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## An evaluation of 4 commercial assays for the detection of SARS-CoV-2 antibodies in a predominantly mildly symptomatic low prevalence Australian population

Michael C. Wehrhahn<sup>a,\*</sup>, Suzanne J. Brown<sup>b</sup>, James P. Newcombe<sup>a,c</sup>, Smathi Chong<sup>d</sup>, Jenny Evans<sup>e</sup>, Melanie Figtree<sup>c</sup>, Laurence Hainke<sup>d</sup>, Linda Hueston<sup>f</sup>, Sadid Khan<sup>g</sup>, Elizabeth Marland<sup>a</sup>, Matthew V.N. O'Sullivan<sup>f,h</sup>, Helen Powell<sup>e</sup>, Jhumur Roy<sup>i</sup>, Lynette Waring<sup>g</sup>, Megan Yu<sup>a</sup>, Jennifer Robson<sup>e</sup>

<sup>a</sup> Department of Microbiology, Douglass Harly Moir Pathology, New South Wales, Australia

<sup>b</sup> Department of Endocrinology, Sir Charles Gairdner Hospital, Western Australia, Australia

<sup>c</sup> NSW Health Pathology, Department of Microbiology, Royal North Shore Hospital, New South Wales, Australia

<sup>d</sup> Department of Microbiology, Clinipath Pathology, Western Australia, Australia

<sup>e</sup> Department of Microbiology, Sullivan Nicolaides Pathology, Queensland, Australia

<sup>f</sup> NSW Health Pathology, Institute of Clinical Pathology and Medical Research, Westmead, New South Wales, Australia

<sup>g</sup> Department of Microbiology, Melbourne Pathology, Victoria, Australia

<sup>h</sup> Faculty of Medicine and Health, University of Sydney, New South Wales, Australia

<sup>i</sup> Department of Microbiology, Clinipath Pathology, South Australia, Australia

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### ABSTRACT

A total of 1080 individual patient samples (158 positive serology samples from confirmed, predominantly mildly symptomatic COVID-19 patients and 922 serology negative including 496 collected pre-COVID) from four states in Australia were analysed on four commercial SARS-CoV-2 serological assays targeting antibodies to different antigens (Roche Elecsys and Abbott Architect: nucleocapsid; Diasorin Liaison and Euroimmun: spike). A subset was compared to immunofluorescent antibody (IFA) and micro-neutralisation. Sensitivity and specificity of the Roche (n = 1033), Abbott (n = 806), Diasorin (n = 1034) and Euroimmun (n = 175) were 93.7%/99.5%, 90.2%/99.4%, 88.6%/98.6% and 91.3%/98.8%, respectively. ROC analysis with specificity held at 99% increased the sensitivity for the Roche and Abbott assays from 93.7% to 98.7% (cut-off 0.21) and 90.2% to 94.0% (cut-off 0.91), respectively. Overall seropositivity of samples increased from a maximum of 23% for samples 0–7 days-post-onset of symptoms (dpos), to 61% from samples 8–14dpos and 93% from those >14dpos. IFA and microneutralisation values correlated best with assays targeting antibodies to spike protein with values >80 AU/mL on the Diasorin assay associated with neutralising antibody. Detectable antibody was present in 22/23 (96%), 20/23 (87%), 15/23 (65%) and 9/22 (41%) patients with samples >180dpos on the Roche, Diasorin, Abbott and microneutralisation assays respectively. Given the low prevalence in this community, two-step algorithms on initial positive results saw an increase in the positive predictive value (PPV) of positive samples (39%–65% to ≥98%) for all combinations. Similarly accuracy increased from a range of 98.5%–99.4% to ≥99.8% assuming a 1% seroprevalence. Negative predictive value (NPV) was high (≥99.8%) regardless of which assay was used initially.

### 1. Introduction

As of December 15, 2020, more than 73 million cases of COVID-19 have been diagnosed causing over 1.6 million deaths worldwide.

While diagnosis has relied largely on SARS-CoV-2 nucleic acid amplification testing (NAAT), for a small number of patients with equivocal or negative NAAT results, serology has been instrumental in clarifying the true infection status of a case. Serology has a greater role in

\* Corresponding author at: 14 Giffnock Ave, Macquarie Park NSW 2113, New South Wales, Australia.

E-mail address: [mwehrhahn@dhm.com.au](mailto:mwehrhahn@dhm.com.au) (M.C. Wehrhahn).

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retrospectively diagnosing COVID-19 especially in asymptomatic cases [1,2]. This in turn improves estimates of attack rate, case fatality rate and reproduction number ( $R_0$ ) in a population [3–5]. Serology may also have prognostic value, with antibody titres found to correlate with severity of infection [4,6–9], and can assist public health investigations of outbreaks [5].

In the longer term, studies may be able to assess whether herd immunity against SARS-CoV-2 has been achieved [3]. If high-throughput commercial Enzyme Linked Immunosorbent Assays (ELISA) or Chemiluminescent Microparticle Immunoassays (CMIA/CLIA) correlate with neutralising antibody (Nab) titres, an immune population may be determined who are lower risk for returning to frontline work [3,5]. At present, serological correlates of immunity post-vaccination are yet to be determined and published studies of Nab following infection with SARS-CoV-2 remain limited [6,10–14].

Commercial assays generally target one of two proteins: the spike or the nucleocapsid protein with uncertainty about which targets are the most sensitive or correlate best with Nab [15,16].

While published studies to date have compared commercial assays head-to-head [17–21] most have been in higher prevalence areas. Australia and New Zealand have been fortunate in largely eliminating transmission in the community but this has presented a unique diagnostic challenge to understand the relative performance of commercial test platforms for use in a very low incidence community. We sought to investigate the reliability of four commercial assays for use within our laboratory network in Australia.

