Title: Evaluation of the clinical performance of seven serological assays for SARS-CoV-2 for use in clinical laboratories

Running head: Evaluation of 7 serological assays for SARS-CoV-2

Authors: Alexis Plaga, Ruhan Wei, Elizabeth Olson, Drew Payto, John Harrington, Pyik That Nwe-Kissig, Michelle Strizzi, Sarah Zilka, Jennifer Ko, Jessica M Colón-Franco

Department of Pathology and Laboratory Medicine, Cleveland Clinic

Corresponding author:

Jessica M Colón-Franco

10300 Carnegie Ave

Cleveland, OH 44195

Telephone: 216-445-2634

Fax: 216-445-0212

Keywords: Serology, SARS-CoV-2, COVID-19, Antibody

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; NAAT, nucleic acid amplification test; POS, post-onset of symptoms; NA, negative agreement; PA, positive agreement; NPV, negative predictive value; PPV, positive predictive value; US Food and Drug Administration, FDA; EUA, Emergency use authorization

ABSTRACT:

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serological assays have emerged as a response to the global pandemic, warranting studies evaluating their clinical performance. This study investigated seven commercially available SARS-CoV-2 serological assays in samples from non-infected individuals and hospitalized patients.

Methods: SARS-CoV-2 qualitative serological assays by Abbott (IgG), Beckman (IgG), DiaSorin (IgG), EUROIMMUN (IgG and IgA), Roche and Bio-Rad (Total) were evaluated using specimens collected pre-December 2019 (n=393), from nucleic acid amplification testing (NAAT) negative patients (n=40), and from 53 patients with COVID-19 by NAAT collected 3-21 days post-onset of symptoms (POS) (N=83). Negative agreement (NA), positive agreement (PA), and positive and negative predictive values (PPV and NPV) at prevalences of 5% and 10% were calculated.

Results:

The overall %NA;95% CI in the negative samples were: Roche 99.8%;99.3-100.2, Beckman 99.8%;98.7-100.0, Abbott and Bio-Rad 99.3%;98.0-99.9, DiaSorin 98.4;97.2-99.6, EUROIMMUN IgG 97.5%;95.5-98.7, and EUROIMMUN IgA 79.7%;75.9-83.5), accounting for positive/equivocal results as false positives. The %PA;95% CI in samples collected 14+days POS (n=24) were: Bio-Rad 83.3%;68.4-98.2, Abbott and Roche 79.2%;62.9-95.4, EUROIMMUN IgA 70.8%;52.6-89.0, Beckman 58.3%;38.6-78.1, DiaSorin 54.2;34.2-74.1, and EUROIMMUN IgG 50.0%;30.0-70.0, accounting for negative/equivocal results as false negatives. NPVs ranged from 97.4-98.9% and 94.7-97.7% for prevalences 5% and 10%, respectively. PPVs ranged from 15.5-94.8% and 27.9-97.4% for prevalences 5% and 10%, respectively.

Conclusion: The Roche and Beckman assays resulted in fewer false positives followed by the Bio-Rad and Abbott assays. While the Bio-Rad assay demonstrated higher antibody detection in

COVID-19-positive patients, PA claims cannot be established with a high level of confidence in our sample population.

Impact statement:

This study evaluated and compared seven commercially available serological assays in three well-categorized cohorts. The data could provide useful insights into the proper utilization of the assays in clinical settings.

INTRODUCTION:

Serological assays for the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), including IgM, IgA, IgG, or total antibodies, are widely available worldwide in a variety of testing formats, making them appealing for clinical and epidemiological use during the ongoing coronavirus disease 2019 (COVID-19) pandemic. However, serological testing has been controversial and there has been confusion about interpretation and applications (1-3). According to several organizations, serology testing is not useful to diagnose acute infection and to establish immunity correlation claims (4, 5). Serological testing may be beneficial in epidemiologic studies, in vaccine development and for verification response, selection of convalescent plasma donors, and to aid in the diagnosis of a subset of patients negative by molecular testing and children with the multisystem inflammatory syndrome (4-6). However, the value of serology testing on patient management decisions is still largely unknown (4, 5) supporting the need to further our understanding of clinical performance and baseline characteristics of serologic assays.

