked for single nucleotide variations. The 5' and 3' gene breakpoints were also determined.

Results

Long-range PCR amplicons

The size of the amplicons from the two LR-PCR assays were checked by agarose gel electrophoresis. For the *RHD* Exon 3-7 assay, *RHD*-positive control and *RHD*DV.10* samples each produced a PCR band between 15,528 and

Table 1. *Long-read sequencing statistics.*

	Targeted *RHD* region	Mapped Reads	Average Coverage
1. *RHD-positive* DNA control	exon 3 to 7	66,780	18,279
2. *RHD*DV.10*	exon 3 to 7	32,820	9,024
3. *RHD*-positive DNA control	exon 7 to 10	42,859	10,158
4. *RHD*DKG*	exon 7 to 10	57,472	11,417

17,000 bp (Figure 5A). For the *RHD* Exon 7-10 assay, *RHD*-positive control and *RHD*DKG* each generated a PCR band between 20,555 and 24,508 bp (Figure 5B). No amplicons were produced by *RHD*-negative DNA control in the two assays as expected.

Long-read sequencing analysis

Long-read sequences generated from four targeted amplicons were aligned to specific regions of *RHD* NG_007494.1 (Figure 6). The summary statistics (total mapped reads and average coverage) for all samples are listed in Table 1.

Nucleotide sequences for *RHD*DV.10* when compared with *RHD* NG_007494.1 identified seven nucleotide variants in exon 5: c.667T>G (p.Phe223Val), c.697G>C (p.Glu223Gln), c.712G>A (p.Val238Met), c.733G>C (p.Val245Leu), c.744C>T (p.Ser248=), c.787G>A (p.Gly263Arg), c.800A>T (p.Lys267Met), and two in exon 6: c.916G>A (p.Val306Ile) and c.932A>G (p.Tyr311Cys) (Figure 7A and 7B). The minimum size of the RHCE insert in *RHD*DV.10* is 3.87 kb (data not shown). For *RHD*DKG*, long-read sequences showed a 1.012 kb gap (grey area) - intron 8, exon 9, and intron 9 - when aligned with *RHD* reference sequence (Figure 7C). For these two samples, the nucleotide variations and gene breakpoints identified by the LRS technique were consistent with previously reported data obtained from SRS and Sanger sequencing.

Sequences from *RHD*-positive DNA control (both the ~16 and ~22 kb fragments) were compared to *RHD* and showed no nucleotide variations in exon 3 to exon 10 and its flanking introns as expected.

Discussion

In this study, long-range PCR amplicons were generated from two *RHD* SVs, *RHD*DV.10* and *RHD*DKG*. Targeted long-read sequencing (LRS) was used to assess its capability to characterise complex *RHD* SVs not easily resolved by the short-read sequencing (SRS) technique. The genetic structure of these *RHD* SVs were accurately characterised by LRS consistent with data obtained from previous studies through a combination of DNA sequencing techniques (Sanger sequencing and SRS) (Lopez *et al* 2016; Lopez *et al* 2018).

A limitation of this study is that only SVs arising from gene conversion and gene deletion were investigated by LRS. Gene duplication, as a molecular mechanism forming SVs was recently reported in *RHD* (Fichou *et al* 2018). This allele called *RHD*weak D type 150*, formed by the duplication of exon 3, is the most common allele causing a weak D phenotype in the Indian population.

DNA-based typing, including MPS, has the advantage over serology-based typing for accurate blood grouping in patients who have been recently transfused, patients who are receiving monoclonal antibody therapy, or patients whose red blood cells are coated with antibodies (Westhoff 2019). In addition, when phenotyping reagents are rare or unreliable, genotyping can help predict blood group phenotype and aid in the identification of clinically significant red cell antibodies (Daniels 2021). A study suggested blood group allele matching for *RHD* could benefit sickle cell patients with partial D by preventing anti-D formation (Takasaki *et al* 2023). Another clinical application for MPS is for non-invasive prenatal testing (NIPT) to predict fetal red blood cell and platelet antigen status (Alford *et al* 2023; McGowan *et al* 2024a; Orzińska *et al* 2022). In alloimmunised pregnant women, it is vital to determine if the fetus is at risk of haemolytic disease enabling early monitoring and treatment to help minimise the clinical impact of HDFN.

MPS technology is now routinely used for blood group determination, however, there are challenges posed by this technology such as data storage and establishing bioinformatics analysis workflow to help analyse and interpret MPS data (Khandelwal *et al* 2023).

Bioinformatics has been identified as an emerging laboratory discipline and the demand for scientists with bioinformatics skills is growing (Belmont and Shaw 2016; Smith 2018). In a 2018 Australian Medical Laboratory Science Workforce Report, 3% of 299 respondents identified bioinformatics as an area of practice (Victorian Government 2018). With MPS as a widely used platform for blood group genotyping, possessing knowledge and

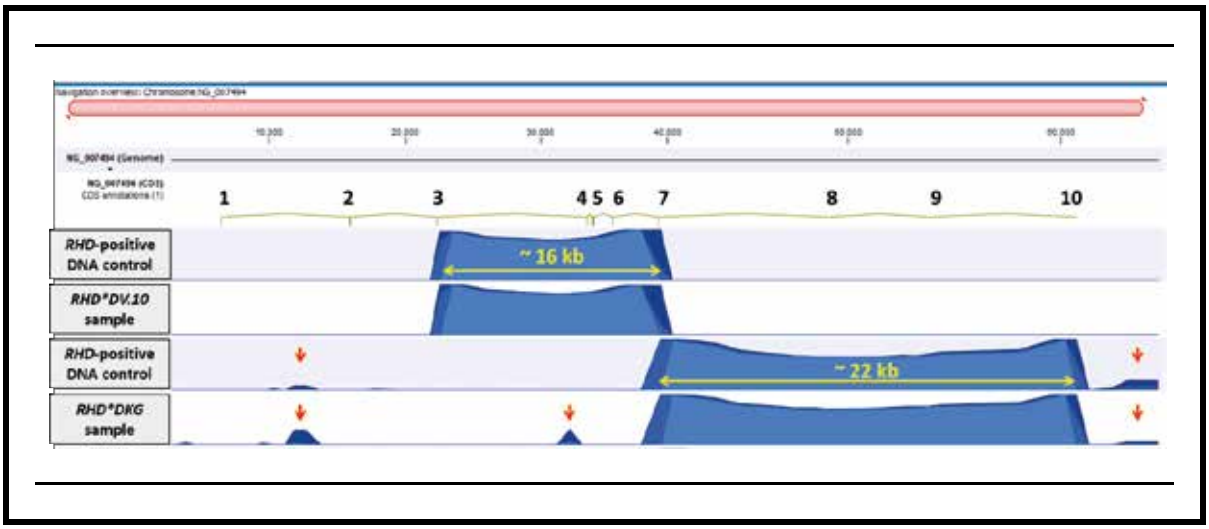


Figure 6. *Overview of long-read sequences aligned to RHD reference sequence.*

RHD exons are numbered from 1–10. Long-read sequences (blue area) from RHD-positive DNA control and the two RHD variants were aligned to RHD reference sequence NG_007494.1. The ~16 kb and ~22 kb long-read sequences spanned the exon 3–7 and exon 7–10 regions of the RHD gene, respectively. Red arrows show sequence reads mapped in the intronic region but outside of the target region. kb = kilobase.

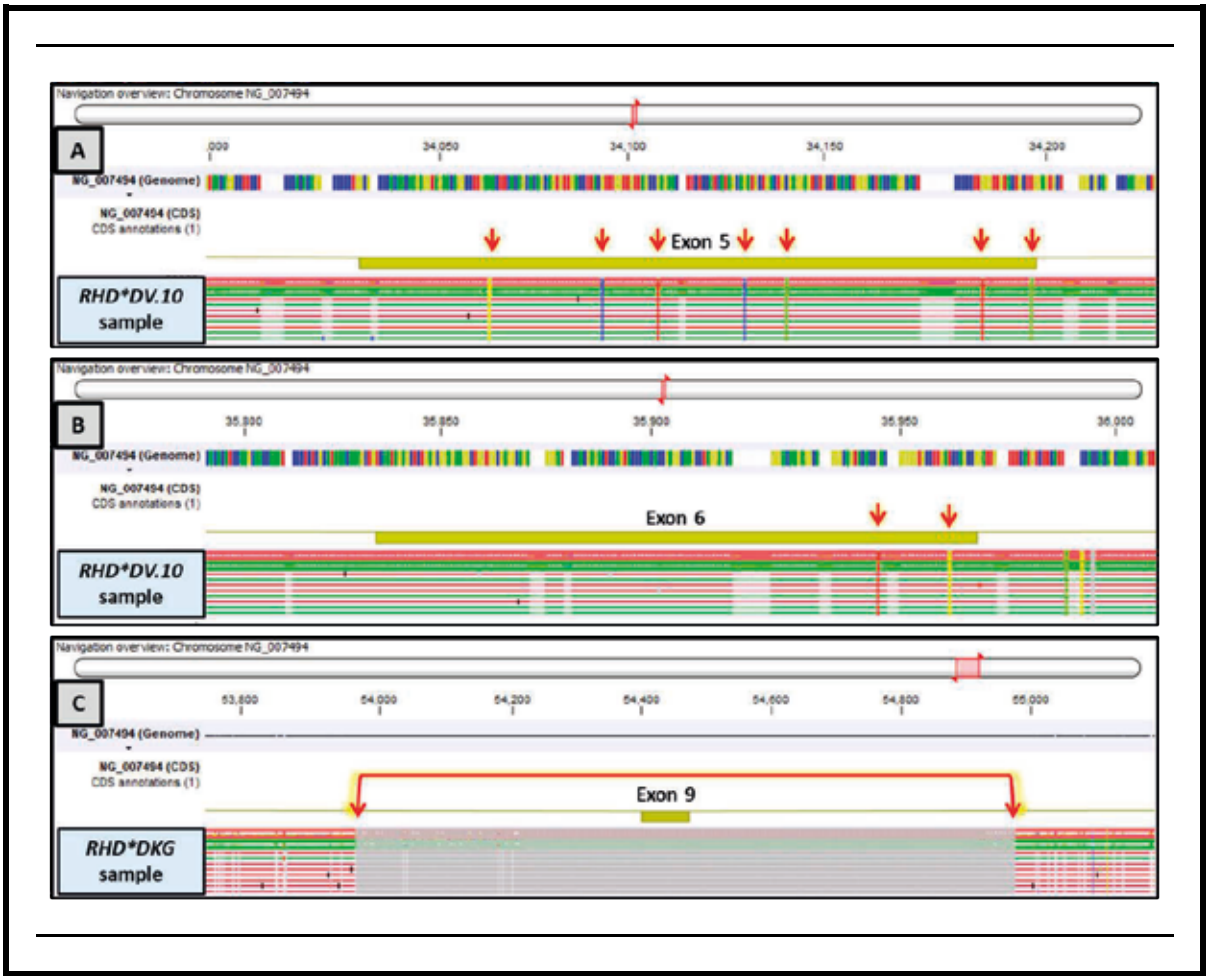


Figure 7. *Long-read sequences mapped to exons 5, 6, and 9 of the RHD reference sequence.*

*RHD*DV.10 long-read sequences mapped to RHD Exon 5 (A) and RHD Exon 6 (B) of the RHD reference sequence. Red arrows in A and B indicate single nucleotide variants. RHD*DKG long-read sequences mapped to RHD Exon 9 (C). Red bracket in 7C points to the deleted region (grey area) in RHD*DKG.*

skills in bioinformatics may be required for future medical laboratory scientists working in a clinical laboratory particularly in transfusion medicine (Owen 2023).

This proof of principle study showed that LRS is a viable tool in defining the genetic structure of *RHD* SVs. Findings from this study, including the development of a bioinformatics analysis pipeline to analyse *RHD* SVs, will help guide the design of customised targeted long-read sequencing panel to investigate SVs formed by *RHD* and *RHCE* genes. This panel can also be expanded to study *GYPA* and *GYPB* genes (MNS blood group system) and *GYPC* gene (Gerbich blood group system), all known to form structural variants (Jaskiewicz *et al* 2018; Wei *et al* 2016).

LRS, as an adjunct to SRS, can help resolve blood types with complex molecular structures necessary to achieve accurate blood type predictions. With increasing use of LRS technology in immunohaematology, the discovery of more novel blood group gene SVs is expected.

Acknowledgement

Australian Governments fund Australian Red Cross Lifeblood to provide blood, blood products and services to the Australian community. We thank the Australian Institute of Medical and Clinical Scientists (AIMS) Research Engagement Grant Scheme (REGS2020006) for funding this study. We also thank Ravi Kalathur (Senior Application Specialist, QIAGEN) for helping us design the bioinformatics analysis workflow.

References

Alford B, Landry BP, Hou S, Bower X, Bueno AM, Chen D, Husic B, Cantonwine DE, McElrath TF, Carozza JA, Wynn J, Hoskovec J, Gray KJ 2023. Validation of a non-invasive prenatal test for fetal RhD, C, c, E, K and Fy(a) antigens. *Sci Rep* 13: 12786.

Beckers EA, Faas BH, Ligthart P, Simsek S, Overbeeke MA, von dem Borne AE, van Rhenen DJ, van der Schoot CE 1996. Characterization of the hybrid RHD gene leading to the partial D category IIIc phenotype. *Transfusion* 36: 567-74.

Belmont JW, Shaw CA 2016. Clinical bioinformatics: emergence of a new laboratory discipline. *Expert Rev Mol Diagn* 16: 1139-1141.

Cameron DL, Di Stefano L, Papenfuss AT 2019. Comprehensive evaluation and characterisation of short read general-purpose structural variant calling software. *Nat Commun* 10: 3240.

Cohn CS, Shaz BH 2023. Blood and Its Components. *JAMA* 330: 1903-1904.

Cone Sullivan JK, Gleadall N, Lane WJ 2022. Blood Group Genotyping. *Clin Lab Med* 42: 645-668.

Daniels G 2013. Variants of RhD--current testing and clinical consequences. *Br J Haematol* 161: 461-70.