## 2. Methodology

Commercial assay testing (Roche Elecsys Anti-SARS-CoV-2 assay targeting IgM/IgA/IgG to nucleocapsid protein, Abbott Architect SARS-CoV-2 IgG targeting nucleocapsid protein, Diasorin Liaison SARS-CoV-2 S1/S2 IgG targeting the S1 and S2 domains of the spike protein, Euroimmun Anti-SARS CoV-2 IgG targeting the S1 domain of the spike protein) was performed at 4 laboratories across 4 states of Australia according to the assays' instructions for use (IFU). Due to limited availability of the Euroimmun assay and residual stored sera, samples from COVID-19 patients were prioritised over specificity samples for testing using this assay. The Institute of Clinical Pathology and Medical Research, NSW performed an in-house immunofluorescent antibody (IFA) assay [22] and a microneutralisation assay [23] as previously described. All data was de-identified and verbal consent of COVID-19 patients was obtained and approval for this study obtained from the Sullivan Nicolaides Pathology Low Risk Ethics Committee.

Sensitivity analysis was performed on stored sera from confirmed patients with COVID-19 diagnosed by NAAT as defined by local guidelines and also household contacts seropositive for IgG by IFA in the absence of NAAT being performed [24,25]. The majority of cases were diagnosed by the Seegene Allplex 2019-nCoV Assay (targeting E, N and RdRP genes) or an in-house developed Taqman assay targeting the E and N genes.

Only the latest sample for each patient was selected. Seropositive samples from confirmed COVID-19 cases had to be positive by at least IFA if tested or in the absence of IFA, at least one commercial assay. A two-step algorithm analysing the different possible combinations of commercial assays to optimise positive predictive value (PPV) was evaluated [26].

The COVID-19 cases and three cohorts used to assess the specificity of the assays under evaluation are depicted in Table 1.

A novel illness severity score (ISS) assessing 19 symptoms and need for hospitalisation, intensive care support and mechanical ventilation (HIM) was developed and evaluated on a subset of patients as depicted in Table 2. Duration of symptoms (DS) was also assessed and capped at 30 days.

Equivocal results for Euroimmun and Diasorin were considered positive for statistical analysis. Sub-analysis was performed with

**Table 1**  
Sensitivity and Specificity samples.

Sensitivity cohort	n (%)	Specificity cohort	n (%)	
COVID-19 cases	Qld 77 (45)	Cohort 1 pre-COVID	411	
	NSW 71 (41)	-antenatal	324 (79)	
	Victoria 14 (8)	-healthy adults 2019	57 (14)	
	WA 11 (6)	-children 2019	30 (7)	
	Qld 67 (42)	Cohort 2*	378	
	NSW 66 (42)	Cross-reactivity (pre-COVID)	235 (62)	
Seropositive COVID-19 cases	Victoria 14 (9)	Cross-reactivity (COVID)	143 (38)	
	WA 11 (7)	Cohort 3 ARI; COVID-19PCR negative	118	
	Total COVID-19 cases	Total specificity samples	907 (84)	Total overall samples 1080 (100)
Total seropositive (% of total overall)	158 (15)	pre-COVID	646 (71)	

NSW New South Wales, Qld Queensland, WA Western Australia.

ARI: Acute respiratory infection.

\* Including but not limited to patients diagnosed with the following respiratory pathogens: influenza A/B n = 55, parainfluenza n = 16, respiratory syncytial virus n = 12, adenovirus n = 10, rhinovirus n = 3, *Mycoplasma pneumoniae* n = 16, *Bordetella pertussis* n = 14, *Chlamydia pneumoniae* n = 10, *Legionella longbeachae* n = 8; non-respiratory pathogens positive for EBV n = 53, CMV n = 43, Parvovirus B19 n = 19, Hepatitis A/B/C n = 26, HIV 5, Ross River virus n = 17; or highly elevated autoantibodies: Rheumatoid factor n = 19, Antinuclear antibody n = 28.

serology values obtained from IFA, 'nucleocapsid assays' (NA), ie Abbott and Roche assays and 'spike assays' (SA), ie Diasorin and Euroimmun.

Receiver operator characteristic (ROC) curve analysis was undertaken to determine the area under the curve (AUC), and sensitivity, specificity and accuracy, along with 95 % confidence intervals (CI), were calculated. Assay cut-off values were optimised by interrogation of ROC results.

Pairwise comparisons of sensitivity and specificity between assays were made. As many of the same samples were assessed by each assay, the diagnostic measures were correlated with each other, and to account for this a procedure using 2000 bootstrap replicates was adopted [27]. Sensitivity was tested while holding specificity at 99 %, then specificity was tested while holding sensitivity at 95 %. A significance threshold of 0.05 was used, with a Bonferroni correction for multiple testing giving an adjusted threshold for 6 tests of  $\alpha' = 0.0083$ .

We evaluated the relationship between IFA and assay values using linear regression analysis, with adjustment for the covariates age and sex. As the IFA distribution was skewed, a normalising square root transformation was applied before subsequent analysis. Associations between the outcomes IFA, NA and SA and the predictors ISS, DS and HIM were similarly explored, with adjustment for the covariates age, sex and age by sex interaction. To correct for the effect of multiple testing on Type I error in this setting, the significance threshold was set at  $\alpha' = 0.05/9 = 0.0056$ . The same associations were also examined in sex-stratified analyses.

Data were analysed in the R statistical computing environment, version 4.0.0, including the package *pROC* [27].