We characterized the clinical performance of seven serologic assays with a focus on specificity in a large cohort of samples collected before 2019 and in samples from nucleic acid amplification testing (NAAT)-negative patients, and in acute disease in samples from hospitalized COVID-19-positive patients.

MATERIALS AND METHODS:

This study was approved by the Cleveland Clinic Foundation Institutional Review Board.

Samples consisted of 84 residual serum/plasma from 53 COVID-19-positive patients by NAAT 3-21 days post-onset of symptoms (POS) (post-test day for 3 with unclear onset), 40 samples

from NAAT-negative pre-surgical patients, and 393 samples collected pre-December 2019 [299 non-crossreactivity: healthy (n=50), pregnant (n=10) and patients with various conditions; 94 cross-reactivity: 29 known serological/autoimmune markers (Supplementary Table 1)]. The assays evaluated were: ARCHITECT SARS-CoV-2 IgG on the Architect (Abbott Diagnostics, Abbott Park, IL, US), Access SARS-CoV-2 IgG on the DxI (Beckman Coulter Diagnostics, Brea, CA, US), LIAISON SARS-CoV-2 S1/S2 IgG (DiaSorin Inc, Stillwater, MN, US) on the Liaison XL, Anti-SARS-CoV-2 ELISA IgG and IgA (EUROIMMUN US Inc, Mountain Lakes, NJ, US) and Platelia SARS-CoV-2 Total Ab (Bio-Rad, Hercules, CA, US) on a QUANTA-Lyser 240 (Inova Diagnostics Inc, San Diego, CA, US), and Elecsys Anti-SARS CoV-2 Total on a cobas 601 (Roche Diagnostics, Indianapolis, IN, US).

Positive agreement (PA) and negative agreement (NA) were calculated relative to NAAT or as negative for the pre-Dec 2019 group. Following the U.S Food and Drug Administration (FDA)'s approach for assays with equivocal results (i.e. Bio-Rad, EUROIMMUN), equivocal results on positive cases were counted as negative for PA, and on negative cases were counted as positive for NA (7); statistics were also calculated in vice versa. Using the former approach, positive and negative predictive values (PPV, NPV) were calculated from the overall %NA and 14+days %PA. Analysis were carried out using © MedCalc software Ltd (Osten, Belgium).

RESULTS

The IgA test had the lowest %NA at 79.7%, while the other assays demonstrated overall %NA of 97.5-99.8% (n=424-433) (Table 1). The %NA was not appreciably different in the pre-December 2019 subcohorts (n=393; 94 in the cross-reactivity cohort). Ninety-seven specimens had

positive/equivocal results by at least 1 assay, 12 by 2 assays (EUROIMMUN IgA, DiaSorin (n=5); EUROIMMUN IgA, EUROIMMUN IgG (n=4)), and 3 by 3 assays (EUROIMMUN IgA, EUROIMMUN IgG, DiaSorin) (Table 1 and data not shown). Only 23 corresponded to the cross-reactivity group (n=94), 14 of which expressed at least 2 known serological/autoimmune makers and 9 expressed more than 2 (Supplementary Table 2). Samples with false positives most commonly expressed EBV IgG, followed by cytomegalovirus IgG, varicella zoster IgG, measles IgG and anti-hepatitis B surface antibody (Supplementary Table 1). Of 10 samples from pregnant women, 3 were positive by the IgA assay, and of 50 samples from healthy individuals, 8 were positive/equivocal by the EUROIMMUN IgA (n=5), EUROIMMUNN IgG (n=2), DiaSorin (n=1) and Bio-Rad (n=1) assays.

Test positivity increased with days POS only for the Abbott, Roche and Bio-Rad assays (Table 2). In samples collected 14-21 days POS (n=24), %PA ranged from 50.0-83.3%. The %PA <60% for the Beckman, DiaSorin and EUROIMMUN IgG assays was unexpected. On days 7-21 (n=60), the Bio-Rad test demonstrated the highest overall %PA. A large seroprevalence study in the US reported variable disease prevalence with most areas still having a prevalence lower than 10% (8). We therefore calculated PVs for 5% and 10% disease prevalence (Table 2). The Roche assay had the highest PPV at prevalences of 5% and 10% (94.8 and 97.4, respectively), and tied NPVs with the Abbott assay, second to the Bio-Rad assay leading with NPVs of 99.1% and 98.2% for the 5% and 10% prevalences, respectively. The IgA assay had an unacceptably low PPV of 27.9% at a 10% prevalence.

