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Evaluation of the analytical performance of four different manufacturer's reagent red blood cells in antibody detection and identification

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Abstract

Objective The detection/identification of clinically significant antibodies to red cell antigens form the foundation for safe transfusion practices. This study aimed to evaluate the diagnostic performance of commercially available 0.8% reagent red blood cells (RRBCs) in Australia. 166 patient-derived plasma samples with a positive indirect antiglobulin test (IAT) were tested using column agglutination technology (CAT) with Immulab, Bio-Rad, Grifols and QuidelOrtho screening and identification RRBCs with the respective manufacturer's proprietary CAT system.

Results False-negative antibody screening and identification results were obtained with Bio-Rad (3/61), Grifols (14/68) and Quidel-Ortho (3/59) RRBCs when tested with the respective manufacturer's proprietary CAT system. Zero false-negative results were observed with Immulab RRBCs when tested with samples across all platforms. The sensitivity of the RRBCs used in this study were calculated to be 95.83% (95%CI 88.30-99.13%) for Bio-Rad RRBCs, 82.50% (95%CI 72.38–90.09%) for Grifols RRBCs and 95.65% (95%CI 87.82–99.09%) for QuidelOrtho RRBCs. The sensitivity of Immulab RRBCs were stratified based on performance in the 3 CAT platforms: Bio-Rad CAT (100%, 95%CI 95.01–100%), Grifols CAT (100%, 95%CI 95.49–100%) and QuidelOrtho CAT (100%, 95%CI 94.79–100%).

Conclusions RRBCs used in antibody detection and identification vary in diagnostic performance and should therefore be carefully considered before being implemented in routine patient testing.

Keywords Reagent red blood cells, Antibody detection, Antibody identification, Analytical performance, Pre-transfusion testing, Blood transfusion

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Introduction

The provision of safe blood for transfusion relies on the detection and identification of clinically significant antibodies against red blood cell (RBC) antigens. RBC alloimmunization occurs as a result of an immune response against foreign RBC antigens following transfusion or pregnancy [1]. Unexpected clinically significant alloantibodies include those against Rh, Kell, Duffy, Kidd, MNS, P1PK and Lewis blood group systems. The consequences of RBC alloimmunization range from asymptomatic serological transfusion reactions to accelerated destruction of transfused donor cells (as observed in hemolytic transfusion reactions (HTRs)), and hemolytic disease of the fetus and newborn (HDFN) [2]. The persistence of alloantibodies varies among individuals and is specificity dependent. Approximately half to two-thirds of alloantibodies become undetectable within 4–5 years in the absence of antigen stimulation, a phenomenon known as antibody evanescence [3–6]. Thus, sensitive detection methods and reagents are essential to minimize the risk of missing antibodies during pretransfusion testing.

An ideal antibody screen should allow the detection of all clinically significant antibodies without capturing clinically insignificant antibodies and non-specific reactions, with acceptable sensitivity and specificity [7]. If an unexpected antibody is detected in an antibody screen, its specificity must be determined. Commercially available antibody identification panels typically consist of RRBCs from 11 unique donors. The selection of donors in a given antibody panel should allow for the identification of single specificities of common alloantibodies, with the exclusion of most others.

In Australia, five manufacturers sell RRBCs for antibody detection and identification in NATA-accredited pathology laboratories: Immulab Pty Ltd, Werfen Australia Pty Ltd (formally Immucor Inc.), Bio-Rad Laboratories Inc., Grifols Australia Pty Ltd and QuidelOrtho Inc. Immulab 0.8% RRBCs are validated and approved for use with all CAT platforms, whilst Bio-Rad, Grifols and Ortho manufacture propriety 0.8% RRBCs approved only for use with their own CAT platform. This study aimed to assess the analytical performance of Immulab, Bio-Rad, Grifols and Ortho 0.8% RRBCs using CAT for the detection and identification of clinically significant RBC antibodies. This evaluation will focus on the analytical performance of these RBCs with respect to various performance measures (sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs) and accuracy).