## 3. Results

There were 173 patients with confirmed COVID-19 by NAAT (n = 169) or IFA (n = 4) who had serology samples evaluated. Of these, 90

**Table 2**  
Illness Severity Score.

	1 point	Score	2 points	Score	3 points	Score
Symptoms	Chills/sweats	<input type="checkbox"/>	Fever	<input type="checkbox"/>	Shortness of breath	<input type="checkbox"/>
	Cough	<input type="checkbox"/>	Rigors	<input type="checkbox"/>		
	Sore throat	<input type="checkbox"/>	Chest pain	<input type="checkbox"/>		
	Rhinorrhoea	<input type="checkbox"/>	Myalgia/ arthralgia	<input type="checkbox"/>		
	Headache	<input type="checkbox"/>				
	Dizziness	<input type="checkbox"/>				
	Tiredness	<input type="checkbox"/>				
	Conjunctivitis	<input type="checkbox"/>				
	Loss of smell	<input type="checkbox"/>				
	Loss of taste	<input type="checkbox"/>				
	Rash	<input type="checkbox"/>				
	Anorexia	<input type="checkbox"/>				
	Nausea/ vomiting	<input type="checkbox"/>				
	Diarrhoea	<input type="checkbox"/>				
Hospital features					Non-ICU 3pts	<input type="checkbox"/>
					ICU 4 pts	<input type="checkbox"/>
					Mechanical ventilation 5pts	<input type="checkbox"/>
Score		/14				/8
Total score						/30

(52 %) were male and the mean age of patients was 50.7 years (range 7–85years). Severity of disease data for 163 patients indicated that 5 (3 %) were asymptomatic, 131 (80 %) were symptomatic but non-hospitalised, 18 (11 %) were hospitalised/non-ICU patients and 9 (6 %) were ICU patients. Thirty day mortality was 0 %.

Of the 173 individual samples from confirmed cases and 907 negative samples, there were 158/1080 (14.6 %) seropositive patients. Time since illness onset available for 147 patients found 139/147 (95 %) were collected ≥10 days-post-symptom onset (dpos). Sensitivity, specificity and accuracy results are presented in Table 3. In the subset of 175 samples common to all assays, there were no differences in AUC among the 4 assays (all p > 0.042), nor in specificity when sensitivity was held

**Table 3**

Diagnostic performance of 4 assays at the published cut-offs for detection of COVID-19 disease (a) in all 1080 samples, of which 158 (14.6 %) tested positive; and (b) in subset of 175 subjects with measurements on all 4 assays, of which 92 (52.6 %) tested positive. Values are expressed as percentages (95 % CI), and as a decimal fraction for AUC.

(a) All samples				
	Roche	Abbott	Diasorin	Euroimmun
N	1033	806	1034	175
Cut-off	1	1.4	12*	0.8*
AUC	0.997 (0.994, 1)	0.994 (0.986, 1)	0.977 (0.961, 0.992)	0.990 (0.980, 1)
Sensitivity	93.7 (89.9, 97.5)	90.2 (85.0, 95.5)	88.6 (83.5, 93.0)	91.3 (84.8, 96.7)
Specificity	99.5 (99.1, 99.9)	99.4 (98.8, 99.9)	98.6 (97.8, 99.3)	98.8 (96.4, 100)
Accuracy	98.6 (98.0, 99.3)	97.9 (96.9, 98.8)	97.1 (96.1, 98.1)	94.9 (91.4, 97.7)
(b) Subset of 175, with values on all 4 assays				
	Roche	Abbott	Diasorin	Euroimmun
N	175	175	175	175
Cut-off	1	1.4	12*	0.8*
AUC	0.999 (0.996, 1)	0.989 (0.976, 1)	0.984 (0.967, 1)	0.990 (0.980, 1)
Sensitivity	93.5 (88.0, 97.8)	89.1 (82.6, 94.6)	87.0 (79.4, 93.5)	91.3 (84.8, 96.7)
Specificity	100 (100, 100)	97.6 (94.0, 100)	97.6 (94.0, 100)	98.8 (96.4, 100)
Accuracy	96.6 (93.7, 98.9)	93.1 (89.1, 96.6)	92.0 (87.4, 95.4)	94.9 (91.4, 97.7)

\* Cut-offs presented for Diasorin and Euroimmun are the equivocal and borderline ranges respectively; positive for Diasorin and Euroimmun is considered ≥15 and ≥1.1 respectively.

at 95 % (all p > 0.15). When specificity was held at 99 %, there was a significant difference in sensitivity only between the Roche and Diasorin assays (p = 0.003).

When assay cutoff values for the Roche and Abbott assays were optimised (1.0 to 0.21 and 1.4 to 0.91, respectively) to achieve 99 % specificity, sensitivity increased for both assays (Table 4).

Twenty-nine samples from 22/158 (14 %) COVID-19 patients were found to be discrepant on ≥1 commercial assays (Table 5). For a family of three COVID-19 cases, one had a positive result only on SA (16–32 dpos), one only on NA (12 dpos) and one with positive results from at least one of SA and NA (17dpos). Falsely negative results were found in 9/29 (31 %), 14/29 (48 %), 14/29 (48 %), and 17/29 (59 %) Euroimmun, Roche, Abbott and Diasorin results respectively. Two patients were only positive by IFA but negative/equivocal on commercial assays (although values for these two patients were above the mean negative value of pre-COVID samples).

Sensitivities by time period are presented in Table 6. Seroconversion occurred by at least 12 dpos on commercial assays (range 5–12dpos). Samples remained positive beyond 180 days in the majority of patients by the Roche (96 %), Diasorin (87 %) and Abbott assays (65 %) (Fig. 1).

The mean cutoff value from the 3 specificity cohorts ranged between 0.08–0.09, 0.06–0.11, 4.3–4.5 and 0.3 (positive cutoff of 1.0, 1.4, 15.0 and 1.1) for Roche, Abbott, Diasorin and Euroimmun assays respectively.

Commercial assays were also compared with 51 positive and 47 negative IFA results with high positive agreement (Diasorin 43/51 (84 %), Abbott 45/51 (88 %), Euroimmun 46/51 (90 %) and Roche 48/51 (94 %)) and negative agreement (Diasorin and Roche 47/47 (100 %) and Abbott and Euroimmun 46/47 (98 %)). From regression analysis after

**Table 4**

Diagnostic performance of 4 assays for detection of COVID19 disease, when specificity is set at 99 %. Values are expressed as percentages (95 % CI).