DISCUSSION

Except the IgA assay, all assays met the >95% specificity criteria required by the FDA for Emergency Use Authorization (EUA) and in agreement with other studies (9-14). The %PA for these seven assays did not meet the FDA's sensitivity standard of 90% and was lower than reported (9-12). Only the Bio-Rad assay had a %PA above 80% in the 14+days POS samples. It detected one more case than the Roche and Abbott assays and 3 more than the IgA assay, but 6-8 more than the other 3 assays. On samples collected <14 days POS, the Bio-Rad assay detected 2 more cases than the Roche Total assay and 3 more than the IgA assay but 7-10 more than the other assays. Therrien *et al* evaluated these assays on samples collected 2-5 weeks POS (n=60) and found the IgA and Bio-Rad assays performed similarly, with sensitivities of 85%;73-95 and 87%;75-94, respectively (9). They reported performance comparable to our findings for the Roche and Abbott assays but superior %PA for the EUROIMMUN IgG, Beckman and DiaSorin assays ranging from 72-78% (vs 50-58% in our study). In samples collected 14 days+ onset of symptoms, Tang and colleagues reported higher sensitivities by 10 and 14% for the Roche and Abbott assays, respectively, and by almost 40% for the EUROIMMUN IgG assay (10, 11). Other studies also reported higher sensitivities for the Beckman, DiaSorin, and EUROIMMUN IgG assays (15, 16). In the context of many unknown factors in the humoral responses of patients with COVID-19, it is difficult to conclusively determine the reason for the low %PA in our study. An obvious limitation of our study is the low number of samples 14+days POS and only up to 21 days. Moreover, the timing of sample collection was subjectively determined from chart review. The observed differences may be a factor of the heterogeneity of the populations studied and an uncharacterized role of co-morbidities, present in many hospitalized patients with COVID-19, in seroconversion. A phenomenon of seronegative non-responding patients (i.e. negative by several assays) has been described and although it is not well characterized yet, it may represent at least

5% of 14+days POS samples (14). In our study, 4/24 samples (16.7%) did not seroconvert by any assay. Lastly, hospitalization rates vary across these studies from approximately 50 to 67% (9, 16), or was not mentioned (10). Our cohort consisted of hospitalized patients (1-38 days), and 50/53 were symptomatic with mild (n=10), moderate (n=11), critical (n=18), and severe (n=11) disease.

Three strategies are recommended by the CDC to improve PPV (4). First, to use assays with >95% specificity, but preferably >99.5%. The most stringent criteria was only met by the Roche and Beckman assays. Second, to test patients with a high pre-test probability. The third recommendation is orthogonal testing (i.e. testing positive samples using another test preferably with a different format or antigen target). For the IgA assay, 12 samples were also positive on at least another assay targeting the viral spike protein (EUROIMUNN IgG and DiaSorin), but none of the assays targeting the nucleocapsid (Abbott, Roche, Bio-Rad) or the receptor biding domain (Beckman). The IgA assay had the lowest PPV, a concerning number of false-positive results, and should not be used in low prevalence settings. In alignment with a study reporting IgA expression in asymptomatic pregnant women (17), 3 of 10 samples from pregnant women were positive for IgA. Although it has been suggested that IgA appears earlier than IgG in patients with COVID-19 (9, 18), our data and longitudinal samples did not support this conclusion (Table 1 and data not shown). It is important to mention that our study calculated PVs using the results from the 14+ POS cohort, which has a very low sample size, and is not well powered to assess PPVs, as indicated by the large 95% confidence intervals.

We found that the Roche, Bio-Rad and Abbott assays performed better 14+days POS in hospitalized patients. Using 20-40 samples for EUA assay validation may not be sufficient to establish robust %PA claims (19, 20). In some cases, extending collection to >7 weeks POS was needed to yield sensitivities near 90% (9). Nonetheless, serology testing is not recommended for diagnosing acute COVID-19 infection (4, 5). We concluded with high level of confidence that the Roche, Beckman, Abbott and Bio-Rad assays detect few false positives and may be useful in seroprevalence studies.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

A. Plaga, provision of study material or patients; D. Payto, administrative support, provision of study material or patients; E. Olson, administrative support; M. Strizzi, administrative support; J. Ko, administrative support, provision of study material or patients.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: M. Strizzi, The Cleveland Clinic Foundation.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: No sponsor was declared.

