Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Interim analysis of the clinical performance of five SARS-Cov-2 serology assays

Since its initial outbreak in December 2019, SARS-CoV-2 has undergone rapid spread causing a global pandemic. Accurate diagnostic testing for SARS-CoV-2 remains a challenge limited by the poor sensitivity of currently available tests, which largely rely on viral RNA detection by nucleic acid amplification testing (NAAT) [1]. Additionally, NAAT-based testing takes upwards of 24 h, and commonly longer, to produce a result, impacting infection control measures and resource utilization. Although NAAT remains the most specific test for diagnosing SARS-CoV-2 infection, errors in sample collection or low viral load can lead to false negative results [2]. NAAT may have limited utility for mass screening for determination of disease prevalence in the community or for vaccine evaluation studies. There is a pressing need for an improved diagnostic algorithm to exclude active infection in current and future viral outbreaks. Serologic testing to detect SARS-CoV-2 neutralizing, spike- and nucleocapsid-specific IgG, IgA and IgM antibodies combinations have shown promise but have been tested in limited number of patients with proven infection [3]. We tested five recently available serologic assays in patients with NAAT proven or suspected SARS-CoV-2 infection. The goal of this study was to compare recently available serologic assays using residual samples from NAAT tested patients to assess the sensitivity and specificity of serologic assays and determine the earliest detection point.

We performed head-to-head comparisons, when sample volume and reagents were not limiting, of commercially available COVID-19 Serology immunoassays [4–6] (DiaSorin SARS-CoV-2 S1/S2 IgG on the Liaison XL, EUROIMMUN Anti-SARS-CoV-2 IgA and IgG on the EUROIMMUN Analyzer-1, the manual Epitope Diagnostics Novel Coronavirus COVID-19 IgM, and the Roche Elecsys Anti-SARS-CoV-2 Total Assay on the Cobas e801). As of the preparation of this letter both DiaSorin and Roche have Health Canada approved clinical diagnostic tests for SARS-CoV-2 [7]. 529 Residual plasma samples from 366 NAAT tested individuals (Roche cobas SARS-Cov-2, reference method) were collected, stored frozen at −20 °C, and evaluated for COVID-19 serologic testing. The sensitivity and specificity of the serologic ELISA based kits were determined based on the NAAT reference test overall and at greater than 14 days (Table 1). In addition, serial samples from the NAAT positive cohort were used to determine the earliest detection point in sera for SARS-CoV-2. All samples were tested in duplicate over the entire ELISA plate to evaluate any potential variability.

When comparing the different serological assays (n = 260 were compared across four assays, n = 100 were compared across five assays), we found good positive agreement between the IgG and Total assays with sensitivity results ranging from 81% to 93% in samples greater than 14 days post positive NAAT. Sensitivity improved to > 95% (which is similar to recent publications [8,9]) for the Roche Total, DiaSorin IgG and EUROIMMUN IgG in samples greater than 28 days post positive NAAT (n = 11 to 61). The EUROIMMUN IgA and Epitope IgM assay show high overall sensitivity like the Roche Total assay in this study but they lack the required clinical specificity both demonstrating false positive results COVID naïve samples (EUROIMMUN IgA 4 of 21, Epitope IgM 1 of 25) unlike the Roche Total assay (0 of 46). This analysis shows that the IgG and Total serologic assays will have clinical utility in determining the prevalence of SARS-CoV-2 within the population (those that have been exposed) beyond those that are shown to be NAAT positive (acute phase with viral load). Given the limitations of both testing methods; for NAAT those could include collection errors and variable viral load, and for serological testing, the timing is critical, due to interindividual biological variability in antibody production. Like other studies evaluating the immunological response to SARS-CoV-2 we found two NAAT positive patients negative for IgG and Total antibodies during the convalescent phase of infection up to 47 days post positive NAAT [10–13]. Based on this analysis we feel that serologic testing should be limited to time points greater than 14 days post symptom onset or positive NAAT testing and recognize that some individuals will not have a detectable serological response. There is limited data showing that IgA response may play a role in patients with severe clinical manifestations of SARS-CoV-2 infection and the resulting immune hyperactivation [14,15].

We feel that is important to share the real world evaluation of these commercially available serology assays using the same patient sample sets to verify manufacture claims and compare across assays.

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Table 1
Clinical performance of SARS-CoV-2 serology assays vs NAAT testing.

DiaSorin SARS-CoV-2 S1/S2 IgG Overall							
		Negative	**Positive**	**Borderline***	**Total**	**Specificity**	**Sensitivity**
NAAT	**Negative**	179	4	0	183	98%	66%
	Positive	24	47	0	71		
	Inconclusive		2	0	2		
	Total	203	53	0	256		
Days after Positive NAAT		**Negative**	**Positive**	**Borderline***	**Total**		
≤ 7		16	16	0	32		50%
8 to 14		4	16	0	20		80%
> 14		4	17	0	21		81%
EUROIMMUN Anti-SARS-CoV-2 IgG Overall							
		Negative	**Positive**	**Borderline***	**Total**	**Specificity**	**Sensitivity**
NAAT	**Negative**	194	5	6	205	95%	75%
	Positive	38	112	3	153		
	Inconclusive	0	3	0	3		
	Total	232	120	9	362		
Days after Positive NAAT		**Negative**	**Positive**	**Borderline***	**Total**		
≤ 7		30	26	1	57		47%
8 to 14		4	29	2	35		89%
> 14		4	56	0	60		93%
EUROIMMUN Anti-SARS-CoV-2 IgA Overall							
		Negative	**Positive**	**Borderline***	**Total**	**Specificity**	**Sensitivity**
NAAT	**Negative**	115	34	22	171	67%	90%
	Positive	11	92	6	109		
	Inconclusive	0	3	0	3		
	Total	126	129	28	283		
Days after Positive NAAT		**Negative**	**Positive**	**Borderline***	**Total**		
≤ 7		4	17	2	23		83%
8 to 14		2	25	2	29		93%
> 14		5	50	2	57		91%
Epitope Diagnostics Novel Coronavirus COVID-19 IgM Overall							
		Negative	**Positive**	**Borderline***	**Total**	**Specificity**	**Sensitivity**
NAAT	**Negative**	99	1	3	103	96%	83%
	Positive	24	57	5	86		
	Inconclusive	0	3	0	3		
	Total	123	61	8	192		
Days after Positive NAAT		**Negative**	**Positive**	**Borderline***	**Total**		
≤ 7		19	27	3	49		61%
8 to 14		4	20	2	26		85%
> 14		1	10	0	11		91%
Roche Elecsys® Anti-SARS-CoV-2 (Total Assay) Overall							
		Negative	**Positive**	**Borderline***	**Total**	**Specificity**	**Sensitivity**
NAAT	**Negative**	135	1	0	136	99%	84%
	Positive	32	174	0	206		
	Inconclusive	0	3	0	3		
	Total	167	178	0	345		
Days after Positive NAAT		**Negative**	**Positive**	**Borderline***	**Total**		
≤ 7		12	24	0	36		67%
8 to 14		9	36	0	45		80%
> 14		11	114	0	125		91%

* Borderline = result cannot be clearly classified as positive or negative; borderline results are evaluated as positive for ELISA assays.

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