Main text

Materials and methods

Study design

Three test scripts were designed to compare the analytical performance of RRBCs from 4 manufacturers. Test script 1 compared the performance of Immulab and Bio-Rad RRBCs in Bio-Rad CAT; test script 2 compared Immulab and Grifols RRBCs in Grifols CAT; and test script 3 compared Immulab and QuidelOrtho RRBCs in QuidelOrtho CAT. The study was designed as such because Immulab RRBCs are validated for use on all CAT platforms, whilst other manufacturer's RRBCs are only approved for use with their own proprietary CAT system. Where volume permitted, samples were tested on all platforms; however, as the objective of the study was to compare the performance of the RRBCs and not the CAT platforms themselves, small-volume samples were randomly allocated to one or more test scripts. In addition, commercially available anti-Fy^a, anti-Fy^b and anti-s polyclonal antisera were sourced and tested at different dilutions in each test script.

Samples

Patient samples with a positive IAT were sourced from various institutions in Australia who used either Immulab, Bio-Rad, Grifols and Ortho 0.8% RRBCs as their primary antibody screening method. Plasma was aliquoted and stored frozen at a maximum temperature of -18°C until commencement of testing.

Serial two-fold dilutions of polyclonal antisera (Immulab polyclonal anti-s, anti-Fy^a and anti-Fy^b) were performed using a diluent of 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Serial dilutions were prepared for each antiserum until a final dilution of 1:256 was obtained. Each dilution for each antisera was tested in all test scripts as per the method below.

Antibody detection and identification

The CAT card, volume of patient plasma, heating block, and centrifuge used are shown in Table 1. All testing was performed in accordance with the Instructions for Use (IFU) provided by each manufacturer [8–13].

All samples with a negative antibody screen required no follow-up action. For samples with a positive screen, an 11-cell antibody identification panel was performed as per the aforementioned steps using the 0.8% reagent panels listed in Table 1. If an antibody screen was positive with only one manufacturers RRBCs, a panel was performed using only the manufacturer of the antibody screening cells by which the antibody was detected.

Analysis of results

The sensitivity, specificity, PPV, NPV and accuracy of the RRBCs were calculated for each test script. The 95%

Table 1 Reagents and equipment used for antibody screening and identification. The CAT card, volume of RRBC, volume of plasma, heating block and centrifuge used in testing were in accordance with the instructions for use (IFUs) published by the various RRBC manufacturers

	CAT card	Screening cells	Panel Cells	Volume of RRBC (µL)	Volume of plasma (µL)	Heating block	Centrifuge
Test Script 1	Bio-Rad ID-Card LISS/Coombs card	Immulab 0.8% Abtectcell III + Bio-Rad 0.8% ID-DiaCell I-II-III	Immulab 0.8% Phenocell Panel B + Bio-Rad 0.8% ID-DiaPanel	50	25	Grifols DG Therm heating block	Bio-Rad ID-Centrifuge 12 S II centrifuge
Test Script 2	Grifols DG Gel Coombs card	Immulab 0.8% Abtectcell III + Grifols 0.8% Perfect Screen 3	Immulab 0.8% Phenocell Panel B + Grifols 0.8% Identisera Diana	50	25	Grifols DG Therm heating block	Grifols DG Spin centrifuge
Test Script 3	Ortho BioVue Anti-IgG, -C3d polyspecific CAT card	Immulab 0.8% Abtectcell III + Ortho 0.8% Surgiscreen	Immulab 0.8% Phenocell Panel B + Ortho 0.8% Panel A	50	40	Ortho BioVue System heating block	Ortho BioVue System centrifuge

Table 2 Samples with discrepant antibody identification results. Only the antibody specificities of samples which contained adequate volume to undergo complete antibody identification are shown

	Test Script 1 (Bio-Rad CAT)		Test Script 2 (Grifols CAT)		Test Script 3 (Ortho CAT)	
	Immulab RRBCs	Bio-Rad RRBCs	Immulab RRBCs	Grifols RRBCs	Immulab RRBCs	QuidelOrtho RRBCs
1	Anti-D	ND				
2	Anti-E	ND				
3	ND	Anti-C^w				
4	Anti-M	ND	Anti-M	ND	Anti-M	ND
5			Anti-D	ND		
6			Anti-D	ND		
7			Anti-D	ND		
8			Anti-D	ND		
9			Anti-D	ND		
10			Anti-E	ND		
11			Anti-e	ND		
12			Anti-Fy^a	ND		
13			Anti-Jk^a	ND		
14			Anti-Jk^a	ND	Anti-Jk^a	ND
15			Anti-M	ND		
16			Anti-D + Anti-C	Anti-D		
17			Anti-E + Anti-Kp^a	Anti-Kpa		
18					Anti-E	ND

ND = no antibody detected

confidence intervals for sensitivity/specificity/accuracy were calculated using the exact Clopper-Pearson method. The 95% confidence intervals for the NPV/PPV were calculated using the standard logit confidence interval method. The calculations were performed using IBM® SPSS Statistics® version 28 and Microsoft Excel® for Microsoft 365.