Daniels G 2021. An overview of blood group genotyping. *Ann Blood* doi: 10.21037/aob-21-37.

Fichou Y, Parchure D, Gogri H, Gopalkrishnan V, Le Marechal C, Chen JM, Ferec C, Madkaikar M, Ghosh K, Kulkarni S 2018. Molecular basis of weak D expression in the Indian population and report of a novel, predominant variant RHD allele. *Transfusion* 58: 1540-1549.

Hyland CA, Millard GM, O'Brien H, Schoeman EM, Lopez GH, McGowan EC, Tremellen A, Puddephatt R, Gaerty K, Flower RL, Hyett JA, Gardener GJ 2017. Non-invasive fetal RHD genotyping for RhD negative women stratified into RHD gene deletion or variant groups: comparative accuracy using two blood collection tube types. *Pathology* 49: 757-764.

ISBT 2023. *International Society of Blood Transfusion (ISBT), Red Cell Immunogenetics and Blood Group Terminology* [Online]. Available: http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/ [Accessed 21 Dec 2023].

Jaskiewicz E, Peyrard T, Kaczmarek R, Zerka A, Jodlowska M, Czerwinski M The Gerbich blood group system: old knowledge, new importance. *Transfus Med Rev* 32: 111-116.

Khandelwal A, Zittermann S, Sierocinski T, Montemayor C 2023. RH genotyping by next-generation sequencing. *Annals of Blood* 8.

Lane WJ, Westhoff CM, Uy JM, Aguad M, Smeland-Wagman R, Kaufman RM, Rehm HL, Green RC, Silberstein LE, MedSeq Project 2016. Comprehensive red blood cell and platelet antigen prediction from whole genome sequencing: proof of principle. *Transfusion* 56: 743-754.

Lopez GH, McGowan EC, McGrath KA, Abaca-Cleopas ME, Schoeman EM, Millard GM, O'Brien H, Liew YW, Flower RL, Hyland CA 2016. A D+ blood donor with a novel RHD*D-CE(5-6)-D gene variant exhibits the low-frequency antigen RH23 D(W) characteristic of the partial DVa phenotype. *Transfusion* 56: 2322-30.

Lopez GH, Turner RM, McGowan EC, Schoeman EM, Scott SA, O'Brien H, Millard GM, Roulis EV, Allen AJ, Liew YW, Flower RL, Hyland CA 2018. A DEL phenotype attributed to RHD Exon 9 sequence deletion: slipped-strand mispairing and blood group polymorphisms. *Transfusion* 58: 685-691.

Mantere T, Kersten S, Hoischen A 2019. Long-Read Sequencing Emerging in Medical Genetics. *Front Genet* 10: 1-14.

McGowan EC, O'Brien H, Sarri ME, Lopez GH, Daly JJ, Flower RL, Gardener GJ, Hyland CA 2024a. Feasibility for non-invasive prenatal fetal blood group and platelet genotyping by massively parallel sequencing: A single test system for

multiple atypical red cell, platelet and quality control markers. *Br J Haematol* 204: 694-705

McGowan EC, Wu PC, Hellberg Å, Lopez GH, Hyland CA, Olsson ML 2024b. A bioinformatically initiated approach to evaluate GATA1 regulatory regions in samples with weak D, Del or D– phenotypes despite normal RHD exons. *Transfus Med Hemother* https://doi.org/10.1159/000538469.

Okuda H, Suganuma H, Kamesaki T, Kumada M, Tsudo N, Omi T, Iwamoto S, Kajii E 2000. The analysis of nucleotide substitutions, gaps, and recombination events between RHD and RHCE genes through complete sequencing. *Biochem Biophys Res Commun* 274: 670-83.

Orzińska A, Kluska A, Balabas A, Piatkowska M, Kulecka M, Ostrowski J, Mikula M, Debska M, Uhrynowska M, Guz K 2022. Prediction of fetal blood group antigens from maternal plasma using Ion AmpliSeq HD technology. *Transfusion*: 62: 458-468.

Owen S 2023. The case for a clinical scientist (transfusion medicine). *Australian Journal of Medical Science* 44: 127-138.

Quantock KM, Lopez GH, Hyland CA, Liew YW, Flower RL, Niemann FJ, Joyce A 2017. Anti-D in a mother, hemizygous for the variant RHD*DNB gene, associated with hemolytic disease of the fetus and newborn. *Transfusion* 57: 1938-1943.

Sandler SG, Chen LN, Flegel WA 2017. Serological weak D phenotypes: a review and guidance for interpreting the RhD blood type using the RHD genotype. *Br J Haematol* 179: 10-19.

Schoeman EM, Lopez GH, McGowan EC, Millard GM, O'Brien H, Roulis EV, Liew YW, Martin JR, McGrath KA, Powley T, Flower RL, Hyland CA 2017. Evaluation of targeted exome sequencing for 28 protein-based blood group systems, including the homologous gene systems, for blood group genotyping. *Transfusion* 57: 1078-1088.

Smith DR 2018. Bringing bioinformatics to the scientific masses: As the demand for high-level bioinformatics is growing, training students in the field becomes ever more important. *EMBO Rep* 19.

Takasaki K, Friedman DF, Uter S, Vege S, Westhoff CM, Chou ST 2023. Variant RHD alleles and Rh immunization in patients with sickle cell disease. *Br J Haematol* 201: 1220-1228.

Thun GA, Gueuning M, Mattle-Greminger M 2023. Long-Read Sequencing in Blood Group Genetics. *Transfus Med Hemother* 50: 184-197.

Veldhuisen B, Van Der Schoot CE, De Haas M 2009. Blood group genotyping: from patient to high-throughput donor screening. *Vox Sang* 97: 198-206.

Victorian Government 2018. Medical Laboratory Science Workforce Report. *In:* Department of Health and Human Services Services. Melbourne.

Wagner FF 2023. Serology and molecular biology of DEL: a narrative review. *Annals of Blood* 8.

Wagner FF, Flegel WA 2000. RHD gene deletion occurred in the Rhesus box. *Blood* 95: 3662-8.

Wagner FF, Flegel WA. 2023. *RhesusBase. Website: http://www.rhesusbase.info* [Online]. Available: http://www.rhesusbase.info/ [Accessed 21 Dec 2023].

Wei L, Lopez GH, Ji Y, Condon JA, Irwin DL, Luo G, Hyland CA, Flower RL 2016. Genotyping for Glycophorin GYP(B-A-B) Hybrid Genes Using a Single Nucleotide Polymorphism-Based Algorithm by Matrix-Assisted Laser Desorption/Ionisation, Time-of-Flight Mass Spectrometry. *Mol Biotechnol* 58: 665-671.

Westhoff CM 2019. Blood group genotyping. *Blood* 133: 1814-1820.

Bringing thrombin generation into the diagnostic setting

Sarah Just[1], Minh Hua[1,2], Abishek Santhakumar[3]

[1] *Haematology Department, New South Wales Health Pathology, Liverpool Hospital, Sydney NSW*

[2] *Department of Medicine, University of New South Wales, Liverpool Hospital Clinical School, Sydney NSW*

[3] *School of Dentistry & Medical Sciences, Charles Sturt University, Wagga Wagga NSW*

Abstract

Abnormal thrombin generation plays a key role in the pathophysiology of abnormal bleeding and thrombosis, with global thrombin generation assays such as the Calibrated Automated Thrombogram (CAT) are widely used in clinical research. Lack of standardisation in methodology has hampered the translation of TG from research into a diagnostic assay. This study aims to standardise pre-analytical and analytical processes and establish reference intervals as the initial step in implementation of TG as a diagnostic test.

*Keywords: **Thrombin generation, reference intervals, Calibrated Automated Thrombogram pre-analytical variables, analytical variables.***

Introduction

Pathological thrombosis or bleeding is associated with significant morbidity and mortality, and abnormal thrombin generation (TG) plays a key role in this pathophysiology (Al Dieri *et al* 2012). Global TG assays such as the CAT allow quantitative assessment of TG, which reflects a better correlate of overall haemostatic function than existing routine clot based assays. The current traditional clotting assays, prothrombin time (PT) and activated partial thromboplastin time (APTT), as used in the diagnostic pathology laboratory, evaluate the clotting cascade in relative isolation and these tests represent a small portion of the overall haemostatic balance of procoagulant and anticoagulant factors involved in an individual (Tripodi 2016). The endpoint for these clotting assays is the formation of a fibrin clot that occurs when approximately 5% of total thrombin generation occurs (Baglin 2005). The remainder of the total thrombin generated is not accounted for in these tests, and thus these tests poorly reflect the overall assessment of the haemostatic profile of the sample (Tripodi 2016). The PT or APTT also cannot assess the prothrombotic state of an individual, which may be facilitated by performing Antithrombin, Protein C, Protein S and Activated Protein C Resistance assays; however these tests are also performed in isolation in an individual. None of these currently available assays, commonly performed in diagnostic laboratories, are a true physiological representation of a patient's overall haemostatic profile in vivo (Tripodi 2016).

Global haemostasis assays such as the TG assay which evaluate the ability of patient plasma to generate and inhibit thrombin, have been used in a multitude of clinical research settings for thrombosis and bleeding risk assessment to determine hypercoagulopathic and hypocoagulopathic states (Castoldi & Rosing 2011). TG assays were first described in 1953 and have been developed into calibrated, automated methods with standardised protocols, including the semi-automated CAT (Hemker *et al* 2003). Fully automated analyser systems that measure TG include ST Genesia system (Stago, Asnieres sur Seine, France), Ceveron Alpha (Technoclone, Vienna, Austria) and Behring Coagulation System BCS XP (Siemens Heathcare, Deerfield, USA). The automation and improvements to the original methods allow these newer TG assays to be transitioned from the research laboratory to the clinical laboratory (Al Dieri *et al* 2012). The main parameters of the thrombin generation curve are the lag time (minutes) which is the time from addition of the trigger to initiation of TG, the thrombin peak (nmol/L) which is the time to reach the peak, the velocity index (VI) calculated from (peak height/(time to peak-lag time), and the area under the curve or endogenous thrombin potential (ETP) (nmol/L thrombin x min), with each parameter having relevance to hyper- and hypocoagulability. In hypercoagulability states there are shortened lag times, high peak height and shortened time

Address correspondence to:
Sarah Just
Email: justsarahcr@gmail.com

to peak, high VI and high ETP values. In contrast, there are prolonged lag times, low peak height and long time to peak, low VI and low ETP values in hypocoagulability states (Tripodi 2016). Currently there are three commercially available assays for TG, all using different wavelengths for measurement, different substrates for thrombin detection, different concentrations of tissue factor as the trigger reagent and varying amounts of phospholipid in their reagents, all of which creates variability in the results generated (Kintagh *et al* 2018). This lack of standardisation in the assays, along with variability in pre-analytical and analytical processes, makes correlation between clinical studies difficult and has hampered the implementation of TG into the diagnostic setting.

This study aims to standardise pre-analytical processes using standardised collection and sample preparation procedures and analytical processes using a commercially available TG assay. Standardised protocol reference intervals for TG parameters (lag time, time to peak, peak height and ETP will be established using normal donor samples, with partitioning for males and females. Standardisation of the pre-analytical and analytical processes in addition to establishment of reference intervals will enable further validation and use of TG in patients with pathological dysfunctional haemostasis to provide a more comprehensive overview of the patient's haemostatic profile.

Materials and Methods

Subject selection

The study was approved by the South-Western Sydney Local Health District Research Ethics Committee, (Project Number HE 17/259) and a reciprocal agreement with Charles Sturt University Human Research Ethics committee, (Protocol H18022)

The study population consisted of 74 healthy adults (31 males and 43 females), median age of 45 y (minimum 22 y and maximum 64 y), with no history of bleeding or thrombosis and not on any known anticoagulant or anti-platelet medications. Participants provided written informed consent prior to participation in the study.

Pre-analytical variables - platelet poor plasma preparation and time after blood collection

Peripheral venous blood was collected aseptically with minimal stasis, using a 21G needle and vacutainer adaptor into plastic 3.0 mL Vacuette tubes (Greiner Bio-One, Austria) containing 3.2% buffered sodium citrate (final concentration 0.105 mol/L).

Platelet-poor plasma (PPP) was prepared by centrifuging the citrate tubes at two different centrifugation conditions (2100g for 20 mins, recommended by the manufacturer for TG) and 1500g for 30 mins (equivalent to current routine sample processing), within 30 mins of blood collection for five normal donors. This was to assess for any differences between the two different centrifugation conditions. All centrifugation was performed at room temperature. After each centrifugation the top two thirds of plasma was separated off into aliquots and stored frozen at -80ºC until ready for testing.

Subsequent samples collected at the same time from the five normal donors were processed using local laboratory centrifugation conditions (1500g for 30 mins), at different time points from collection, 2 h, 4 h and 6 h to determine the effect of time from collection on TG parameters. Once centrifuged, the top two thirds of plasma was separated into aliquots and stored frozen at -80ºC until ready for testing.

For establishment of reference intervals, the current routine sample processing conditions were used (1500g for 30 mins), and PPP prepared within two h of collection with the top two thirds of plasma separated off into aliquots and stored frozen at -80ºC until ready for testing.

Thrombin generation assay

TG was assessed using a CAT system with a Fluoroscan Ascent fluorometer and dispensing system (Thermofisher Scientific, Australia) according to the manufacturer's instructions (Thrombinoscope BV, Maastricht, Netherlands).

TG was performed using commercially produced reagents from Diagnostica Stago (Asnieres sur Seine, France), PPP reagent (5pM tissue factor and 4uM phospholipids), FluCa kit (fluorogenic substrate and calcium chloride in a buffer solution) and thrombin calibrator reagent. For each sample tested, thrombin calibrator and thrombin generation wells were measured in triplicate. Fluorescence readings were measured at 390 nm and 430 nm every 30 secs for 40 mins.