References

1. Farnsworth CW, Anderson NW. SARS-CoV-2 serology: much hype, little data. Clin Chem 2020;66:875–7.
2. Andersson M, Low N, French N, Greenhalgh T, Jeffery K, Brent A, Ball J, et al. Rapid roll out of SARS-CoV-2 antibody testing-a concern. BMJ 2020 Jun 24;369:m2420. Epub 2020/06/26 as doi: 10.1136/bmj.m2420.
3. Damluji AA, Christenson RH, deFilippi C. Clinical Application of Serologic Testing for Coronavirus Disease 2019 in Contemporary Cardiovascular Practice. J Am Heart Assoc 2021 Feb 23:e019506. Epub 2021/02/24 as doi: 10.1161/JAHA.120.019506.
4. Centers for Disease Control and Prevention. Interim Guidelines for COVID-19 Antibody Testing. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html. (Accessed December 2020).
5. Hanson KE, Caliendo AM, Arias CA, Englund JA, Lee MJ, Loeb M, Patel R, et al. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19. Clin Infect Dis 2020 Jun 16. Epub 2020/06/20 as doi: 10.1093/cid/ciaa760.
6. Henderson LA, Canna SW, Friedman KG, Gorelik M, Lapidus SK, Bassiri H, Behrens EM, et al. American College of Rheumatology Clinical Guidance for Multisystem Inflammatory Syndrome in Children Associated With SARS-CoV-2 and Hyperinflammation in Pediatric COVID-19: Version 2. Arthritis Rheumatol 2020 Dec 5. Epub 2020/12/06 as doi: 10.1002/art.41616.
7. U.S. Food and Drug Administration. Independent Evaluations of COVID-19 Serological Tests. https://open.fda.gov/apis/device/covid19serology/. (Accessed February 2021).
8. Bajema KL, Wiegand RE, Cuffe K, Patel SV, Iachan R, Lim T, Lee A, et al. Estimated SARS-CoV-2 Seroprevalence in the US as of September 2020. JAMA Intern Med 2020 Nov 24. Epub 2020/11/25 as doi: 10.1001/jamainternmed.2020.7976.
9. Therrien C, Serhir B, Belanger-Collard M, Skrzypczak J, Shank DK, Renaud C, Girouard J, et al. Multicenter Evaluation of the Clinical Performance and the Neutralizing Antibody Activity Prediction Properties of ten high throughput serological assays used in Clinical Laboratories. J Clin Microbiol 2020 Dec 10. Epub 2020/12/12 as doi: 10.1128/JCM.02511-20.
10. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, Farnsworth CW. Clinical performance of two SARS-CoV-2 serologic assays. Clin Chem 2020;66: 1055–62.
11. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, Farnsworth CW. Clinical performance of the Roche SARS-CoV-2 serologic assay. Clin Chem 2020;66: 1107–9.
12. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, Jerome KR, et al. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. J Clin Microbiol 2020 Jul 23;58 8. Epub 2020/05/10 as doi: 10.1128/JCM.00941-20.
13. U.S. Food and Drug Administration. InVitro Diagnostic EUAs. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas. (Accessed February 2021).