Results

A total of 166 patient plasma samples were subjected to antibody detection and identification across all three test scripts.

Diagnostic accuracy of immulab, bio-rad, grifols and quidelortho RRBCs

In test script 1, concordant specificities were observed in 57/61 samples (93.4%). Four samples produced discrepant results (Table 2). Three false-negative reactions were observed with Bio-Rad RRBCs despite the presence of donors with a homozygous expression of the corresponding antigen: one anti-D, one anti-E and one anti-M. These specificities were positively identified using Immulab RRBCs in Bio-Rad CAT. In one sample containing anti-Cw, the specificity was identified using Bio-Rad RRBCs but screened negative using Immulab RRBCs because

of the lack of Cw+cells. As this result was expected (i.e., not a false-negative), the data point was omitted for both Immulab and Bio-Rad RRBCs performance calculations.

In test script 2, concordant specificities were observed in 54/68 samples (79.4%). Fourteen false negatives were observed using the Grifols RRBCs as shown in Table 2, accounting for all discrepancies. The false-negative results comprised a range of antibody specificities: five anti-D, two anti-E (one of which was in a sample containing anti-E+anti-Kpa), one anti-C (in a sample containing anti-D+anti-C), one anti-e, two anti-Jk^a, two anti-M and one anti-Fy^a; these were identified using Immulab RRBCs in Grifols CAT.

In test script 3, concordant specificities were observed in 56/59 samples (94.9%). Three false negatives were observed using QuidelOrtho RRBCs as shown in Table 2, accounting for all discrepancies. The false negatives had the following specificities: one anti-E, one anti-Jk^a and one anti-M. These specificities were positively identified using Immulab RRBCs in QuidelOrtho CAT.

The sensitivity, specificity, PPV, NPV, and accuracy were calculated for all the RRBCs and are presented in Table 3.

Antibody titration

Serial two-fold dilutions of commercially available polyclonal antisera (anti-s, anti-Fy^a and anti-Fy^b) were prepared and each dilution underwent antibody detection in each test script. Direct comparisons of reaction strength were made as the number of homozygous and heterozygous cells in each batch of RRBCS for each specificity was comparable between manufacturers, with the exception of test script 3 where the Ortho 0.8% Surgiscreen contained a heterozygous Fy(a+b+) cell and Immulab 0.8% Abtectcell III contained only homozygous cells. As shown in Fig. 1, Immulab 0.8% RRBCs demonstrated higher sensitivity than comparator RRBCs in detecting antibodies with these specificities at low titers.

Discussion

The false-negative antibody identification results encountered with Bio-Rad, Grifols and Ortho RRBCs negatively affected the sensitivity/NPV of these RRBCs. Importantly, these false-negative results occurred despite the presence of antigen-positive cells, including cells with homozygous expression of the corresponding antigen, where relevant. The NPV statistic is subject to change depending on the prevalence of antibodies in the patient population. Given that the present study utilized samples that were antibody-positive, the NPV was highly sensitive to false-negative results. In a study population more representative of the true Australian population (i.e. where most individuals are not alloimmunized), the NPV statistic would be markedly different. For instance, a 2018 study by Orlando et al., found the sensitivity of Bio-Rad 0.8% RRBCs to be 90.0% (95%CI 0.879–0.918) and the NPV 99.9% (95%CI 0.993–1.00) when tested on an automated systems using Bio-Rad CAT [7]. While the sensitivity of Bio-Rad RRBCs is comparable to the current study, the NPV for Bio-Rad RRBCs was significantly higher. This can be attributed to the use of a sample population that was largely negative for antibodies to RBC antigens (12/986 samples contained antibodies). Similarly, Sawierucha et al. in 2018 examined the analytical performance of Bio-Rad and Ortho 0.8% RRBCs using a population of 1000 samples, which again were mostly antibody-negative (17/1000