TG parameters assessed were lag time, time to peak, peak height and area under the curve (ETP),

Coagulation parameters

To assess normal routine haemostatic profile of the donors, coagulation parameters (PT, APTT, fibrinogen and D-dimer) were performed on a STA-R model R Evolution coagulation analyser (Diagnostica Stago, Asnieres sur Seine, France) using standard methods and reagents: STA Neoplastine for PT, CI Plus, TriniCLOT S for APTT, STA Liquid Fibrinogen for fibrinogen and STA Liatest DDI Plus for the D-Dimer. All reagents were purchased from Diagnostica Stago.

Statistical analysis

Comparisons of results for different pre-analytical conditions of PPP preparation and time after blood collection were performed using repeated measures one way analysis of variance (ANOVA) multiple t-tests with statistical significance determined using Tukey's method for each TG parameter. Comparisons between reference interval groups (males and females and all normals) was performed using unpaired t-test, and comparisons across age groups was performed using one way ANOVA, multiple comparisons. A p-value of < 0.05 was considered significant in all comparisons.

Data for establishment of reference intervals is presented as mean +/- standard deviation (SD) and mean +/- 2SD with the lower reference limit as the 2.5th percentile and the upper limit as the 97.5th percentile, along with coefficient of variation (CV %). Data was also assessed for normality (if values are showing a Gaussian distribution) using D'Agostino & Pearson normality test. Statistical analysis was performed using GraphPad Prism software (Version 7.0, La Jolla, CA, USA).

Results

Pre-analytical variables - platelet poor plasma preparation and time after blood collection

There was no significant difference seen in TG parameters, lag time, time to peak, peak height or ETP, across the two centrifugation protocols and at the four different time points from collection with the five normal donors tested (Figure 1a to 1d).

Reference intervals and inter-individual variability

A total of 74 normal donors were tested and data calculated for mean +/- standard deviation (SD) and mean +/- 2SD to generate reference intervals (lower limit 2.5th and upper limit 97.5th percentiles) and inter-individual variability (CV %) for the TG parameters (lag time, time to peak, peak height and ETP,). The mean age for participants was 43.9 y with minimum age 22 y and maximum age 64 y. All parameters passed the normality test except peak height (p 0.0001) (Table 1).

The group was further divided into males (n=31) and females (n=43) with no significant differences seen between the reference intervals for each TG parameter. For the 31 males, mean age was 42.1 y with minimum age 22 y and maximum age 61 y. All parameters passed the normality test except Peak height (p 0.0068). For the 43 females, mean age was 45.1 y with minimum age 22 y and maximum age 64 y. All parameters passed the normality test except Peak height (p 0.0025) (Table 2).

There was no statistical difference seen between male and female reference interval values for all TG parameters. There was also no significant difference compared to the normal reference interval data (Figure 2a to 2d).

The group was divided into age ranges, 20 - 29 y (n=10), 30 - 39 y (n=10), 40 - 49 y (n=14) and 50 - 64 y (n=6). The

Table 1. *Reference Intervals All Normal samples - (Range Mean +/- SD, Range 2.5th and 97.5th percentiles and CV %)*

All Normal samples	Lag Time (min)	Time to Peak (min)	Peak Height (nM)	ETP (nM.min)
Range (Mean +/- SD)	2.86 – 3.79	5.934 – 8.322	220.3 – 381.9	1648.8 – 2463.2
Range (2.5th - 97.5th percentiles)	2.36 – 4.30	5.08 – 9.09	185 - 519	1296 - 2957
CV %	13.9	16.8	26.8	19.8

Table 2. *Reference Intervals Males and Females - (2.5th and 97.5th percentiles and CV %)*

Males	Lag Time (min)	Time to Peak (min)	Peak Height (nM)	ETP (nM.min)
2.5th - 97.5th percentiles	2.44 – 4.11	5.33 – 10.44	183.1 – 523.2	1344 - 2824
CV %	12.9	17.1	24.9	15.5
Females				
2.5th - 97.5th percentiles	2.33 – 4.33	4.78 – 9.60	188.9 – 523	1287 – 3278
CV %	14.8	16.1	28.0	22.6

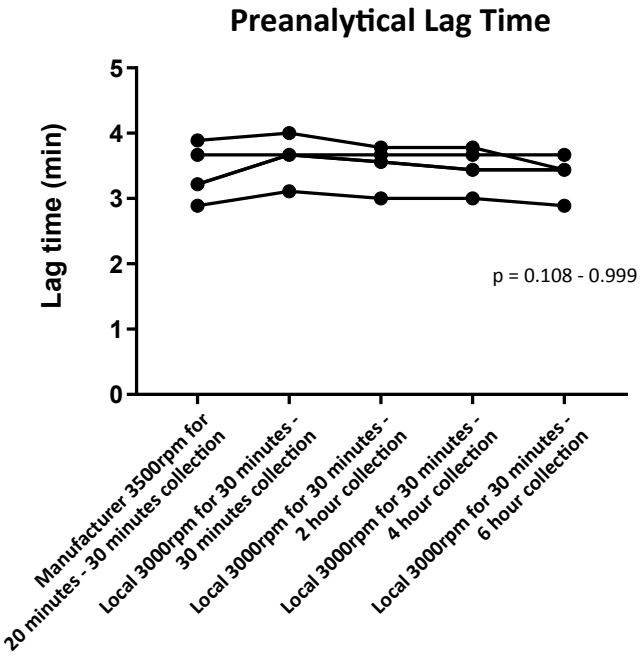


Figure 1a. *Pre-analytical variable effects on thrombin generation parameter – Lag time for five normal donors across two different centrifugation protocols and different times from collection.*

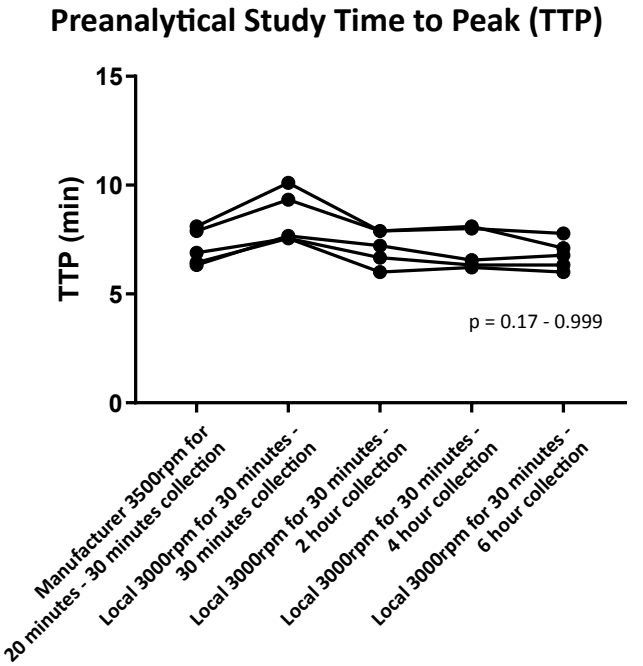


Figure 1b. *Pre-analytical variable effects on thrombin generation parameter – TTP for five normal donors across two different centrifugation protocols and different times from collection.*

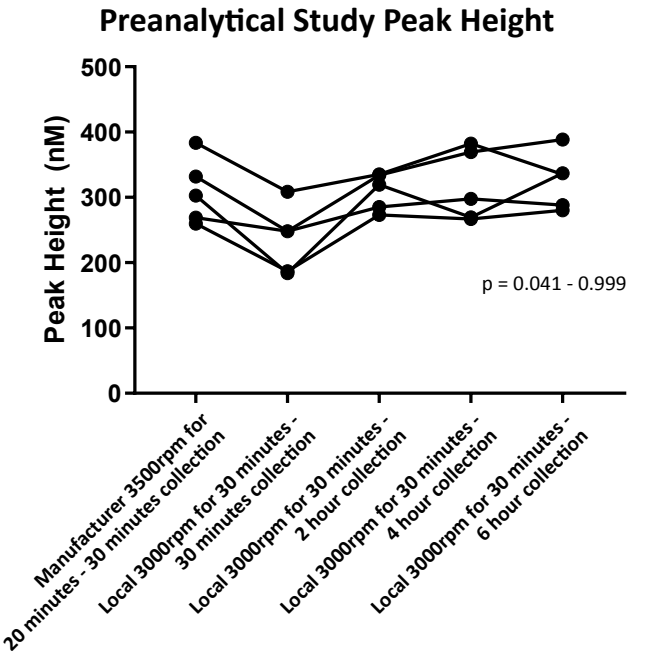


Figure 1c. *Pre-analytical variable effects on thrombin generation parameter – Peak Height for five normal donors across two different centrifugation protocols and different times from collection.*

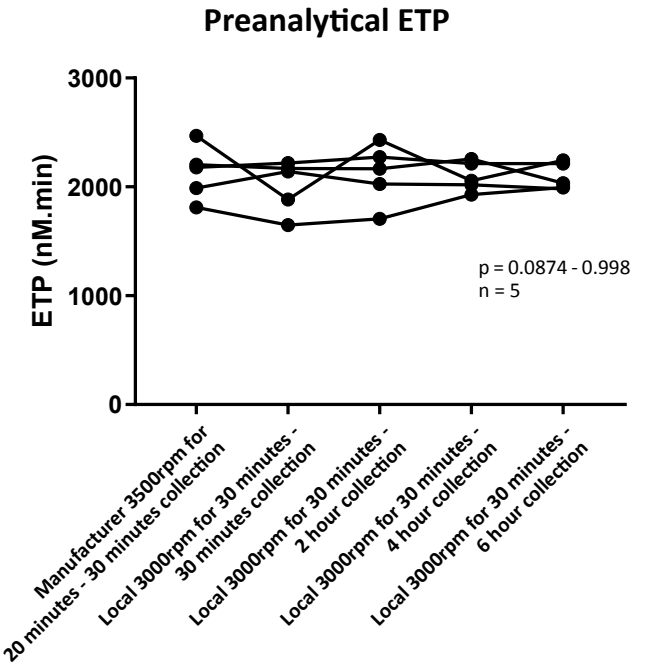


Figure 1d. *Pre-analytical variable effects on thrombin generation parameter – ETP for five normal donors across two different centrifugation protocols and different times from collection.*

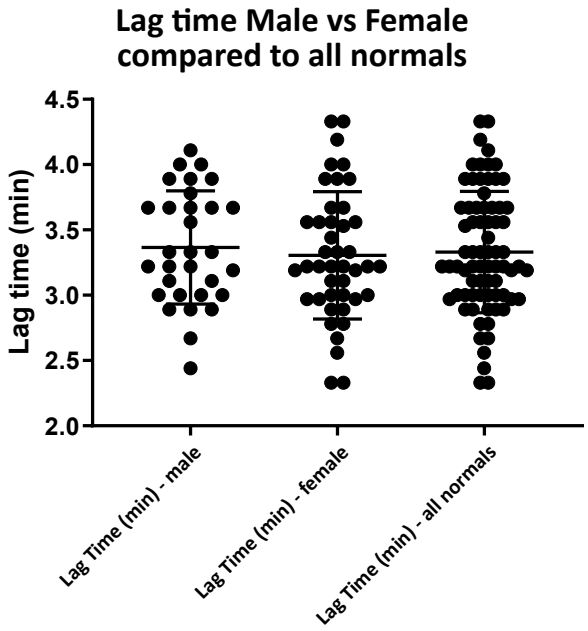


Figure 2a. *Thrombin generation parameter - Lag time for Males vs Females compared to Normal samples*

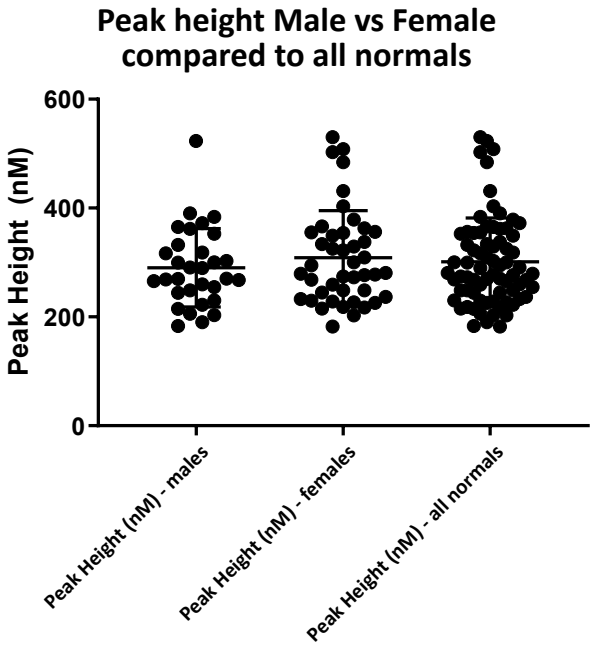


Figure 2c. *Thrombin generation parameter - Peak height for Males vs Females compared to Normal samples*

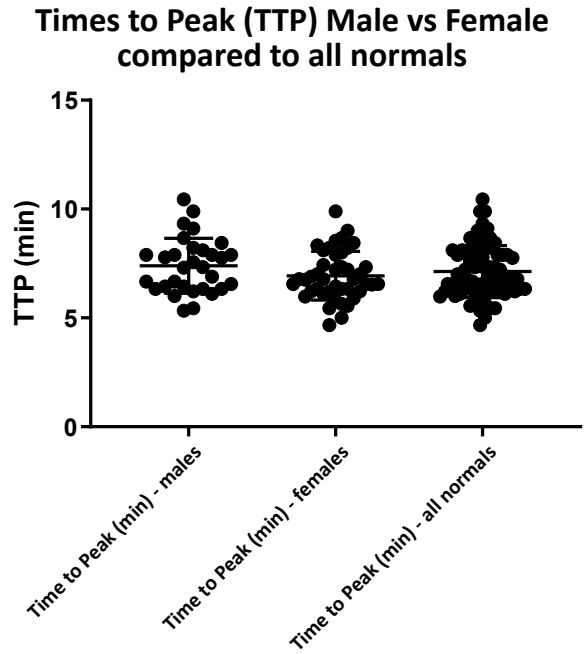


Figure 2b. *Thrombin generation parameter – Time to Peak (TTP) for Males vs Females compared to Normal samples*