14. Oved K, Olmer L, Shemer-Avni Y, Wolf T, Supino-Rosin L, Prajgrod G, Shenhar Y, et al. Multi-center nationwide comparison of seven serology assays reveals a SARS-CoV-2 non-responding seronegative subpopulation. EClinicalMedicine 2020 Dec;29:100651. Epub 2020/11/26 as doi: 10.1016/j.eclinm.2020.100651.
15. Gattinger P, Borochova K, Dorofeeva Y, Henning R, Kiss R, Kratzer B, Muhl B, et al. Antibodies in serum of convalescent patients following mild COVID-19 do not always prevent virus-receptor binding. Allergy 2020 Jul 30. Epub 2020/08/01 as doi: 10.1111/all.14523.
16. Trabaud MA, Icard V, Milon MP, Bal A, Lina B, Escuret V. Comparison of eight commercial, high-throughput, automated or ELISA assays detecting SARS-CoV-2 IgG or total antibody. J Clin Virol 2020 Nov;132:104613. Epub 2020/09/18 as doi: 10.1016/j.jcv.2020.104613.
17. Hausler S, Weigl M, Ambrosch A, Gruber R, Seelbach-Gobel B, Fill Malfertheiner S. Peripartal anti-SARS-CoV-2-IgA/IgG in asymptomatic pregnant women during regional SARS-CoV-2-outbreak. J Perinat Med 2021 Feb 23. Epub 2021/02/26 as doi: 10.1515/jpm-2021-0001.
18. Wheeler SE, Shurin GV, Keetch C, Mitchell G, Kattel G, McBreen J, Shurin MR. Evaluation of SARS-CoV-2 prototype serologic test in hospitalized patients. Clin Biochem 2020 Dec;86:8-14. Epub 2020/08/29 as doi: 10.1016/j.clinbiochem.2020.08.008.
19. Parikh BA, Farnsworth CW. Laboratory evaluation of SARS-CoV-2 in the COVID-19 pandemic. Best Pract Res Clin Rheumatol 2021 Jan 12:101660. Epub 2021/02/02 as doi: 10.1016/j.berh.2021.101660.
20. Theel ES, Couturier MR, Filkins L, Palavecino E, Mitchell S, Campbell S, Pentella M, et al. Application, Verification, and Implementation of SARS-CoV-2 Serologic Assays with Emergency Use Authorization. J Clin Microbiol 2020 Dec 17;59 1. Epub 2020/10/07 as doi: 10.1128/JCM.02148-20.

Table 1: Negative agreement (NA) performance for serological assays

	Assays	Pre-December 2019 %NA (95% CI) Negative/n			NAAT-negative %NA (95% CI)	Overall %NA (95% CI)
		Non cross-reactivity	Cross-reactivity	Total		
Positive = Positive and Equivocal results	Abbott IgG	99.3 (97.6-99.2) 297/299	98.9 (96.7-101.0) 93/94	99.2 (98.4-100.0) 390/393	100.0 (91.2-100.0) 40/40	99.3 (98.0-99.9) 430/433
	Beckman IgG	100.0 (98.7-100.0) 292/292	98.9 (96.8-101.0) 91/92	99.7 (99.2-100.2) 383/384	100.0 (91.2-100.0) 40/40	99.8 (98.7-100.0) 423/424
	DiaSorin IgG	98.7 (96.6-99.6) 294/298	96.8 (93.3-100.4) 91/94	98.5 (96.9-99.5) 385/392	100.0 (91.2-100.0) 40/40	98.4 (97.2-99.6) 425/432
	Roche Total	99.7 (98.2-100.0) 298/299	100.0 (98.8-100.0) 299/299	99.7 (99.2-100.2) 392/393	100.0 (91.2-100.0) 40/40	99.8 (99.3-100.2) 432/433
	Bio-Rad Total	99.3 (97.6-99.9) 297/299	98.9 (96.7-101.0) 93/94	99.2 (98.4-100.0) 390/393	100.0 (91.2-100.0) 40/40	99.3 (98.0-99.9) 430/433
	EU IgG	97.7 (95.2-99.1) 292/299	97.9 (95.0-100.8) 92/94	97.7 (96.9-99.5) 384/393	95.0 (88.2-101.8) 38/40	97.5 (95.5-98.7) 422/433
	EU IgA	80.6 (75.7-84.9) 241/299	84.0 (76.6-91.4) 79/94	81.4 (77.6-85.3) 320/393	62.5 (47.5-77.5) 25/40	79.7 (75.9-83.5) 345/433
Positive = Positive results	Bio-Rad Total	***99.7 (98.2-100.0) 298/299***	98.9 (96.7-101.0) 93/94	***99.5 (98.8-100.2) 391/393***	100.0 (91.2-100.0) 40/40	***99.5 (98.9-100.2) 431/433***
	EU IgG	***98.7 (96.6-99.6) 295/299***	97.9 (95.0-100.8) 92/94	***98.5 (96.7-99.4) 387/393***	95.0 (88.2-101.8) 38/40	***98.2 (96.4-99.2) 425/433***
	EU IgA	***83.3 (78.6-87.3) 249/299***	***88.3 (80.0-94.0) 83/94***	***84.7 (81.2-88.3) 332/393***	***67.5 (53.0-82.0) 27/40***	***82.9 (79.4-86.5) 359/433***