	Roche	Abbott	Diasorin	Euroimmun
N	1033	806	1034	175
Published Cut-off	1	1.4	12*	0.8*
99 % Specificity Cut-off	0.210	0.91	16.7	1.55
Sensitivity	98.7 (93.7, 100)	94.0 (83.5, 98.5)	86.1 (67.7, 92.4)	83.7 (72.8, 97.8)
Accuracy	99.0 (98.2, 99.2)	98.2 (96.4, 98.9)	97.0 (94.2, 98.0)	91.0 (85.2, 98.4)

\* Cut-offs presented for Diasorin and Euroimmun are the equivocal and borderline ranges respectively; positive for Diasorin and Euroimmun is considered ≥15 and ≥1.1 respectively.

**Table 5**  
Discrepant results in 22 COVID-19 positive patients.

Patient	dpos	Roche value	Roche Cat	Abbott value	Abbott Cat	Euroimmun Cat	Euroimmun Cat	Diasorin value	Diasorin Cat
D3	39	12.3	P	3.25	P	1.35	P	5.86	N
D6	16	1.4	P	3.73	P	0.62	N	4.79	N
D22	33	3.81	P	1.52	P	0.93	EQ	6.33	N
D28	42	2.02	P	1.01	N	0.64	N	6.44	N
D29	41	3.97	P	2.07	P	0.76	N	4.63	N
D33	40	0.223	N	0.53	N	3.1	P	43.4	P
D36	36	59.9	P	6.55	P	1.4	P	9.32	N
D42	41	1.23	P	0.66	N	0.99	EQ	7.57	N
D50	43	1.59	P	0.77	N	0.99	EQ	7.62	N
D70	39	0.0872	N	0.03	N	0.94	EQ	3.8	N
D71	48	23.9	P	3.03	P	0.34	N	71.5	P
D72	57	23.5	P	3.32	P	0.5	N	25.2	P
S12	16	0.166	N	0.6	N	2.262	P	24.9	P
S12	25	0.228	N	0.73	N	4.282	P	31.9	P
S12	32	0.393	N	0.64	N	4.409	P	43.5	P
S13	17	0.792	N	1.86	P	1.705	P	23.3	P
S14	12	1.7	P	2.42	P	0.6665	N	3.96	N
S49	13	1.23	P	0.83	N	0.5651	N	5.98	N
S49	22	0.39	N	0.85	N	1.207	P	9.07	N
S49	29	0.613	N	0.9	N	1.489	P	12.8	EQ
S50	17	0.495	N	1.29	N	2.337	P	20	P
S54	19	0.644	N	2.2	P	2.694	P	36.1	P
S57	23	0.672	N	2.34	P	0.7569	N	6.52	N
S57	31	2.55	P	3.01	P	0.9117	EQ	8.42	N
S57	40	7.15	P	3.49	P	0.8868	EQ	9.14	N
S62	33	6.79	P	3.97	P	1.909	P	11.8	N
S63	15	0.88	N	0.35	N	0.9439	EQ	9.8	N
S63	27	0.752	N	0.42	N	2.57	P	20	P
S72	26	0.665	N	3.27	P	0.7775	N	21.4	P

dpos: days post onset of symptoms; Cat: Categorisation; P: Positive, N: Negative, EQ: Equivocal; Results highlighted in blue indicate samples that were only positive on nucleocapsid-specific assays while those highlighted in yellow indicate samples that were only equivocal/positive on spike-specific assays.

**Table 6**  
Sensitivity of various assays for detection of SARS-CoV-2 antibodies by increasing time intervals; All Positive: positive by at least one assay; dpos: days post onset of symptoms.

dpos	n (%)	All Positive n (%)	Roche n (%)	Abbott n (%)	Euroimmun n (%)	Diasorin n (%)	IFA n (%)
1–7	32 (13)	12/32 (38)	3/32 (9)	4/27 (15)	5/22 (23)	6/32 (19)	–
8–14	23 (9)	15/23 (65)	14/23 (61)	8/19 (42)	5/16 (31)	10/23 (44)	1/1
>14	190 (78)	187/190 (99)	171/190 (90)	154/182 (85)	103/111 (93)	171/190 (90)	–
>28	148 (60)	147/148 (99)	139/148 (94)	122/144 (85)	69/74 (93)	133/148 (90)	72/75 (96)
>60	40 (16)	40/40(100)	39/40 (98)	28/40 (70)	–	36/40 (90)	22/25 (88)
>180	23 (9)	23/23 (100)	22/23 (96)	15/23 (65)	–	20/23 (87)	20/23(87)
<b>Total</b>	<b>245</b>	<b>214/245 (87)</b>	<b>188/245 (77)</b>	<b>168/228 (74)</b>	<b>113/149 (76)</b>	<b>187/245 (76)</b>	<b>–</b>

adjustment for age, there was a stronger association between IFA and SA values (Diasorin:  $R^2 = 59\%$ ; Euroimmun:  $R^2 = 61\%$ ); than between IFA and NA values (Roche:  $R^2 = 10\%$ ; Abbott:  $R^2 = 23\%$ ).

Thirty microneutralisation results from 27 patients were available (range 32–203 dpos). Fourteen patients (52%) had detectable Nab (6/6 samples collected <180 dpos, 9/22 (41%) samples ≥180 dpos). Significantly higher titres were found in those who were hospitalised, while samples collected ≥180 dpos had lower titres ( $p < 0.003$ ). A minority of patients (1/14, by Roche; 2/14 by Diasorin and 3/14 by Abbott) were negative by all commercial assays but had detectable Nab. Increasing assay values only on the SA correlated with presence of Nab. All 8 patients with an assay value >80 AU/mL by Diasorin were found to have detectable Nab with higher values in males than females (Fig. 2).