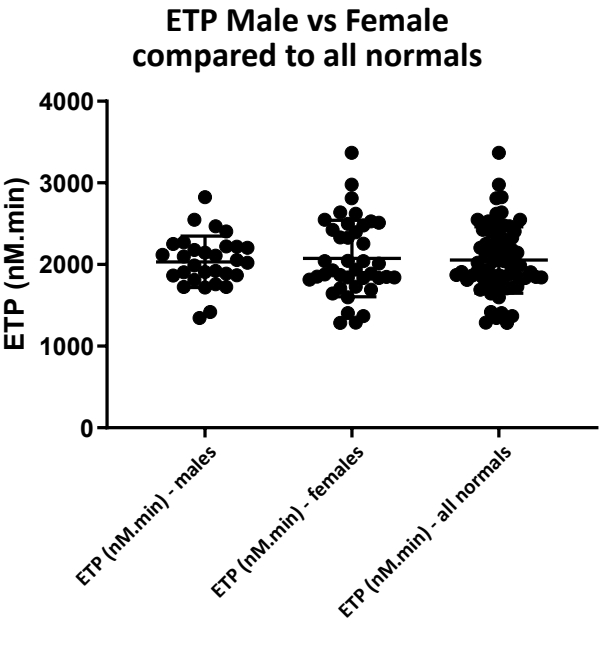


Figure 2d. *Thrombin generation parameter - ETP for Males vs Females compared to Normal samples*

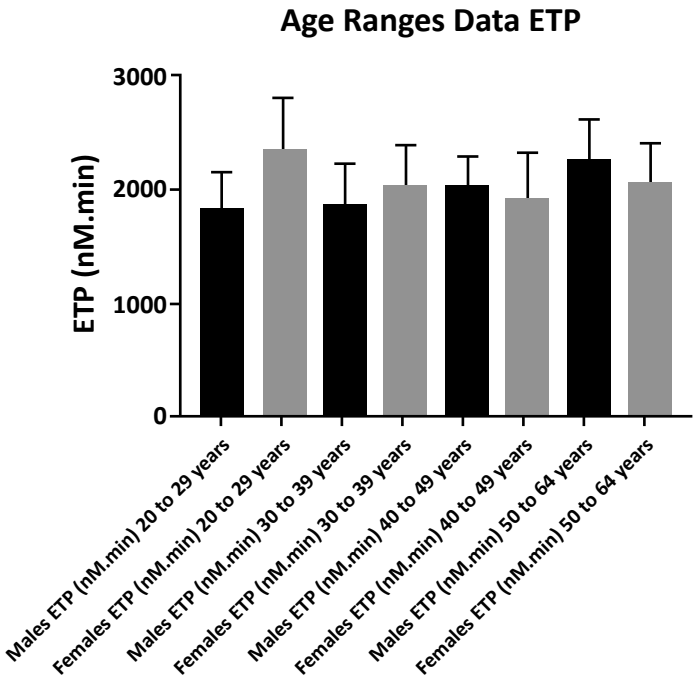


Figure 3. *Thrombin generation parameter – ETP mean differences across the different age group ranges for males and females*

thrombin parameter, ETP was analysed in this subgroup analysis. There was no significant difference seen between male and female ETP parameters, across any of the age group ranges. There were marked variations in the mean differences seen across different groups ranging from a maximum of – 519.8 in males 20 - 29 y compared to females 20 - 29 y and minimum difference of –4.23 with females 30 - 39 y compared to males 40 - 49 y (Figure 3).

Discussion

Routine coagulation assays have standardised procedures to minimise the effect from pre-analytical and analytical variables on results, with documented Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI H21-A5 2008) for collection and processing of plasma for haemostasis testing. TG assays use much lower concentrations of coagulation trigger reagents, tissue factor and phospholipids which potentially make them even more sensitive to these pre-analytical and analytical variables (Tripodi 2016). This study aimed to minimise the known variables affecting TG by development of standardised pre-analytical procedures.

Pre-analytical variables, including collection technique and processing and storage of the sample prior to testing, affect TG parameters causing variability in results when comparing across centres (Baglin 2005). Contact activation at time of collection can be minimised with the addition of corn trypsin inhibitor (CTI) to the blood collection tube, but there has been no agreement on whether this has a significant effect on TG parameters with conflicting data from several studies (Luddington & Baglin 2004; Spronk *et al* 2009; Dargaud *et al* 2017; Baglin 2011). Assays using low concentration tissue factor (1pM) showed better sensitivity to detect hypo- and hyper-coagulability by addition of CTI at blood sampling with variability reduced from 25% to less than 5% (Luddington & Baglin 2004). Spronk *et al* (2009) discussed other studies that have shown neglible effect on addition of CTI to plasma samples prior to testing, using tissue factor concentrations > 1 pM, but have observed reduced contact activation by using venepuncture rather than winged collection infusion sets (Spronk *et al* 2009). The scientific subcommittee for the control of anticoagulation for the International Society on Thrombosis and Haemostasis (ISTH) recommends using CTI to minimise contact activation when measuring TG using low levels of tissue factor (< 1 pM) for testing in haemophiliacs and hypocoagulable states (Dargaud *et al* 2017). This reduces assay imprecision and eliminates the need for immediate sample preparation and testing (Baglin 2011). Addition of CTI would however also require the use of special blood collection tubes, compromising general test usage. In our study, tissue factor was utilised at a concentration of 5 pM, thus avoiding the need for CTI in collection tubes and utilised venepuncture collection into routine sodium citrate tubes.

Standardised collection tubes and technique were used and our study assessed plasma processing by centrifugation using the local centrifugation protocol compared to the manufacturer recommended centrifugation protocol. The local protocol is used to prepare plasma samples for coagulation testing at a later date with plasma stored at --80ºC. The local procedure produces PPP with residual platelet counts of less than 3 x 10^9/L prior to freezing (confirmed by local validation studies). Residual platelets in the sample should be < 10 x 10^9/L prior to freezing (CLSI H21-A5 2008) as subsequent freezing and thawing leads to release of phospholipids from platelets which affect thrombin generation parameters by additional phospholipids and platelet microparticles in the test system. Sufficiently high centrifugation speeds and time and even a double centrifugation step as recommended by CLSI (CLSI H21-A5 2008) and the manufacturers remove this variable. It was confirmed that the local single spin centrifugation (1500g for 30 mins) compared to the recommended manufacturer centrifugation (2100g for 20 mins) exhibited no significant differences in TG parameters for the five normal donors tested. This is due to low residual platelet counts in PPP and removal of larger platelet microparticles using the local centrifugation protocol. Rodgers *et al* (2014) evaluated several pre-analytical variables and found the effect of each varied depending on the concentration of tissue

factor used in the CAT assay with low concentrations (1 pM TF) particularly sensitive to centrifugation method compared to high concentrations (5 pM TF) (Rodgers *et al* 2014). They assessed 10 normal donors, using four different centrifugation methods (single spin (1400g for 10 mins), double spin (1400g for 10 mins twice), enhanced single spin (2600g for 15 mins) and enhanced double spin (1400g for 10 mins then 20000g for 3 mins)) as well as four different time points for plasma separation and showed no difference in TG parameter ETP or peak height across the different centrifugation parameters using PPP reagent 5 pM TF. This is similar to what our study found with ETP but not peak height. Loeffen *et al* (2012) compared the effects of collection tube types and systems, centrifugation methods, whole blood stability and plasma stability and the effects on TG parameters for 12 normal donors. They observed collection tube type had the main influence on TG variability due to contact activation differences, with venepuncture collection technique using conventional needles least likely to cause haemolysis and release of phospholipids affecting TG. Our study used routine sodium citrate collection tubes with blood collected using venepuncture with standard needles as the standardised process to minimise the variables as mentioned by Loeffen *et al* (2012).

Further pre-analytical variables were reviewed affecting TG results, which include time taken from blood collection to processing of the sample for testing, due to the half-life of labile coagulation factors (CLSI H21-A5 2008; Dargaud *et al* 2017). There was no significant difference in TG parameters using the local centrifugation protocol at 30 minutes after blood collection compared to two h, four h and six h after blood collection for the five normal donors tested. This demonstrates that delays in centrifugation and processing of samples up to six h after blood collection is acceptable without affecting results. Rodgers *et al* (2014) found that there was no effect on any TG parameters with either 5pM or 1 pM tissue factor, up to six h after collection in samples collected into plastic collection tubes without CTI (Rodgers *et al* 2014).

Analytical variability is another factor in TG assays causing high inter- and intra-laboratory variation, with several international multicentre studies addressing this issue. The high inter-centre variability ranges from 58 to 118% (Dargaud *et al* 2007; Dargaud *et al* 2012). Dargaud *et al* (2007) attempted to reduce this variability by using a standardised protocol (commercial reagents and standard testing procedures) along with normalisation of results against mean ETP values of normal plasmas (Dargaud *et al* 2007). Using the standardised protocol across all testing sites with the same set of plasmas, the inter-centre variability was reduced from 39-109% to 4.4-23.2%. Our study also used commercially available TG reagents (PPP reagent - 5pM TF) and a standardised analytical procedure (manufacturer recommended) to reduce the effect of analytical variation. De Smedt *et al* (2011) found TG is extremely sensitive to temperature variations and preheating conditions of the assay (De Smedt *et al* 2011). The effect of temperature variation was minimised in our study by using commercial software (Thrombinoscope) which standardises pre-warming of the plate in the fluorometer for 10 minutes prior to the assay being started and ensures the system temperature is maintained at 37ºC for all experiments. Equipment and reagent variability affects diagnostic assays across multiple testing sites contributing to the high CV% in TG results seen in multicentre studies. Currently three commercially available thrombin generation systems are on the market, with variations across the systems in reagents or equipment used (Kintagh *et al* 2018). The equipment and reagent variability were minimised in our study by utilising the Thrombinoscope system (Diagnostica Stago, France), which is the most widely reported in the literature (Dargaud *et al* 2012).

Another recommendation to minimise variability caused by analytical procedures across multiple laboratories is normalisation of test results using either the mean of normal plasma results or a reference plasma (Dargaud *et al* 2007; Dargaud *et al* 2010). A study by Dargaud *et al* (2010) looked at several different commercial lyophilised reference plasmas to normalise TG results across five testing centres (Dargaud *et al* 2010). The use of normalised results reduced inter-centre variability but still some variation was seen in ETP results. This seemed to be dependent on the reference plasma used and was most likely due to the different concentrations of clotting factors seen in each reference preparation. We assessed the use of commercial reference plasmas run in conjunction with our normal donors (one lyophilised, the other a frozen normal pooled plasma) and found similar variability in the TG results from the two reference plasmas. A suitable normal reference plasma for TG could not be found therefore results were not normalised against reference plasma prior to establishing reference intervals. Another multicentre study by Dargaud *et al* (2012) applied standardisation by using identical equipment, standardised reagents and reference plasma to normalise results in combination with educational training to minimise pre-analytical variables and ensure consistent testing procedures (Dargaud *et al* 2012). They found the standardised protocol with educational training reduced the inter-centre variability to acceptable limits (<10%) for TG parameters to enable CAT to be used in clinical trials. Perrin *et al* (2015) performed a multicentre study across 34 sites utilising external reference plasma and locally prepared reference plasmas to normalise results (Perrin *et al* 2015). They found that the local plasma, due most

likely to differences in plasma preparation, was not able to reduce lab to lab variability and often increased inter-lab CVs, however the external reference plasma reduced CV's between laboratories after normalisation of results. They also stated that normalisation corrects for batch-to-batch variability of reagents, reconstitution issues with reagents and preheating or not of the plates.

Our study adopted the Thrombinoscope system with standardised commercial reagents to minimise analytical variability. An internal quality control (IQC) (commercial normal pooled plasma (NPP) was tested with each TG run and analytical acceptability for the run was determined if CV < 10% for this plasma. This is similar to Bagot *et al* (2015) who showed that use of an IQC can reduce inter laboratory variation and that normalisation of results was not required if a suitable IQC was used (Bagot *et al* 2015). This study also stated that normalisation of results alone is not testing run acceptability and could potentially introduce errors in normalisation data.

Recommended guidelines for establishing reference intervals state a minimum of 20 samples from healthy normal individuals for verifying intervals and 120 samples for establishing reference intervals with partitioning of individuals to account for variations due to age, sex and other conditions which may affect the assay (CLSI EP28-A3C 2010). Our study chose to partition based on sex and age and had less than 120 samples. Monagle *et al* (2010) showed changes in TG across different age ranges, with reported changes in levels of alpha-2-macroglobulin inhibiting thrombin in children and neonates compared to adults (Monagle *et al* 2010). Haid *et al* (2006) reported TG parameters showed an age-dependency across both children and older patients with shorter lag times, shorter time to peak and increased ETP seen in older patients (> 35 y), which effected biological variability of the assay (Haid *et al* 2006). Our study assessed the overall, male and female reference ranges across a range of adult ages, 22 to 64 y (mean age 43.9 y). When comparing male and female reference intervals to overall reference intervals we found no significant differences across all three groups. In the smaller sub analysis, comparing variation in TG parameters across the age groups (20 - 29, 30 - 39, 40 - 49 and 50 - 64 y) we found no significant difference in TG parameters across the different age groups. There were no individuals less than 22 y, so this may explain why we found no difference in TG parameters across our different age groups.

Bloemen *et al* (2017) performed a study on inter-individual variability and normal ranges in 129 healthy individuals, following CLSI recommendations for calculation of reference intervals, and found no effect of age on TG parameters (Bloemen *et al* 2017). They noted that oral contraceptive use affected TG parameters with increased TG parameters seen although these were still within the reference ranges established on the total population tested. They chose to include individuals on oral contraceptives, since excluding them would derive narrow reference ranges and since individuals on oral contraceptives are still considered to be healthy. We also chose to follow this same protocol and did not assess whether females were on oral contraceptives.