EU, EUROIMMUN; n, sample size; CI, Confidence interval

Table 2: Positive agreement (PA), positive predictive value (PPV) and negative predictive value (NPV) performance for serological assays

	Assays	% Positive Agreement (95% CI) Positive/n				5% Infection Rate (95% CI)		10% Infection Rate (95% CI)	
		Day 3-6	Day 7-10	Day 11-13	Day 14+	PPV, %	NPV, %	PPV, %	NPV, %
Positive = Positive results	Abbott IgG	30.0 (1.6-58.4) 3/10	52.9 (29.2-76.7) 9/17	65.6 (49.2-82.1) 21/32	79.2 (62.9-95.4) 19/24	85.7 (65.7-95.0)	98.9 (97.7-99.5)	92.7 (80.1-97.6)	97.7 (95.2-98.9)
	Beckman IgG	30.0 (1.6-58.4) 3/10	47.1 (23.3-70.8) 8/17	62.5 (45.7-79.2) 20/32	58.3 (38.6-78.1) 14/24	92.9 (64.2-99.0)	97.9 (96.6-98.7)	96.5 (79.1-99.5)	95.6 (93.1-97.2)
	DiaSorin IgG	40.0 (9.6-70.4) 4/10	35.3 (12.6-58.0) 6/17	62.5 (45.7-79.2) 20/32	54.2 (34.2-74.1) 13/24	63.8 (43.6-80.0)	97.9 (96.6-98.7)	78.8 (62.0-89.4)	95.1 (92.6-96.8)
	Roche Total	40.0 (9.6-70.4) 4/10	58.8 (35.4-82.2) 10/17	75.0 (60.0-90.0) 24/32	79.2 (62.9-95.4) 19/24	94.8 (71.6-99.2)	98.9 (97.7-99.5)	97.4 (84.2-99.6)	97.7 (95.2-99.0)
	Bio-Rad Total	40.0 (9.6-70.4) 4/10	64.7 (42.0-87.4) 11/17	78.1 (63.8-92.4) 25/32	83.3 (68.4-98.2) 20/24	86.4 (66.9-95.2)	99.1 (97.9-99.6)	93.0 (81.0-97.7)	98.2 (95.6-99.2)
	EU IgG	30.0 (1.6-58.4) 3/10	35.3 (12.6-58.0) 6/17	65.6 (49.2-82.1) 21/32	50.0 (30.0-70.0) 12/24	61.9 (41.3-78.9)	97.4 (96.2-98.2)	77.4 (59.8-88.8)	94.7 (92.2-96.4)
	EU IgA	40.0 (9.6-70.4) 4/10	52.9 (29.2-76.7) 9/17	75.0 (60.0-90.0) 24/32	70.8 (52.6-89.0) 17/24	15.5 (11.8-20.1)	98.1 (96.5-99.0)	27.9 (22.0-34.7)	96.1 (92.9-97.9)
Positive= Positive and Equivocal results	Bio-Rad Total	***50.0 (19.0-81.0) 5/10***	***76.5 (56.3-96.6) 13/17***	78.1 (63.8-92.4) 25/32	83.3 (68.4-98.2) 20/24				
	EU IgG	30.0 (1.6-58.4) 3/10	***41.2 (17.8-64.6) 7/17***	***71.9 (56.3-87.5) 23/32***	***54.2 (34.2-74.1) 13/24***				
	EU IgA	40.0 (9.6-70.4) 4/10	52.9 (29.2-76.7) 9/17	***78.1 (63.8-92.4) 25/32***	***75.0 (57.7-92.3) 18/24***				

EU, EUUROIMMUN; n, sample size; CI, confidence interval

Bolded data represents the %PA that changed if equivocal results were considered positive.

Predictive values were calculated using the overall %NA including positive and equivocal results as false positives.