Eighteen possible two-step algorithms (including optimised sensitivities for the Roche and Abbott assays) were analysed assuming a population prevalence of 1% based on recent data indicating low seroprevalence in our community [28]. PPV after the second assay improved from a range of 0.39–0.65 to ≥0.98 for all combinations. The highest PPV was obtained from the combination of the Roche and Abbott

assays (0.996). Accuracy increased from a range of 0.985–0.994 to ≥0.998. The most accurate combination was the optimised Roche assay followed by the Euroimmun assay (0.999). Calculations for the Roche followed by Diasorin combination are illustrated in Fig. 3.

Twenty pairs of household contacts (HHC) (spouses/partners/family members) providing 54 samples collected within 4 dpos of each other, with range of collection : 7-219 dpos, were matched with 18 pairs of unrelated patients (URP). No significant difference was found between the HHC and URP within-pair assay differences for IFA or the 4 commercial assays, even when this was limited to the subset of patients with probable same exposure (Table 7).

The constructed ISS, evaluated in 62 seropositive patients, ranged from 0 to 17/30 with a mean value of 9. The presence of HIM was more commonly demonstrated in males than females (13/35 vs 2/27). Mean DS was 20 days (range 1 to ≥30 days). ISS was not found to be significantly associated with any serology outcomes. DS showed a significant positive association only with SA ( $p = 0.001$ ) and HIM was significantly positively associated only with IFA and SA (both  $p \leq 0.001$ ). There was a divergence between male and female groups for Diasorin, Euroimmun,

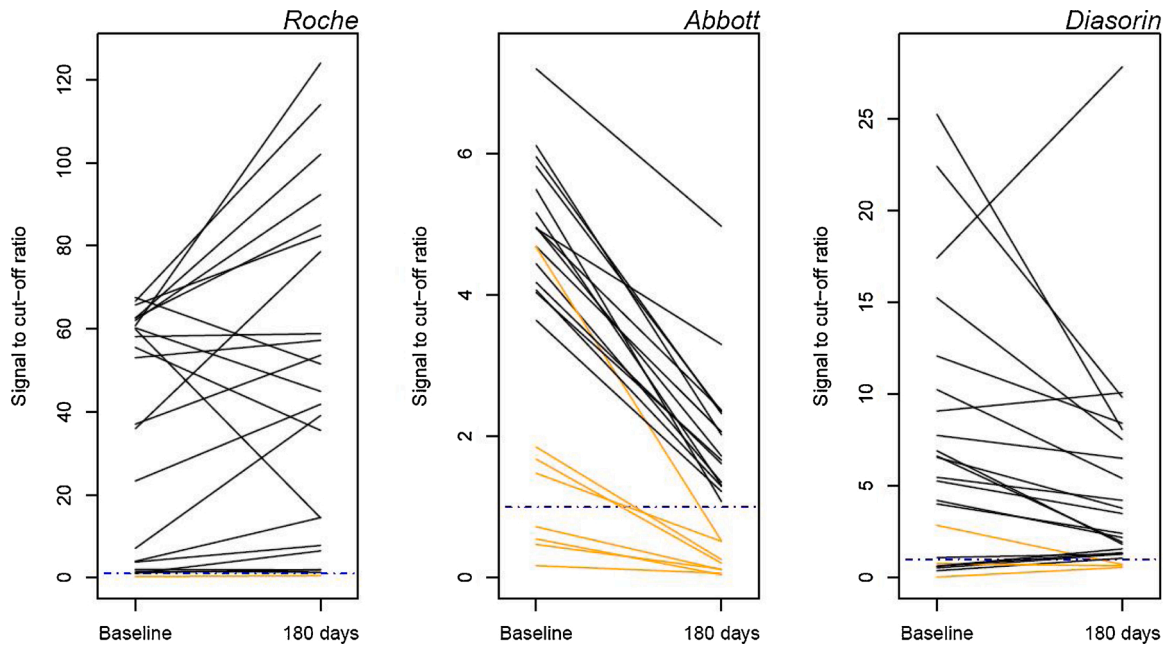


Fig. 1. Trajectory of serology values in patients with samples collected at approximately 42 dpos (31-48) and after 180 dpos (183-219) in 23 patients. The dashed blue line represents the assay threshold for positivity.

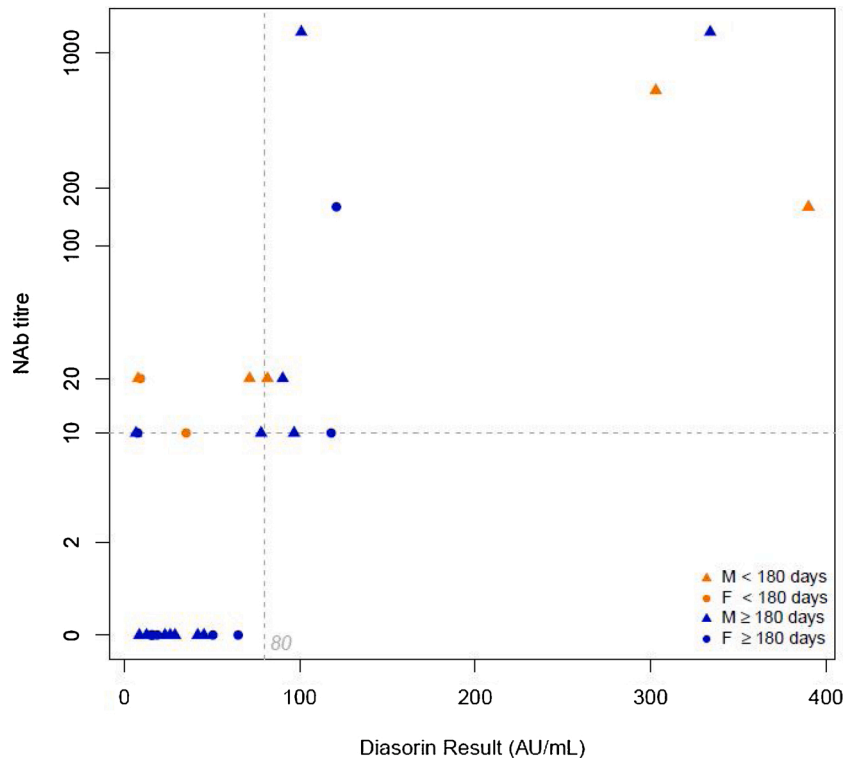


Fig. 2. Correlation of Diasorin assay values with Neutralising antibody titres in 23 patients.