Table 3 Diagnostic accuracy of 0.8% Immulab, Bio-rad, Grifols and QuidelOrtho RRBCs. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of Immulab RRBCs compared to Bio-rad (test script 1), Grifols (test script 2) and QuidelOrtho (test script 3) 0.8% RRBCs with 95% confidence intervals (95% CI)

	Test Script 1: Immulab and Bio-Rad		**Test Script 2: Immulab and Grifols**		**Test Script 3: Immulab and Ortho**	
	Immulab 0.8% Abtectcell III	**Bio-Rad 0.8% ID-DiaCell I-II-III**	**Immulab 0.8% Abtectcell III**	**Grifols 0.8% Perfect Screen 3**	**Immulab 0.8% Abtectcell III**	**QuidelOrtho 0.8% Surgiscreen**
True-positive	72	69	80	66	69	66
False-positive	0	0	0	0	0	0
True-negative	15	15	8	8	24	24
False-negative	0	3	0	14	0	3
Sensitivity (%) (95% CI)	100 (95.01–100)	95.83 (88.30–99.13)	100 (95.49–100)	82.50 (72.38–90.09)	100 (94.79–100)	95.65 (87.82–99.09)
Specificity (%) (95% CI)	100 (78.20–100)	100 (78.20–100)	100 (63.06–100)	100 (63.06–100)	100 (85.75–100)	100 (85.75–100)
PPV (%) (95% CI)	100	100	100	100	100	100
NPV (%) (95% CI)	100 (78.20–100)	83.33 (58.58–96.42)	100 (63.06–100)	36.36 (17.20–59.34)	100 (85.75–100)	88.89 (70.84–97.65)
Accuracy (%) (95% CI)	100 (95.85–99.97)	96.55 (90.25–99.28)	100.00 (95.89–100)	84.09 (74.75–91.02)	100 (96.11–100)	96.77 (90.86–99.33)