There are various formulae for determining reference intervals, with the most widely used mean +/- 2SD (2.5th and 97.5th percentiles) assuming a Gaussian distribution, using non-parametric statistics (CLSI EP28-A3C 2010) and this was used in our study. The sample size (n=74) is slightly less than the recommended (n=120) for parametric methods but is sufficient for non-parametric to determine 2.5th and 97.5th percentiles. Large reference interval studies are often not possible in individual laboratories and verification of intervals requiring less individuals may be done using multicentre studies to achieve a large enough sample size with standardised pre-analytical and analytical protocols (Ozada 2016). The standardised protocols can be used across multicentre laboratories to further increase the sample size and verify these reference intervals for TG parameters.

Whilst our study was being performed, a survey was sent out by a scientific sub-committee working group of the ISTH to understand the differences in performance of TG testing including pre-analytical steps and TG protocols. De Laat-Kremers *et al* (2020) concluded from this survey data that a standardised protocol and data normalisation should lead to better reproducibility and allow for comparison of data across laboratories (De Laat-Kremers *et al* 2020). The standardised protocol should include pre-analytical variables (collection tubes, time from collection to processing, centrifugation protocols) and the practical part of the TG assay. In follow-up to this survey, Ninivaggi *et al* (2021) made several recommendations for measurement of TG aimed at harmonising between methods and laboratories to support the application of TG in patient diagnosis and management (Ninivaggi *et al* 2021). Based on these recommendations, our study did adhere to the suggested pre-analytical factors including blood collection, plasma handling and processing and sample storage. In terms of the recommendations for analytical components, the use of commercial reagents according to manufacturer, no pre-dilution of sample and the temperature and preheating steps followed as per the manufacturer. Ninivaggi *et al* (2021) recommended for semi-automated and automated systems to use the software accompanying the device, which in our study was the Thrombinoscope software (Ninivaggi *et al* 2021). They also discouraged the use of reference values from published studies due to variability between TG

procedures and recommended establishment of reagent, age and analyser specific reference values according to the CLSI guidelines (CLSI EP28-A3C), and this approach was also used in our study.

Some of the limitations of our study are the demographics and selection criteria for the population of healthy individuals. The inclusion criteria were minimal; no history of thrombosis or bleeding (subjective to donor knowledge) and not on any anticoagulant or anti-platelet medications. The donors were also laboratory staff, who may not reflect the same demographics of a clinical population or general community. The number of males to females was slightly reduced (31 males to 43 females) and there were unequal numbers across the different age groups. There was no statistically significant difference in TG parameters in the different age groups, but this may need to be confirmed with a larger number of individuals across the different age groups.

Conclusion

This study is a step forward towards bringing TG into the diagnostic setting, with establishment of reference intervals using standardised pre-analytical and analytical processes for TG testing. Future directions include verification of the current study reference intervals with multicentre studies and clinical studies assessing patients with pathological dysfunctional haemostasis and TG parameters to correlate assay results to clinical outcomes. The current study utilised a tissue factor concentration of 5 pM (as a screening test), nevertheless further investigations are warranted on low tissue factor concentration (1 pM) and high tissue factor concentration (10 pM) for assessing hypocoagulable individuals and those on anticoagulant therapy.

Acknowledgements

This study was supported by the Haematology Research Trust fund, Liverpool Hospital, Sydney, Australia.

Conflict of Interest

All authors have no conflict of interest and no competing interests.

References

Al Dieri R, de Laat B, Hemker C. Thrombin generation: what have we learned? *Blood Reviews*. 2012; 26: 197-203.

Baglin T. The measurement and application of thrombin generation. *British Journal Haematology.* 2005; 130: 356-661.

Baglin T. Using the laboratory to predict recurrent venous thrombosis. *International Journal of Laboratory Haematology*. 2011; 33: 333-342.

Bagot CN, Leishman E. Establishing a reference range for thrombin generation using a standard plasma significantly improves assay precision. *Thrombosis Research.* 2015; 136, 139 – 143.

Bloemen S, Huskens D, Konings J, et al. Interindividual Variability and Normal Ranges of Whole Blood and Plasma Thrombin Generation. *Journal Applied Laboratory Medicine. 2017; Sept (2): 150 – 164.*

Castoldi E, Rosing J. Thrombin generation tests. *Thrombosis Research. 2011*; 3: 521-525.

CLSI EP28-A3C Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third edition. *Clinical and Laboratory Standards Institute (CLSI). 2010*; Wayne, Pennsylvania, USA.

CLSI H21-A5. Collection, transport and processing of blood samples for testing plasma-based coagulation assays and molecular hemostasis assays: approved guideline fifth edition. *Clinical and Laboratory Standards Institute (CLSI). 2008*; Wayne, Pennsylvania, USA

Dargaud Y, Luddington R, Gray E, et al. Effect of standardization and normalization on imprecision of calibrated automated thrombography: an international multicentre study. *British Journal Haematology.* 2007; 139: 303 – 309.

Dargaud Y, Luddington R, Gray E, Lecompte T, et al. Standardisation of thrombin generation test – which reference plasma for TGT? An international multicentre study. *Thrombosis Research. 2010*; 125, 353-356.

Darguad Y, Wolberg AS, Luddington R, et al. Evaluation of a standardised protocol for thrombin generation measurement using the calibrated automated thrombogram: An international multicentre study. *Thrombosis Research. 2012*; 130: 929-934.

Dargaud Y, Wolberg AS, Gray E, Negrier C, Hemker HC. Proposal for standardized preanalytical and analytical conditions for measuring thrombin generation in haemophilia: communication from the SSC of the ISTH. J*ournal Thrombosis Haemostasis. 2017*; 15: 1704 – 1707.

De Smedt E, Hemker HC. Thrombin generation is extremely sensitive to preheating conditions. *Journal Thrombosis Haemostasis. 2011*; 9: 233- 234.

De Laat-Kremers RMW, Ninivaggi M, Devreese KMJ, de Laat B. Towards standardisation of thrombin generation assays: Inventory of thrombin generation methods based on results of an International Society of Thrombosis and Haemostasis Scientific Standardization Committee survey. *Journal Thrombosis Haemostasis. 2020*;18: 1893-1899.

Haidl H, Cimenti C, Leschnik B, Zach D, Muntean W. Age-dependency of thrombin generation measured by calibrated automated thrombography (CAT). *Thrombosis and Haemostasis. 2006; 95* (5), 772-775.

Hemker H, Giesen P, AlDieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiology of Haemostasis and Thrombosis. 2003*; 33: 4-15.

Kintagh J, Mongale P, Ignjatovic, V. A review of commercially available thrombin generation assays. R*esearch Practice Thrombosis Haemostasis. 2018*; 2: 42 – 48.

Loeffen R, Kleinegris MCF, Loubele STBG, et al. Preanaltycial variables of thrombin generation: towards a standard procedure and validation of the method. *Journal Thrombosis Haemostasis. 2012*; 10: 2544-2554.

Luddington R, Baglin T. Clinical measurement of thrombin generation by calibrated automated thrombography requires contact factor inhibition. *Journal of Thrombosis and Haemostasis. 2004*; 2, 1954-1959.

Mongale P, Ignjatovic V, Savoia H. Hemostasis in neonates and children: pitfalls and dilemmas. *Blood Reviews. 2010*; 24, 63-68.

Ninivaggi M, deLaat-Kremers RMW, Tripodi A, Wahl D, Zuily S, Dargaud Y, ten Cate H, Ignjatovic V, Devreese KMJ, de Laat B. Recommendations for the measurement of thrombin generation: Communication from the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies. *Journal Thrombosis Haemostasis. 2021*;19: 1372-1378.

Ozada Y. Reference intervals: current status, recent developments and future considerations. *Biochemia Medica. 2016*; 26(1): 5-16.

Perrin J, Depasse F, Lecompte T. Large external quality assessment survey on thrombin generation with CAT: further evidence for the usefulness of normalisation with an external reference plasma. *Thrombosis Research. 2015*; 136, 125-130.

Rodgers SE, Wong A, Gopal RD, Dale BJ, Duncan EM, McRae SJ. Evaluation of pre-analytical variables in a commercial thrombin generation assay. *Thrombosis Research. 2014*; 134: 160-164.

Spronk H, Dielis A, Panova-Noeva M, et al. Monitoring thrombin generation: is addition of corn trypsin inhibitor needed? *Thrombosis and Haemostasis. 2009*; 10, 1156-1162.

Tripodi A. Thrombin generation assay and its application in the clinical laboratory. *Clinical Chemistry. 2016*; 62 (5): 1-9.

Implementation of risk control measures to manage storage of corrosive substances in the Australian Standard AS ISO 15189:2023 accredited medical laboratory

Dennis Mok[1], Naira Eloyan[2], Sharfuddin Chowdhury[3], Rana Nabulsi[4], Donna Marie Gillespie[5], Tai The Diep[6], María del Rocío González Guerrero[7], Georgi Koutin[8], Ritwik Palit[9], Thuy Ha Nguyen[10]

[1] *Medical Management Consulting, Birkdale, Australia*

[2] *Scientific Center of Drug and Medical Technology Expertise, Yerevan, Armenia*

[3] *King Saud Medical City, Riyadh, Saudi Arabia*

[4] *Dubai Academic Health Corporation, Dubai, United Arab Emirates*

[5] *Chicago, United States*

[6] *Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom*

[7] *Rio Tinto Hospital, Huelva, Spain*

[8] *Role 2B Medical Treatment Facility, Camp Film City, Pristina, Kosovo*

[9] *Mater Pathology, St. Vincent's Private Hospital Northside, Chermside, Australia*

[10] *Quality Control Center for Medical Laboratory, Hanoi Medical University, Ha Noi, Viet Nam*

Introduction

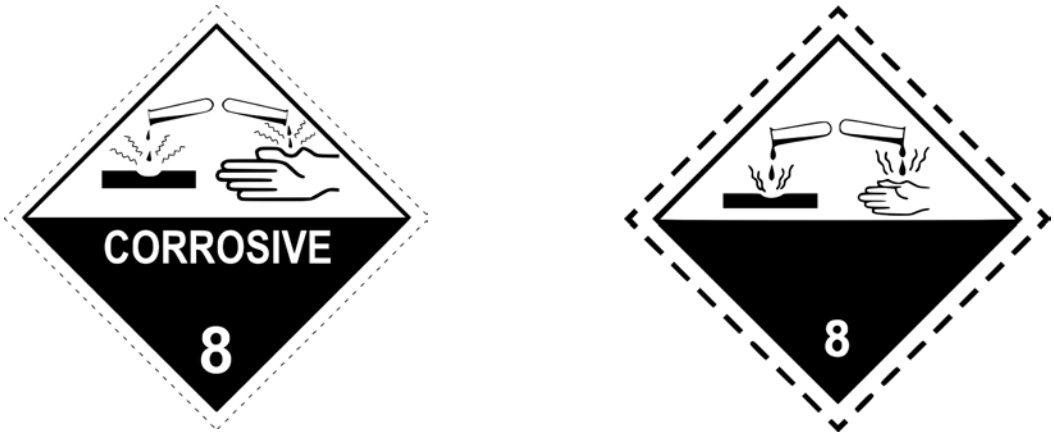
Class 8 dangerous goods (corrosive substances) are hazardous chemicals routinely used in the medical laboratory. The medical laboratory must ensure the safety of laboratory personnel by implementing relevant and reasonably practicable operational control measures to support the risk management (Australasian Health Infrastructure Alliance 2022) (Clause 5.6 of AS ISO 15189:2023). The main objective of this paper is to enhance the awareness of requirements relating to the provision of operational risk control measures relevant to using indoor storage cabinets for Class 8 dangerous goods storage. Selected organisations were identified to provide relevant information to support the medical laboratory and these were: American Society for Health Care Engineering (ASHE), American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE), the International Labour Organization (ILO), the International Organization for Standardization; National Transport Commission (Australia), Safe Work Australia; Standards Australia; the United Nations Economic Commission for Europe (UNECE) and the World Health Organization.

Contemporary issues

The risk control measures (Subclause 6.3.1 of AS ISO 15189:2023) relating to the storage of corrosive substances in chemical storage cabinets receive scant attention from laboratory personnel. The issue is mainly due to the current guidance documents providing ambiguous recommendations and requirements for laboratory personnel to follow. In addition, the imported chemical storage cabinets that are available may not be constructed to specifications of Standards Australia (Subclause 5.6.4 of AS 3780:2023) and may not be supplemented with the Class 8 dangerous goods (corrosive substances) marking to comply with the specification of the National Transport Commission (Australia) (National Transport Commission 2023). It is important to note that marking requirements of the National Transport Commission (Australia) must be complied with, not those of Standards Australia (AS 1216—2006) and not those of the UNECE (UNECE 2023) (Figure 1). The implementation of additional requirements other than those stated in Table 1 may be required depending on the operational control measures required to reduce the exposure risks (Sveinbjornsson and Gizurarson 2022; Doa 2023). Implementation of relevant control measures should be in alignment with the medical laboratory good professional practice commitment in Clause 5.5 a) of AS ISO 15189:2023 (Guest *et al* 2014).

Siting considerations

The medical laboratory must site chemical storage cabinets containing corrosive substances in locations accessible only to authorised laboratory personnel and with reasonable airflow ventilation to eliminate potential unsafe chemical vapours (Subclause 8.5 of ISO 15190:2020). The ASHRAE and the ASHE list relevant air change rates for various areas of the medical laboratory (Section 7.1 of ANSI/ASHRAE/ASHE Standard 170-2021).



a) Marking for Class 8 dangerous goods according to the National Transport Commission (Australia).

b) Marking for Class 8 dangerous goods according to the United Nations Economic Commission for Europe.

Figure 1. Markings for Class 8 dangerous goods (corrosive substances). *Two commonly found markings for Class 8 dangerous goods in medical laboratories are associated with the National Transport Commission (Australia) and the United Nations Economic Commission for Europe respectively. The National Transport Commission (Australia) marking should be used in AS ISO 15189:2023 accredited medical laboratories to support good professional practice in Australia.*

The medical laboratory must also ensure the siting considerations of two or more chemical storage cabinets are in alignment with relevant Australian Standards AS 2243.2:2021 and AS 3780:2023. Interpretation of implementation requirements relating to siting decisions depend on the individual circumstances of the medical laboratory and may require support from a competent third party to ensure conformity and consistency (Alizamir *et al* 2021).