\* One was added to all NAb values to enable the use of a logarithmic scale, however the vertical axis is marked to reflect the original NAb values.

SA and IFA values (Fig. 4). In females, age was found to be significantly positively associated with IFA and all assay values (all  $p \leq 0.004$ ); while HIM, DS and ISS were not. By contrast, in males, age was not associated with any outcome (all  $p > 0.05$ ); HIM showed positive associations only with SA and IFA (both  $p \leq 0.004$ ); and DS and ISS were not significantly associated with any assay outcomes.

Mean/median ratios of SA:NA values for Diasorin:Roche, Diasorin:

Abbott, Euroimmun:Roche and Euroimmun:Abbott were significantly higher in hospitalised compared with non-hospitalised patients and highest in those in ICU (Fig. 5).

#### 4. Discussion

This evaluation found satisfactory performance and comparability of

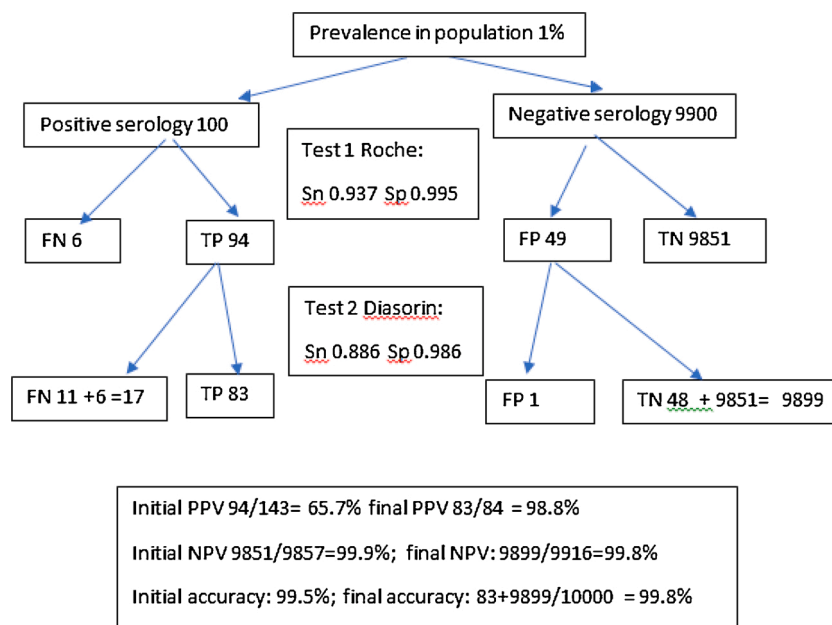


Fig. 3. An example of a 2-step SARS-CoV-2 serology algorithm.

Table 7

Comparison of mean assay difference (diff) between household contact (HHC) pairs and matched unrelated patient pairs (URP); values for continuous variables are presented as mean (standard deviation).

	HHC	HHC (same exposure)	URP	P-value	
				HHC v URP	HHC (same exposure) v URP
Number of patients	40	22	36		
Number of patient pairs	20	11	18		
Number of samples	54	30	36		
% Male	48	41	47		
Age mean	51.6 (17.1)	50.9 (17.0)	51.4 (15.5)	0.952	0.912
dpos diff mean	1.8 (1.6)	1.5 (1.4)	8.0 (6.1)	0.005	0.061
Roche pairs	27	15	18		
Roche mean diff	22.4 (19.6)	22.4 (19.8)	32.9 (28.8)	0.285	0.360
Abbott pairs	27	15	18		
Abbott mean diff	2.3 (2.1)	2.9 (2.4)	2.2 (2.1)	0.839	0.343
Diasorin pairs	27	15	18		
Diasorin mean diff	52.2 (60.1)	50.2 (51.8)	63.7 (63.1)	0.616	0.514
Euroimmun pairs	17	8	9		
Euroimmun mean diff	2.9 (2.0)	2.8 (1.8)	2.6 (2.6)	0.689	0.817