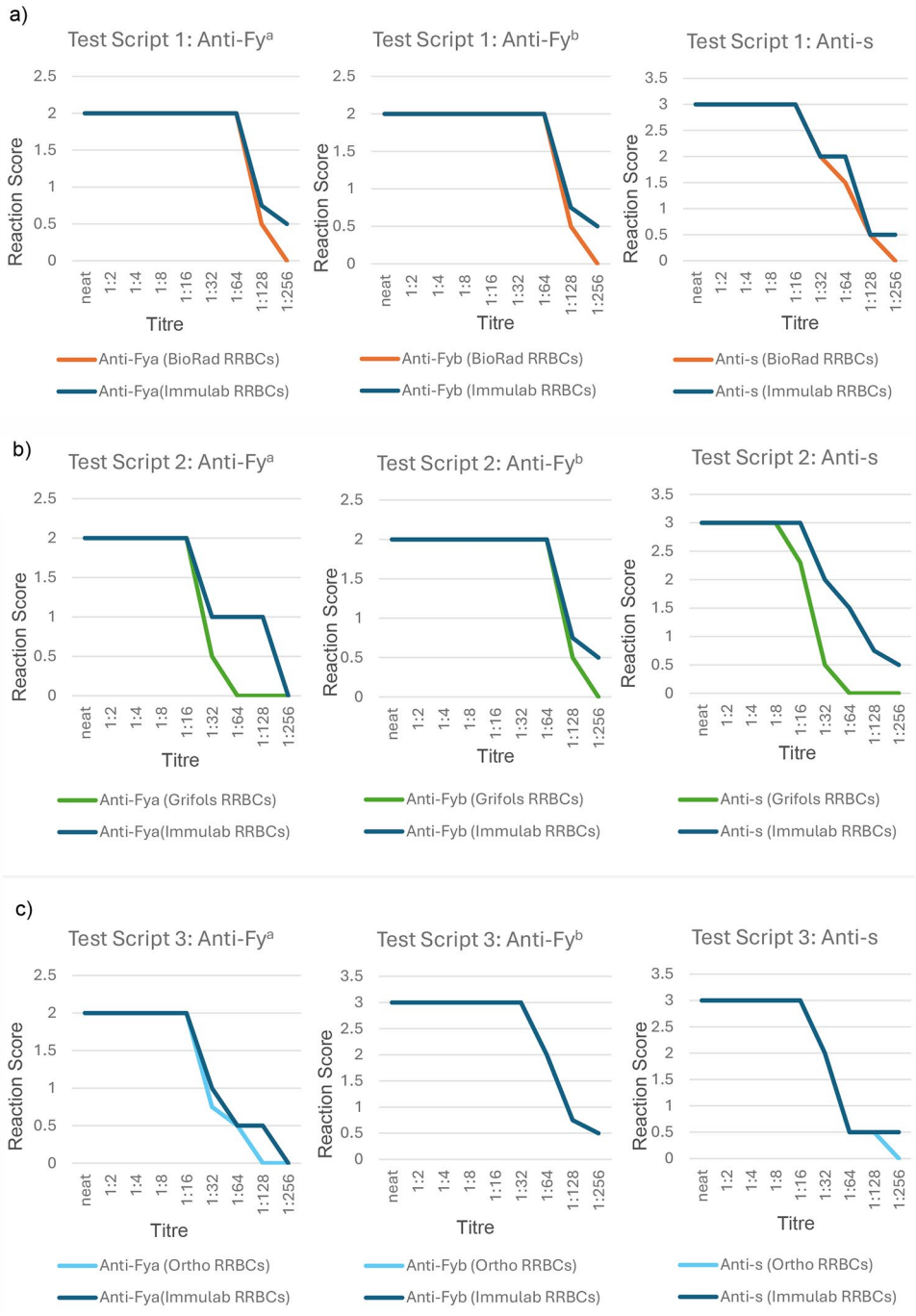

Fig. 1 Differences in mean reaction strength of polyclonal antisera (anti-Fy^a, anti-Fy^b and anti-s) when titrated using doubling dilutions. Reactions represented on a 0–4 haemagglutination scale. Differences are shown between (a) Immulab 0.8% Abtectcell III and Bio-Rad 0.8% ID-DiaCell I-II-III when tested in Bio-Rad polyclonal AHG CAT (b) Immulab 0.8% Abtectcell III and Grifols Perfect Screen 3 when tested in Grifols polyclonal AHG CAT and (c) Immulab 0.8% Abtectcell III and Ortho Diagnostics 0.8% Surgiscreen when tested in Ortho BioVue polyclonal AHG CAT

samples contained antibodies) [5]. The calculated sensitivities (Bio-Rad RRBCs = 86.36%, Ortho RRBCs = 90.9%) were again comparable to those in the current study [5], though the NPVs were markedly higher due to the differences in study design. In the current study, the specificity and PPV statistics were the same for all the RRBCs (100%). This is attributed to the study design in which samples were selected based on expected antibody positivity (no false positives were encountered). Thus, the results of this study may not be directly comparable to other studies if the study design and sample characteristics are different. However, by utilizing a study

population predominantly positive for antibodies, the current study is able to amplify differences in RRBC performance whilst minimizing research wastage.

Several manufacturing variables may contribute to differences in RRBC performance. For example, the constituents used to create 0.8% suspensions of red cells may vary among manufactures, including diluents, preservatives, and antimicrobial agents. Differences in these constituents may affect the performance of these reagents in CAT. The use of local donors for detecting and identifying antibodies in the Australian population may confer unique advantages to Immulab RRBCs compared with imported Bio-Rad, Grifols and QuidelOrtho RRBCs. Although strict standards and guidelines exist for the storage, transport and traceability of blood in Australia [14], RRBCs made from local donors may be less prone to fluctuations in cold-chain maintenance by avoiding the need for extended periods of time in transit. Red cells subjected to conditions outside of strict cold-chain maintenance may display increased osmotic fragility, hemolysis, and bacterial growth in rare circumstances [15]. Thus, there may be a potential benefit in the use of local donors for RRBCs when detecting low-titre antibodies in the Australian population. Further research is needed to determine the exact implications of this logistical factor on RRBC performance.

Clinical significance of RRBC performance: should patient safety be a concern?