Further considerations

The medical laboratory should also take further notes from the ILO, Safe Work Australia, and the World Health Organization. The ILO provides prevention and control measure recommendations relating to storage of hazardous chemicals (ILO 1993; ILO 2001). Safe Work Australia provides relevant information on how to manage health and safety risks associated with storing hazardous chemicals (Safe Work Australia 2020b). The World Health Organization provides recommendations on the requirements for appropriate storage cabinets for hazardous reagents and chemicals (World Health Organization 2020).

The medical laboratory must, to the extent that is reasonably achievable, make provisions to ensure the relevant risk control measures for Class 8 dangerous goods (corrosive substances) are identified unmistakably, implemented adequately, and displayed clearly for hazard communication to laboratory personnel.

Acknowledgments

The authors would like to thank: Sharita Meharry, BAppSc MMLS (Hons), Senior Lecturer in Biomedicine and Medical Diagnostics, School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand; Helen Neilson, BSc (Hons) CQP MCQI RCT, Quality Manager, NHS Greater Glasgow and Clyde, Glasgow, United Kingdom; and Victoria Tait, BSc (Hons) CQP MCQI, Quality Assurance Specialist, Qualitait, Duns, United Kingdom.

Table 1. Risk control measures for storage of Class 8 dangerous goods (corrosive substances). *An action list for medical laboratories to ensure the relevant risk control measures for corrosive substance storage are identified, implemented, and displayed for hazard communication.*

Areas	Action list	References
Location of storage	Ensure the storage locality is situated in a secure location.	Subclause 8.5.1 a) of ISO 15190:2020
	Ensure the storage locality is accessible only to authorised laboratory personnel.	Subclause 6.3.2 a) of AS ISO 15189:2023 Subclause 8.5.1 b) of ISO 15190:2020
	Ensure the storage locality has adequate ventilation. Note 1. The minimum ventilation rate is not specified in Subclause 8.5.1 d) of ISO 15190:2020. Note 2. The American Society of Heating, Refrigerating and Air Conditioning Engineers and the American Society for Health Care Engineering have specified relevant air change rates to various areas of medical laboratories in Section 7.1 of ANSI/ASHRAE/ASHE Standard 170-2021.	Subclause 8.5.1 d) of ISO 15190:2020
	Ensure the storage locality is away from direct heat, sunlight or highly variable temperatures.	Subclause 8.5.1 e) of ISO 15190:2020
Quantities of storage	Observe the storage cabinet acceptable limits (≤ 1000 kg or ≤ 1000 L) according to appropriate packing densities [Packing Group I (≤ 50 kg or ≤ 50 L) and Packing Group II (≤ 250 kg or ≤ 250 L)]. Note. The maximum quantity of Class 8 dangerous goods kept in a single cabinet is specified (≤ 1000 kg or ≤ 1000 L) in Subclause 5.6.3 of AS 3780:2023; however, another specification (≤ 250 L) is in Subclause 7.3.3.1 of AS 2243.2:2021.	Subclause 5.6.3 of AS 3780:2023
Method of storage (indoor storage cabinet)	Store Class 8 dangerous goods in appropriate locations (acids are to be stored in a dedicated acid cabinet).	Subclause 8.5.2 a) of ISO 15190:2020
	Store Class 8 dangerous goods in appropriate locations (in compartments or devices capable of containing spills on lower shelves or cabinets).	Subclause 8.5.2 c) of ISO 15190:2020
	Store Class 8 dangerous goods in appropriate locations (not stored on tops of chemical storage cabinet, on the floor, or on bench tops and in chemical fume hoods).	Subclause 8.5.2 e) of ISO 15190:2020
Chemical storage cabinet storage considerations	Store Class 8 dangerous goods in design specified storage cabinet (Subclause 5.6.4 of AS 3780:2023).	Subclause 6.3.4 of AS 2243.2:2021
	Store Class 8 dangerous goods in appropriate locations (inside a closable cabinet or on a sturdy shelf that is secured to the wall or floor).	Subclause 8.5.1 c) of ISO 15190:2020
	Store Class 8 dangerous goods in appropriate chemical storage cabinets.	Subclause 6.3.1 of AS ISO 15189:2023 Subclause 6.3.3 c) of AS ISO 15189:2023

Chemical storage cabinet siting considerations	Site the storage cabinet at appropriate locations (not jeopardising emergency escape). Note. The distance of ≥ 3 m is recommended between any chemical storage cabinet and escape doors in Subclause 6.3.6 (b) of AS 2243.2:2021.	Subclause 5.6.6 (a) of AS 3780:2023
	Site the storage cabinet at appropriate locations (in proximity to a provision for the washing of hands). Note. The distance is not specified in Subclause 4.6.6 (b) of AS 3780:2023.	Subclause 5.6.6 (b) of AS 3780:2023
	Site multiple storage cabinets (≥ 2) in any area or building with specified quantities (Subclause 5.6.3 of AS 3780:2023) or with specified separating distance (≥ 5 m). Note 1. The medical laboratory is recommended to keep ≤ 3 chemical storage cabinet(s) in a room with each chemical storage cabinet located ≥ 3 m apart in Subclause 6.3.6 of AS 2243.2:2021. Note 2. The medical laboratory is to keep ≤ 3 chemical storage cabinet(s) in a 250 m^2 area with each chemical storage cabinet to have an air space of ≥ 3 m in Subclause 7.3.3.2 of AS 2243.2:2021. Note 3. The medical laboratory is to have an air space between chemical storage cabinets of ≥ 3 m in Subclause 8.5.1 of AS 2243.2:2021. The air space is to remain clear and accessible. Note 4. The medical laboratory is recommended to have a separating distance of ≥ 3 m for Class 8 dangerous goods to Class 2.1, Class 2.2, Class 3, Class 4.2, Class 5.1, and Class 5.2 dangerous goods to avoid adverse chemical reactivities (Safe Work Australia 2020a). Note 5. The mandatory separating distance between chemical storage cabinets of ≥ 5 m is stated in Subclause 5.6.6 (c) of AS 3780:2023 and ≥ 3 m is stated in Subclause 7.3.3.2 of AS 2243.2:2021 and Subclause 8.5.1 of AS 2243.2:2021; however, the recommended separating distance of ≥ 3 m is stated in Subclause 6.3.6 of AS 2243.2:2021.	Subclause 5.6.6 (c) of AS 3780:2023
	Site the storage cabinet at appropriate locations (not located one above the other).	Subclause 6.3.6 (a) of AS 2243.2:2021
	Site the storage cabinet at appropriate locations (not located where they can jeopardise emergency escape).	Subclause 6.3.6 (b) of AS 2243.2:2021
	Site the storage cabinet at appropriate locations (not under stairs or in corridors).	Subclause 6.3.6 (c) of AS 2243.2:2021
	Maintain the integrity of consumables by appropriate storage conditions.	Subclause 6.3.1 of AS ISO 15189:2023 Subclause 6.3.3 a) of AS ISO 15189:2023 Subclause 6.6.2 of AS ISO 15189:2023
Chemical storage cabinet (segregation in storage)	Store Class 8 dangerous goods separately from incompatible goods or goods with which they might react dangerously (acids and alkalis, acids and hypochlorites, acids and cyanides, acids and Class 4.3 dangerous goods, oxidising acids and combustible materials, and incompatible acids).	Subclause 7.6.9 of AS 2243.2:2021
	Store Class 8 dangerous goods separately from incompatible goods or goods with which they might react dangerously.	Subclause 5.6.2 of AS 3780:2023

Display of hazard identification information	Display an appropriate Class 8 dangerous goods marking on the storage cabinet door. Note. The Class 8 dangerous goods marking prepared by the National Transport Commission (Australia) is to be used.	Subclause 6.5 of AS 2243.2:2021
	Display an appropriate Class 8 dangerous goods marking with an appropriate nominal length (sides of ≥ 100 mm). Note 1. The Class 8 dangerous goods marking prepared by the National Transport Commission (Australia) is to be used. Note 2. The Class 8 dangerous goods marking is clearly visible when the cabinet doors are closed.	Subclause 4.6.7 (c) of AS 3780:2023
Display of relevant chemical storage cabinet markings	Display the name and address of the storage cabinet manufacturer or the distributor for imported models. Note. The marking is clearly visible when the cabinet doors are closed.	Subclause 5.6.7 (a) of AS 3780:2023
	Display the storage cabinet maximum storage capacity. Note. The marking is clearly visible when the cabinet doors are closed.	Subclause 5.6.7 (b) of AS 3780:2023

References

Australasian Health Infrastructure Alliance 2022. *Australasian health facility guidelines: part B - Health facility briefing and planning: 0550 – Pathology unit.* Revision 7.0. St. Leonards: Australasian Health Infrastructure Alliance. Section 3.7.1, Safety; 26-27.

National Transport Commission (Australia) 2023. *Australian Code for the Transport of Dangerous Goods by Road & Rail.* 7th ed. Melbourne: National Transport Commission (Australia). Section 5.2.2.2.2, Specimen labels; 696-708.

United Nations Economic Commission for Europe 2023. *Recommendations on the transport of dangerous goods: Model Regulations.* 23rd rev. ed. Vol. II. New York: United Nations Economic and Social Council, United Nations Economic Commission for Europe. Section 5.2.2.2.2, Specimen labels; 171-177.

Sveinbjornsson B, Gizurarson S 2022. *Handbook for laboratory safety.* Amsterdam: RELX Group, Elsevier. Corrosives—acids and bases; 53-54.

Doa MJ 2023. Chemical safety. In: Rumble JR Jr, editor-in-chief; Bruno TJ, Doa MJ, associate editors. *CRC handbook of chemistry and physics: a ready reference book of chemical and physical data.* 104th ed. Boca Raton: Taylor & Francis Group, CRC Press. Chemical safety; 15-1-15-2.

Guest DE 2014. Commitment. In: Flood PC, Freeny Y, volume editors. *Wiley Encyclopedia of management.* 3rd ed. Vol. 11, Organizational behaviour. Chichester: John Wiley & Sons; 57-60.

Alizamir S, Kim S-H, Muthulingam S 2021. Compliance as operations management. In: van Rooij B, Sokol DD, editors. *The Cambridge handbook of compliance.* Cambridge: University of Cambridge, Cambridge University Press; 81-92.

International Labour Organization 1993. *Safety in the use of chemicals at work.* Geneva: International Labour Organization, International Labour Office. Section 6.7, Control measures for the storage of hazardous chemicals; 28-29.

International Labour Organization 2001. *Ambient factors in the workplace.* Geneva: International Labour Organization, International Labour Office. Chapter 4, Hazardous substances; 21-26.

Safe Work Australia 2020a. *Managing risks of storing chemicals in the workplace.* Canberra: Safe Work Australia. Table 3, Recommended segregation types; 17.

Safe Work Australia 2020b. *Managing risks of storing chemicals in the workplace.* Canberra: Safe Work Australia. Chapter 4, Controlling risks; 8-12.

World Health Organization 2020. *Laboratory design and maintenance.* Geneva: United Nations, World Health Organization. Subsection 2.2.2, Chemicals; 4.

The fifth human malaria – a case of *Plasmodium knowlesi*

Lisa Ellison[1], Ting Lin[1], Robyn Wells

[1] Haematology Department, Pathology Queensland Central Laboratory, Brisbane, Qld

Abstract

This case study describes a *Plasmodium knowlesi* infection in a 27-year-old French national male who had been in the Philippines for one week and then in Malaysia for one month. After being in Australia for eight days, he presented to the Emergency Department with fevers, headache and dark urine.

Tests requested were a malaria screen, full blood count (FBC), electrolytes and liver function tests (ELFTs) and hepatitis A.

A small number of parasites were found on the thick and thin film and a preliminary diagnosis of a mixed infection of *Plasmodium falciparum* and *Plasmodium malariae* was made due to the morphological features of those parasites. Confirmatory PCR testing was performed but those species were not detected. Further testing was performed by two laboratories that had primers for *Plasmodium knowlesi* and it was eventually confirmed to be a *P. knowlesi* infection.

The patient was hospitalised and treated successfully.

Introduction

Over 20 species of malaria are known to infect non-human primates but until recently only four were thought to regularly infect humans *(Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale*). Over the last decade *Plasmodium knowlesi* has been recognised as a significant cause of malaria infection in Southeast Asia due to the work of two scientists, Janet Cox-Singh and Balbir Singh. They noted in 2008 that many of the infections in this region were diagnosed as *P. malariae* due to the morphological features of the parasite, but the incidence of this species in this area was known to be historically low. The other anomaly that triggered their investigation was that the parasite counts in these cases were high and *P. malariae* is associated with a low parasite count that is rarely >5000/µl (Garnham 1966, Cox-Singh *et al* 2008). This is partly due to the erythrocytic cycle of *P. malariae* taking 72 hours. Molecular techniques enabled the correct identification of *P. knowlesi* in these cases and the high parasite load is due to *P. knowlesi* parasites replicating every 24 hours (Figure 1). This short life cycle rapidly increases the parasitaemia and leads to the development of symptoms and complications (Daneshvar *et al* 2009).

Address correspondence to:
Robyn Wells
Email: contact@aims.org.au

It occurs naturally in the Macaque monkey population in the forested areas of Malaysia, Borneo, Myanamar, Singapore, the Philippines, Indonesia, Thailand, Taiwan and Vietnam, and is transmitted by the *Anophleles* mosquito species. There have been fatalities caused by *P. knowlesi* infection due to the high parasite loads and subsequent hepatorenal dysfunction and/or acute respiratory distress syndrome.

Case report

A 27-year-old male travelled to the low areas of the Philippines for a week and then spent four weeks in Sabah. He had no vaccinations or taken any malaria prophylaxis.

Symptoms started with fever, headaches, and dark urine after he had been in Australia for eight days. On examination in the Emergency Department, he was found to be alert and orientated, not distressed, not icteric, not pale or febrile, no enlarged lymph nodes but spleen palpable with some discomfort and tenderness.