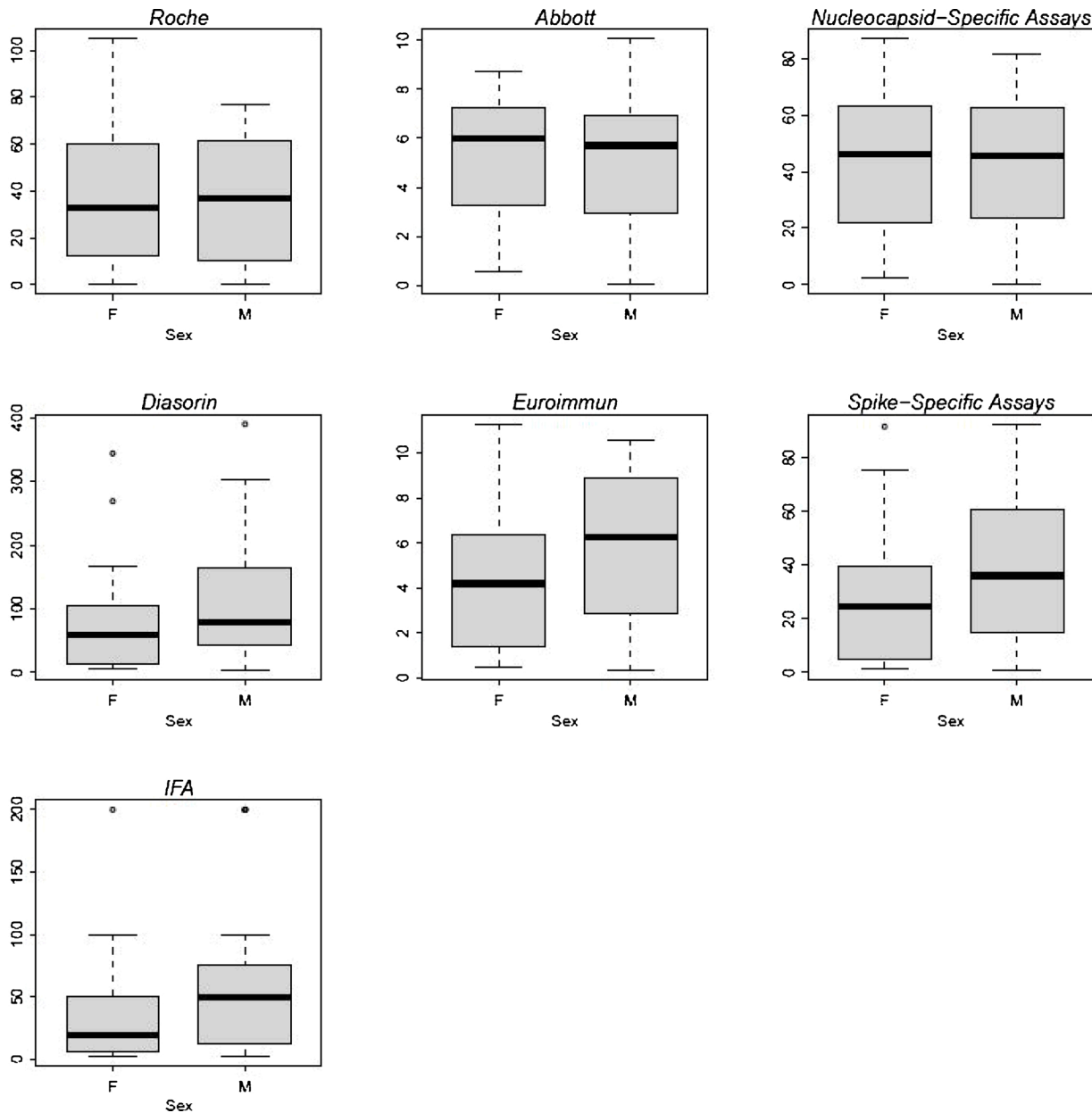
the tested commercial assays. Using all available samples, specificity was >98.5 % (range 98.6–99.5 %), and sensitivity/accuracy was high (range 88.6–93.7 %/ 94.9–98.6 % respectively). Sensitivity/specificity varied by 0.2–1.1 %/0.5–1.8% respectively when analysis was confined to samples that were measured by all 4 assays. The area under the curve (AUC) was >97 % for all assays, indicating high discriminatory power. When analysed by time period, sensitivity increased from a maximum of 61 %<14 dpos to 93 %>14 dpos and 98 %>60 dpos. The positive cases in our dataset experienced predominantly mild to moderate COVID-19 and may explain the reduced sensitivities when compared with those

in the assay IFUs.

Discordant results by commercial assays were found in a minority (14 %) of our patients as has been found in other studies [12,17] and may relate to assay design or immune response. Also of note was the difference in persisting seropositivity between the Abbott and Roche assays on samples ≥180 dpos despite targeting the same nucleocapsid protein in keeping with the indirect ‘sandwich assay’ design of the Roche assay that preferentially detects more mature antibody. This finding supports the use of more than one assay with differing target antigens or design as has been recommended locally and by others [8,29] to allow serological testing to address a variety of clinical and public health questions.

This is also the only currently available study providing large numbers in the Oceania region where COVID-19 case numbers are relatively limited. Reassuringly, the high specificities of the assays found in this study corresponded closely to those reported by the manufacturers (≤0.5 % difference). We found that initial false positive results can be further reduced by adopting a two-step approach utilising a second assay as found by others [21,30]. Only NA (Roche and Abbott) gained sensitivity by lowering of the positive cutoff without a significant reduction in specificity. A two-step algorithm may also increase the detection of low level antibody only detectable by one type of assay. In one of our laboratories, the use of the Roche assay as the initial screening assay has been enhanced by reflexing any results >0.2 cutoff index to the Diasorin assay. In the other laboratory in our network performing serology, positive results on the Diasorin were reflex tested on the Abbott assay. While these two combinations were not the most accurate or provided the highest PPV, they resulted in the most efficient laboratory workflow using assays targeting two different antigens and resulted in a PPV/accuracy of 98.4 %/99.9 % and 99.0 %/99.8 % respectively. In the near future, with the advent of vaccines that largely target the spike protein, there may be a need to differentiate the development of antibodies and immunity following infection (NA + SA positive) or vaccination (SA positive only) providing further reason for utilising assays that target both antigens. However, careful evaluation of the performance of available assays is required to maximise PPV as was found by Ripperger et al. where their in-house nucleocapsid-specific assay was not suitably discriminatory for use in their two-step algorithm [30].