In the current study, statistically significant differences in the sensitivity and NPV of Immulab, Bio-Rad, Grifols and QuidelOrtho RRBCs were identified. However, the clinical implications of these differences remain unclear. Antibodies not detected by Bio-Rad, Grifols and/or QuidelOrtho RRBCs range from having specificities to Rh, Duffy, Kidd and MNSs blood group system antigens; all of which are clinically significant and may be implicated in HTRs and HDFN. The Serious Hazards of Transfusion (SHOT) reports from 2019 to 2021 show that anti-Jk^a was the most common antibody specificity involved in HTRs in the UK [16–18], followed by anti-Fy^a, anti-C, anti-E, anti-S, and anti-c. The Blood Matters Serious Transfusion Incidents Reporting (STIR) system in Victoria, Australia, report similar findings [19]. Given the outcomes of the most recent STIR and SHOT reports [16–19], the significance of failing to detect alloantibodies to clinically significant RBC antigens should not be overlooked.

A key recommendation in the most recent STIR report is the development of a national red cell alloantibody database [19]. As different pathology providers may not share a patient's antibody history, patients may be at risk of HTRs if an antibody can no longer be detected. This may be particularly true if different RRBCs are used by different health services; RRBCs with lower sensitivities and overall performance may be more likely to miss clinically significant antibodies at low titers. In the current study, serial dilutions of polyclonal anti-s, anti-Fy^a and anti-Fy^b antisera were used to mimic antibody evanescence. At every dilution, Immulab RRBCs performed equally, if not better, than Bio-Rad, Grifols and Ortho RRBCs in the detection of these antibodies. These findings suggest that Immulab RRBCs may detect low-titer red cell antibodies with greater sensitivity.

Limitations

Not all samples were able to undergo testing in all platforms owing to insufficient sample volume. Because the objective of the study was to compare the RRBCs used and not the test platform itself, this limitation does not impact analytical performance calculations but rather, limits interpretation between CAT platforms. Despite the use of all RRBCs before the expiration date, differences in manufacturing cycles and RRBC 'freshness' is a potential limitation. This limitation is largely accounted for by using three to four batches of RRBCs from each manufacturer and performing testing twice weekly.

Conclusions

Immulab 0.8% RRBCs demonstrated greater sensitivities and NPVs compared to the equivalent Bio-Rad, Grifols and QuidelOrtho RRBCs in Bio-Rad, Grifols, and QuidelOrtho CAT, respectively. No false-negative results were observed using Immulab RRBCs. In contrast, antibody detection testing performed using Bio-Rad, Grifols, and QuidelOrtho RRBCs resulted in several examples of false-negative antibody detection outcomes. Antibodies not detected by these RRBCs ranged from having specificities to Rh, Duffy, Kidd and MNS blood group system antigens; all of which are clinically significant and may be implicated in HTRs and HDFN. Although the clinical implications of these differences remain unclear, these results should trigger further research on the performance of RRBCs used for pre-transfusion testing.

Abbreviations

BSA	Bovine serum albumin.
CAT	Column agglutination technology
HDFN	Haemolytic disease of the fetus and newborn
IAT	Indirect antiglobulin test
PBS	Phosphate buffered saline
PPV	Positive predictive value
RBC	Red blood cell
RRBC	Reagent red blood cell
NPV	Negative predictive value
SHOT	Serious Hazards of Transfusion
STIR	Serious Transfusion Incidents Reporting
STR	Serological transfusion reaction

Author contributions
Conceptualization: C.M. G., F. J. M., D.E.J., J.G. Investigation and Data curation: J.G. Formal Analysis: J. G. Supervision: F.J.M., D.E.J. Writing – original draft: J.G., D.E.J. Writing – review and editing: J.G., F.J.M., C.M.G., D.E.J.

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Data availability
Data is provided within the manuscript tables and figure.

Declarations

Ethics approval and consent to participate
Ethical approval was not required for this study in accordance with local and national guidelines, namely the *National Health and Medical Research Council Act 1992* (Australia), as no patient information was collected. Written informed consent from participants was not required in accordance with these guidelines, and the study adhered to the tenets of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
Jessica Guglielmino and Claire Grattidge are employed by Paragon Care Scientific and Diagnostic Group, Victoria, Australia.Note the project was carried out at RMIT University with resources, equipment and oversight by RMIT staff to avoid any conflict of interest.

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