He was to be admitted to hospital pending the blood culture and malaria screen results.

Results

The initial result from the thick and thin blood films was very occasional late trophozoites and gametocytes of a *Plasmodium* species present suggestive of *P. malariae* (but *P. knowlesi* cannot be excluded) and parasitised cells <0.01%.

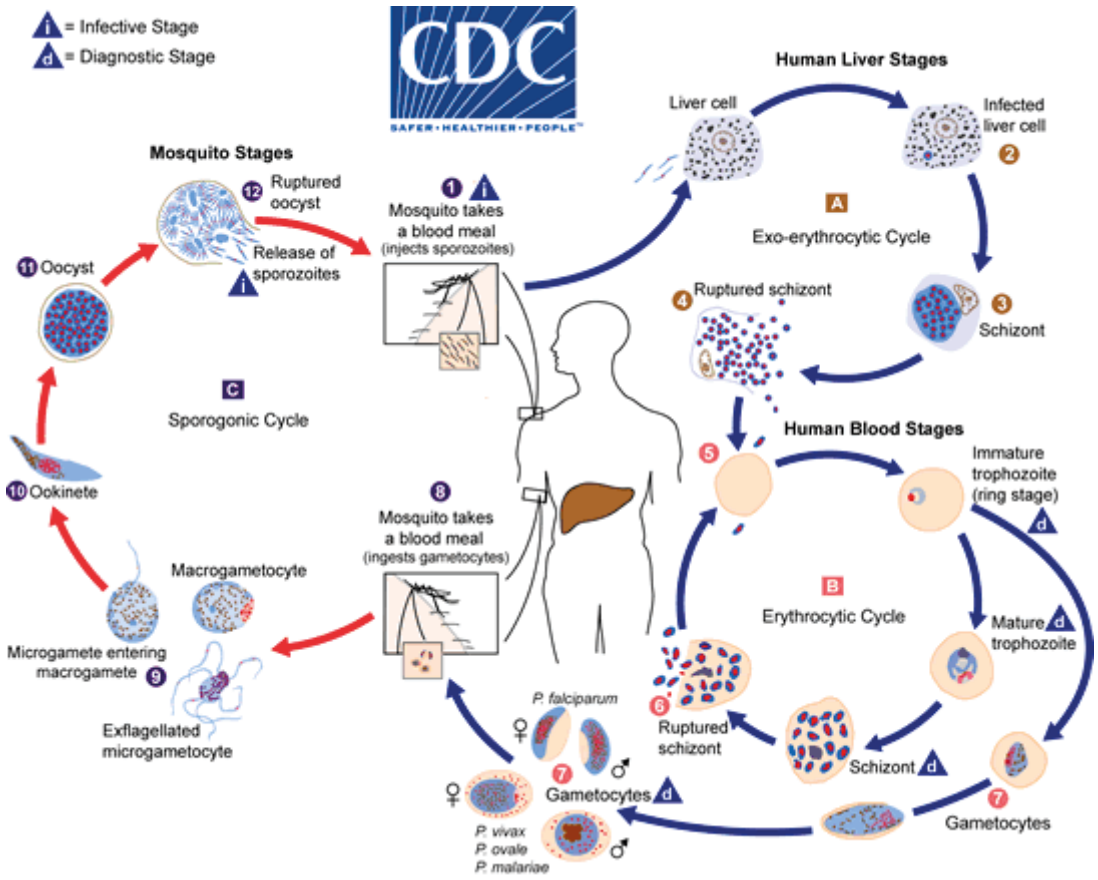


Figure 1. *Life cycle of malaria*
https://www.cdc.gov/malaria/images/graphs/life_cycle/Malaria_LifeCycle_1.gif

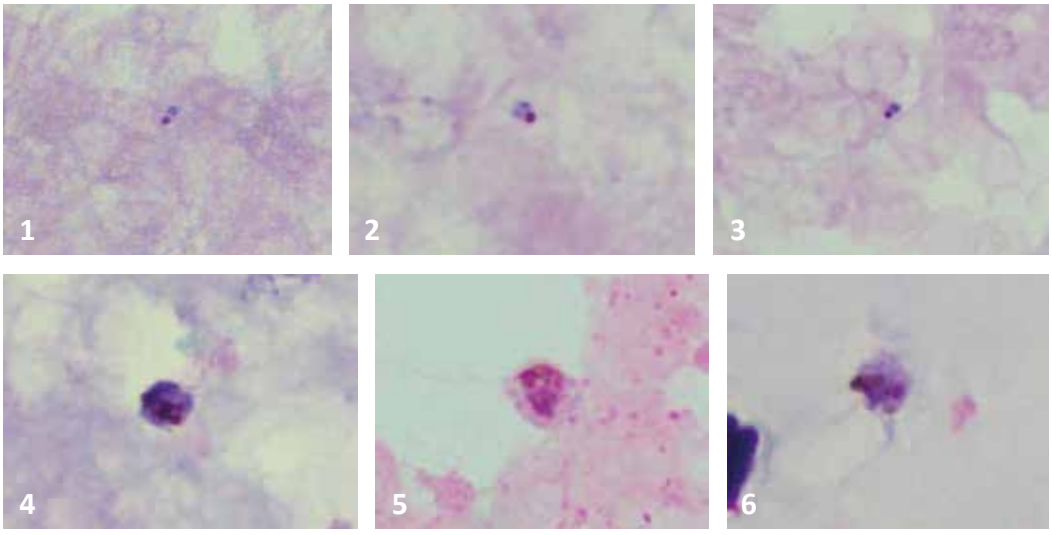


Figure 2 (1-6). *Parasites in the thick film. Giemsa stain, pH 7.2*

The blood film was was then sent to a second laboratory where it was suspected to be a mixed infection of *P. falciparum* and *P. malariae* due to the morphology (Figures 2 and 3).

Due to the irregular morphology of the parasites in the thin and thick films, the sample was sent for PCR testing but the four primers for *P. falciparum, P. vivax, P. malariae* and *P. ovale* did not detect any of those species in the sample.

It was then sent to another laboratory that had primers for *P. knowlesi* with the results shown in Table 1.

As can be seen in the first run the controls were invalid, on the second run there was a faint positive for *P. vivax* but all were negative for the third run.

Table 1. *PCR results from a second laboratory*

	Run 1	Run 2	Run 3
P.falciparum	Negative	Negative	Negative
P.vivax	Negative	Faint Positive	Negative
P.ovale	Negative	Negative	Negative
P.malariae	Negative	Negative	Negative
P.knowlesi	Negative	Negative	Negative
Controls	Invalid	Valid	Valid

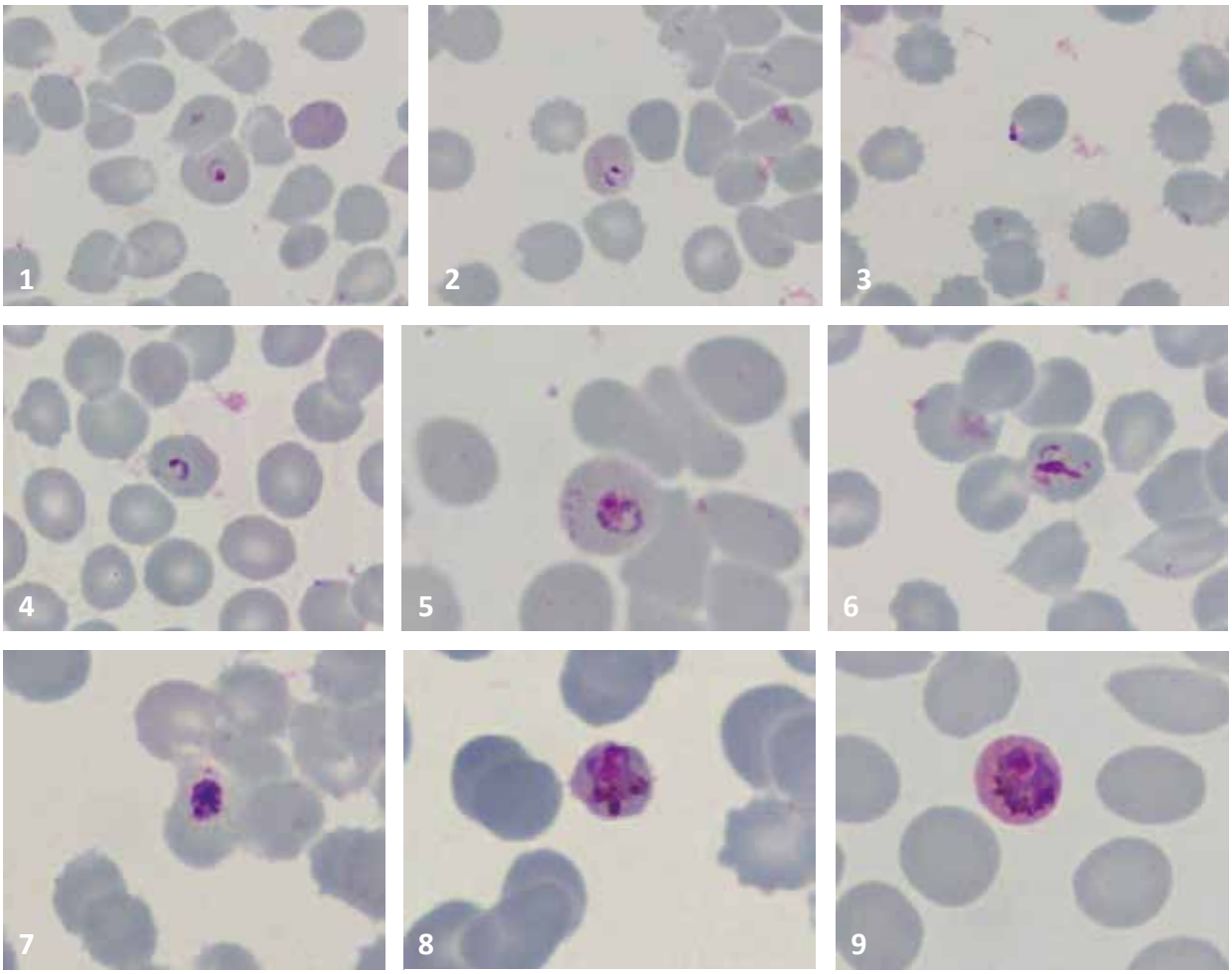


Figure 3 (1-9). *Parasites in the thin film. Giemsa stain pH 7.2*

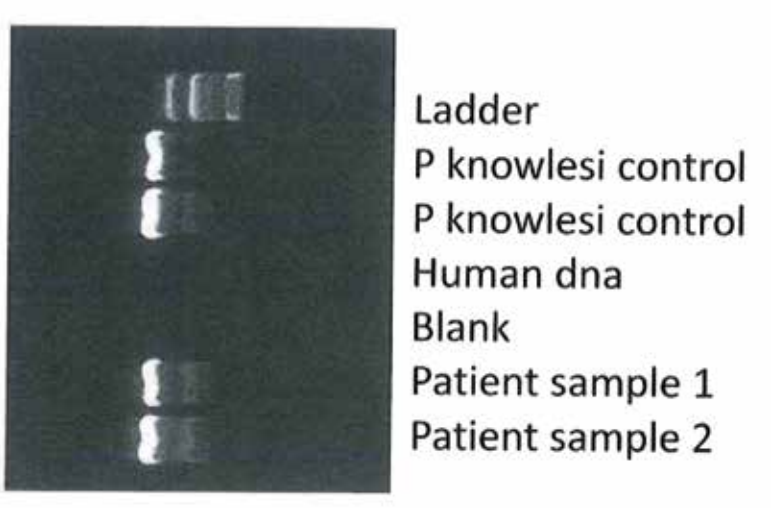


Figure 4. *PCR results from interstate laboratory.*

It was suspected that there were issues with the primers at this laboratory so the sample was sent interstate and run again. This time there were definitive results (Figure 4) confirming that it was indeed a case of *P. knowlesi*.

Treatment

The patient commenced treatment with Riamet – a combination of the two medications artemether and lumefantrine which is used to treat uncomplicated malaria caused by *P. falciparum* that is not treatable with chloroquine (WHO 2015).

Treatment with primaquine to eliminate hypnozoite forms, characteristic of *P. vivax*, was not prescribed as patient was diagnosed as *P. malariae/P. knowlesi.*

Discussion and conclusion

This case illustrates the challenges sometimes faced of determining the infecting species of malaria. The early trophozoites of *P. knowlesi* look similar to *P. falciparum* but as the parasites grow and mature they resemble *P. malariae*. One of the characteristics of *P. knowlesi* is high parasite numbers but this is not always seen and this makes the diagnosis difficult for even experienced morphologists.

The confusing PCR results added to this challenge and at one stage it was considered to be another type of "monkey malaria" (*P. cynomolgi, P. brasilianum, P. eylesi, P. inui, P. schwetzi or P. simium*) but with the confirmatory PCR results from the interstate laboratory, morphology of the parasites and the travel history, a confident diagnosis of *P. knowlesi* could be made.

References

Cox-Singh J, Davis TME, Lee KS, Shamsul S, Matusop A, Ratnam S, Rahman HA, Conway DJ, Singh B. 2008. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life-threatening. *Clin Infect Dis* 46:165-171

Daneshvar C, Davis TME, Cox-Singh J, Rafa'ee MZ, Zakaria K, Divis PCS, Singh B 2009. Clinical and laboratory features of *Plasmodium knowlesi infection. Clin Infect Dis* 49:852-860

Garnham PCC 1966. *Malaria parasites and other haemosporidia.* Oxford: Blackwell Scientific Publications

Guidelines for the treatment of Malaria, 3rd edition (WHO) 2015 https://www.afro.who.int/publications/guidelines-treatment-malaria-third-edition#:~:text=The%20core%20principles%20underpinning%20this,of%20resistance%3B%20and%20appropriate%20weight%2D Accessed 02/02/2024

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.

Journal-based CPD No. 98
Page 1 of 2

Questions relating to the article *'Application of targeted long-read sequencing on RHD Gene structural variants'* at page 87 of this issue.