This is also one of only a few studies that have correlated commercial



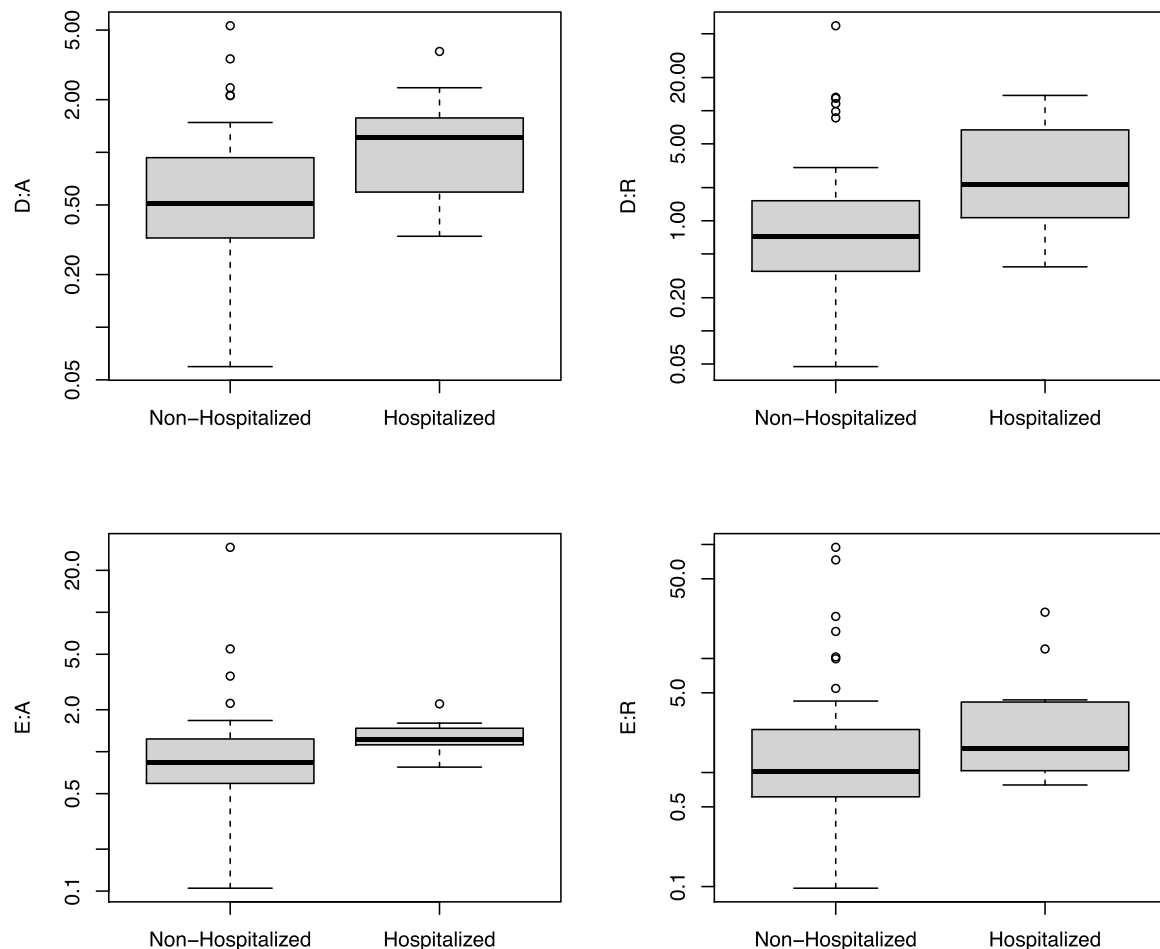
**Fig. 4.** Boxplot of assay values by sex for individual assays and grouped by target antigen (SA and NA) and IFA in the subset of subjects who have an IFA value. 0= female, 1=male.

assay results with Nab and the only one we are aware of comparing it with reference laboratory IFA results [11]. Overall concordance of negative and positive samples with IFA was high (92–97 %) and the high sensitivity of IFA in samples  $\geq 28$  dpos (96 %) was comparable to that reported recently by members of our group (91 % for samples  $\geq 14$  dpos) [22]. Nab, currently considered the best potential correlate with protective immunity, was still detectable in 9/22 (41 %) of those with samples collected more than 6 months post-illness. Nab also corresponded to IFA results and SA values. Taking into account the higher proportion (67 %) of late collected samples ( $\geq 180$  dpos) of predominantly mildly infected patients in our study, we also found a similar proportion of samples with Diasorin values  $> 80$  AU/mL associated with Nab titres of  $\geq 1:160$  (5/9, 56 % vs 87 % in the Diasorin IFU). A negative commercial assay result does not exclude the possibility of detectable Nab and possible protective immunity and caution should be taken when interpreting negative results  $> 6$  months following a compatible illness due to the possibility of false negative serology particularly in

young females where antibody levels were found to be lowest in both SA and IFA.

There are clear differences in mortality between males and females, despite similar incidence of infection, and this may relate to a differing immune response which is reflected in lower antibody levels [31]. While our novel ISS alone did not correlate with assay values and was likely limited by the retrospective design and associated recall and reporting bias, HIM was found to be significantly associated with higher assay values in the IFA and SA in males. Similar findings were found in one large study where higher antibody levels were seen in older and sicker patients while lower levels were seen in females suggesting gender-specific immune responses towards SARS-CoV-2 [32]. In contrast to other studies that showed lower spike:nucleocapsid antibody ratios in hospitalised and deceased patients [33,34], our study found the higher/highest ratios in the hospitalised/intensive care patients compared with those managed in the community. This may relate to both sampling later in the illness and ultimate recovery of patients in our





**Fig. 5.** Boxplots of spike:nucleocapsid median assay values for the different combinations in non-hospitalised vs hospitalised patients; D:A Diasorin:Abbott, D:R Diasorin:Roche, E:A Euroimmun:Abbott, E:R Euroimmun:Roche.

study, with strong spike-specific antibody responses having been shown to correlate with survival [33].

Since greater inoculum of SARS-CoV-2 has been shown in hamster models to lead to more severe disease [35], by comparing assay values from COVID-19 patients of the same household with matched unrelated patients, we postulated that the household group were more likely to have been exposed to a similar inoculum of SARS-CoV-2 from the same source and therefore might return similar assay values. However, this was not found to be the case and may relate to probable varying inoculum despite the same source/setting in addition to differing host factors amongst household members especially those who were genetically unrelated.

Limitations of this study include its restricted demography relating only to Australian patients and the small sample size of patients with long term results ( $\geq 180$  dpos).

In conclusion, we demonstrate that currently available commercial assays perform well with the majority of samples taken more than 6 months following infection still positive with associated neutralising antibody detected in almost half. In low prevalence areas such as Australia, the use of a two-step algorithm increases the PPV of a positive result while maintaining high NPV/accuracy.

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#### Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

#### Declaration of Competing Interest

The authors report no declarations of interest.

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