1	Accurate blood typing for blood donors and patients is necessary for safe blood transfusion and reduces the risk of alloimmunisation.	True/False
2	Patients with a D– or D+ variant phenotype, who receive D+ red blood cells can form anti-D.	True/False
3	D variants on RBCs can be classified as weak D, partial D and Del.	True/False
4	Short-read sequences for RHD and RHCE are prone to mis-mapping because the two genes share 93.8% sequence homology.	True/False
5	The RH system has 65 blood group antigens encoded by RHD, RHCE, or hybrids of RHD/RHCE.	True/False
6	Anti-D is known to cause haemolytic disease of the fetus and newborn and haemolytic transfusion reaction.	True/False
7	Long-read sequencing (LRS) can produce sequence reads of more than 15 kb.	True/False
8	In this study, targeted LRS was used to assess its capability to characterise complex RHD SVs not easily resolved by the SRS technique.	True/False
9	RHD*DV.10 and RHD*DKG are known to carry RHD gene structural variations.	True/False
10	The RHD and RHCE genes are 57,831 bp and 57,295 bp long, respectively.	True/False

Name: _____

Email: _____

Please photocopy this page or print it from the electronic AJMS which is stored in the AIMS 'Member Centre' under the heading 'Journal' at www.aims.org.au. ***Circle your answers, then scan, and enter them as activities in the APACE diary under 'My CPD Record'.***

https://www.aims.org.au/online-diary

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.

Journal-based CPD No. 99
Page 2 of 2

Questions relating to the article '*Bringing thrombin generation into the diagnostic setting*' at page 95 of this issue.

1	None of the currently available assays, commonly performed in diagnostic laboratories, are a true physiological representation of a patient's overall haemostatic profile in vivo.	True/False
2	Abnormal thrombin generation (TG) plays a key role in the pathophysiology of abnormal bleeding and thrombosis.	True/False
3	The TG assay evaluates the ability of patient plasma to generate and inhibit thrombin.	True/False
4	In hypocoagulability states there are shortened lag times, high peak height and shortened time to peak, high VI and high ETP values.	True/False
5	In hypercoagulability states there are prolonged lag times, low peak height and long time to peak, low VI and low ETP values	True/False
6	Delays in centrifugation and processing of samples up to six hours after blood collection is acceptable without affecting results.	True/False
7	Lack of standardisation in methodology has hampered the translation of TG from research into a diagnostic assay.	True/False
8	The Calibrated Automated Thrombogram allows quantitative assessment of TG.	True/False
9	Pre-analytical variables, including collection technique and processing and storage of the sample prior to testing, affect TG parameters.	True/False
10	Reference intervals demonstrated significant differences between sex and age-related groups	True/False

Name: _____

Email: _____

Please photocopy this page or print it from the electronic AJMS which is stored in the AIMS 'Member Centre' under the heading 'Journal' at www.aims.org.au. ***Circle your answers, then scan, and enter them as activities in the APACE diary under 'My CPD Record'.***

https://www.aims.org.au/online-diary

The APACE (Australasian Professional Acknowledgement of Continuing Education) scheme is a voluntary programme that recognises continuing education, formal courses and a wide range of professional activities which contribute to your professional growth.

The healthcare industry is undergoing rapid change. We are expected to keep our knowledge and skills up to date to enable us to perform to the highest professional standard. The APACE scheme provides a method by which your professional activities are recognised.

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

Australian Institute of Medical Scientists

Immunohaematology Quality Assurance Program

Meet your accreditation requirements with the AIMS Immunohaematology Quality Assurance Program (QAP)

- Designed to suit both small and large laboratories;
- Includes blood grouping, antibody screening/identification and compatibility testing;
- Runs bi-monthly starting in July at the beginning of each financial year;
- The reports provide participants' own results along with graphical representation of results of their peers (de-identified) allowing for easy comparison and analysis.

For enrolment enquiries please contact:
aimsqap@aims.org.au

Instructions to authors

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.

Manuscripts that do not fully comply with the following 'Instructions to Authors' may be returned for revision before they are considered for publication.

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.

Papers published in the *AJMS* are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the *AJMS* and those accepted become the property of the *AJMS*.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

Articles should be submitted in electronic format to programs@aims.org.au. If an article is too large to be submitted by email, it should be submitted on an or USB stick.

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title page

The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, ...). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.

Abstract & keywords

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.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus (see http://www.nlm.nih.gov/mesh/). Keywords should be given below the Abstract.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible, observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance. Do not include a separate section for conclusions, these should be given in the discussion.

Introduction

Clearly state the purpose of the article leading the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials & methods

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.

Results

Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

Discussion

Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However, avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

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

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:

(i) Research in this area (Jones 1999) …

(ii) It has been successfully demonstrated that (Smith and Brown 1981; Auteur 1995; Scienziato *et al* 2007).

(iii) Following further investigation, Wetenschapper (2002 highlighted the difficulties inherent in…

Where there are three or more authors, acknowledge only the first author, e.g. (Smith *et al* 2007). For two authors the following style should be used: (Smith and Brown 2007).

The reference list should be in the format described below. Journal titles should be abbreviated in Index Medicus format (see: ftp://nlmpubs.nlm.nih.gov/online/journals/ljiweb.pdf) using standard abbreviations from the ISSN List of Title Word Abbreviations (see: http://www.issn.org/en/node/344) All authors should be given in the reference list.

Do not use abstracts as references. "Unpublished observations" and "personal communications" may not be used as references, although references to written, not verbal, communications may be cited (in parentheses) in the text. Include in the references manuscripts accepted but not yet published, designate the journal followed by "in press" (in parentheses). Information from manuscripts submitted but not yet accepted should be cited in the text as "unpublished observations" (in parentheses).

Examples of the correct form for references are given below:

Journal Reference:

Stein MK, Downing RW, Rickels K 1978. Self-estimates in anxious and depressed outpatients treated with pharmacotherapy. *Psychol Rep* 43: 487-492.

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.

Tables

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:

* † ‡ § ¶ ** ††

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.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a USB stick. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

○ ● ⏄ ▲ □ ■

In most instances, figures will be reduced to one column in width. All letters and numbers should be drawn to be at least 1.5 mm high after reduction, symbols at least 1.0 mm. Titles for illustrations belong in the legends for illustrations and not on the illustrations themselves.

Photomicrographs must have internal scale markers and the magnification must be stated. Symbols, arrows, or letters used in the photomicrographs should contrast with the background.

Cite each figure in the text in consecutive order, e.g. "Figure 1 illustrates …" or "… as shown (Figure 2)". If a figure has been published, acknowledge the original source and submit with the manuscript written permission from the copyright holder to reproduce the material. Permission is required, regardless of authorship or publisher, except for documents in the public domain.

Legends for illustrations

When symbols, arrows, numbers, or letters are used to identify parts of illustrations, identify and explain each one in the legends. The figure legend must contain a boldface (a) name ("Figure" + arabic figure number) and (b) substantive title.

Abbreviations

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

Avoid abbreviations in the title. The full term for which an abbreviation stands must precede its first use in the text unless it is a standard abbreviation for a unit of measurement.

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

Abbreviation or Symbol	Standard Units of Measurement
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.]

Medical Training Solutions

Online Training and Competency Assessment for the Clinical Lab and Point of Care

Features/Benefits

- Streamlined tracking and documentation;
- High quality multimedia content developed with the University of Washington;
- Over 50 hours of Continuing Education (CE) credit;
- Create your own tests;
- Upload and track your existing documents;
- Used by over 1,500 laboratories.

As an AIMS member you have exclusive access to the Medical Training Solutions (MTS) online clinical laboratory continuing education programme. Please note only current financial members of AIMS are eligible to access MTS. Free Student members do not have access to this resource.

Overview

AIMS purchases a yearly subscription to the Medical Training Solutions (MTS) online clinical laboratory continuing education programme as one of your member benefits. AIMS members receive unlimited access to the laboratory training and competency assessments from MTS.

MTS is accessible online from work and at home so it is most convenient to you. The laboratory training and competency assessment provides training, continuing education and rotating competency assessment tests on clinical laboratory procedures in an engaging format.

MTS adds new training courses periodically. At the moment there are more than 60 courses in the Training Library and over 90 presentations in the Lecture Library.

https://www.aims.org.au/member-resources/medical-training-solutions-mts/medical-training-solutions

http://www.medtraining.org/

The *Training Library* covers topics in:

- Safety
- Specimen Collection
- Microscopy
- Molecular Techniques
- Chemistry
- Haematology
- Coagulation
- Microbiology
- Body Fluids
- Blood Banking
- Immunology
- and more!!

Australian Institute of Medical and Clinical Scientists

Fellowship of AIMS

The AIMS Fellowship is an attractive and highly competitive option to academic post graduate degrees. The Fellowship is recognised by the Department of Health for meeting the requirements for supervision of category GX and GY laboratories.

TRANSFUSION SCIENCE
CLINICAL BIOCHEMISTRY
HAEMATOLOGY
ANATOMICAL PATHOLOGY
IMMUNOLOGY
MICROBIOLOGY
GENERAL (including Core Laboratory)

Qualification for the Fellowship is by EXAMINATION in one of the eight disciplines.

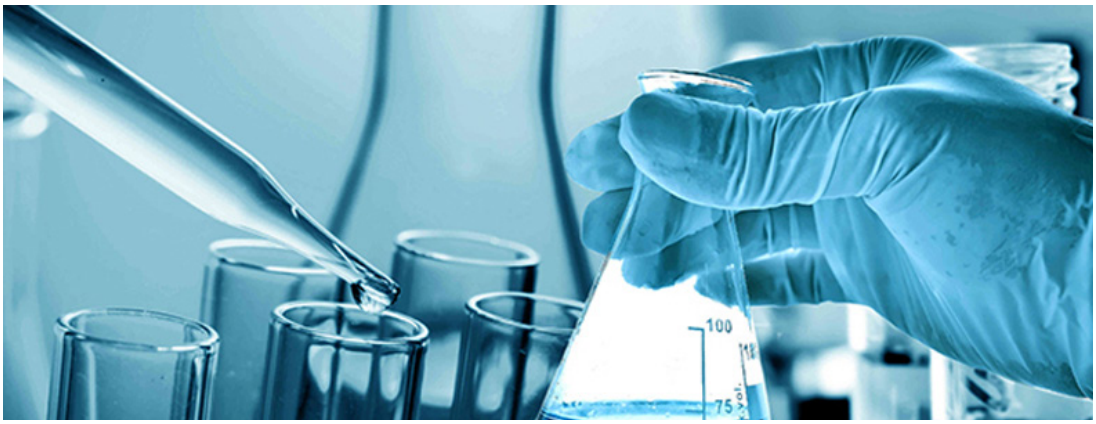
Candidates for the Fellowship must have been members for a minimum of two years and must meet certain other criteria.

The Fellowship program is modular - candidates must complete:

- two compulsory modules
- two elective modules
- a viva voce examination
- a scientific diseration OR a successful relevant research degree thesis completed within the last two years (eg Honours, Masters, PhD); OR a relevant paper published in a peer reviewed journal.

To enrol in the Fellowship program or for further information please contact the AIMS National Programs Manager:

Ph: +61 7 3876 2988
E mail: programs@aims.org.au



Australian Institute of Medical and Clinical Scientists

cmls
Australian Council for the Certification of the Medical Laboratory Scientific Workforce

qualified ✓
competent ✓
certified ✓

Changes to Certification arrangements for the Medical Laboratory Science Profession

From April 2023, the Australian Council for the Certification of the Medical Laboratory Scientific Workforce (CMLS) Board are no longer accepting applications for certifications directly. Instead, professional bodies operating CMLS approved CPD schemes will be able to issue certification on behalf of the Council for their members who meet the requirements for certification as detailed on the CMLS website.

What this means for AIMS members utilising APACE

AIMS Members using the APACE scheme to track their professional development activities can now apply to be certified through the AIMS National Office.

AIMS National Office will now issue Certification to APACE users who have:

- Completed their required CPD activities;
- Been issued their APACE certificate;
- Provided a competency assessment signed by your employer **as part of your AIMS Membership**.

AIMS Members will have access to their APACE record and submission system in the AIMS Members' Area. To get started, follow the step-by-step guide detailed at: https://www.aims.org.au/apace/certification-cmls.

Why become Certified?

Your status as a certified medical laboratory professional is a public guarantee that you are qualified, competent, and continuing your professional development.

If you would like more information on Certification, contact the AIMS National Office via email at: programs@aims.org.au.

CALL FOR SUBMISSIONS

SUBMIT YOUR ARTICLE TO THE AJMS TODAY

Submit your article to our journal to be shared with the Medical Science community.

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
- Molecular biology

Areas of general interest to medical laboratory scientists will also be considered, including:

- Toxicology
- Epidemiology
- Public and Community Health
- Professional and Management Issues

Papers published in the AJMS are in the form of original articles, case studies, discipline updates, opinion pieces and review articles.

All successful articles will be peer reviewed and published online and print.

We look forward to reading your work!

EMAIL YOUR ARTICLE TO

programs@aims.org.au

AIMS

NATIONAL SCIENTIFIC MEETING

MOVING FORWARD

21-23 AUGUST 2024

ADELAIDE CONVENTION CENTRE

MEET THE KEYNOTE SPEAKERS!

Vienna Tran

MBBS (Hons.), DAME

Registrar, Australian College of Rural and Remote Medicine

David Ross

MBBS, PhD, FRACP, FRCPA

Consultant Haematologist, SA Pathology

Christopher McCudden

PhD, DABCC, FACB, FCAB

Clinical Biochemist, Ottawa Hospital

Mark Shephard

Founding Director of the International Centre for

Point-of-Care Testing, Flinders University

REGISTRATION NOW OPEN

Register now for the AIMS National Scientific Meeting to secure the Earlybird rate!

DOWNLOAD THE PROGRAM

The Scientific Meeting program has been released

Download now!

WWW.AIMSNSM2024.COM

CONTACT THE MEETING ORGANISERS

All Occasions Group

conference@aomevents.com 08 8125 2200