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Journal of Clinical Virology 129 (2020) 104512

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation

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ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Serology
IgG
IgA
Neutralisation

ABSTRACT

There is an urgent need for reliable high-throughput serological assays for the management of the ongoing COVID-19 pandemic. Preferably, the performance of serological tests for a novel virus should be determined with clinical specimens against a gold standard, i.e. virus neutralisation. We compared the performance of six commercial immunoassays for the detection of SARS-COV-2 IgG, IgA and IgM antibodies, including four automated assays [Abbott SARS-COV-2 IgG (CE marked), Diasorin Liaison® SARS-COV-2 S1/S2 IgG (research use only, RUO), and Euroimmun SARS-COV-2 IgG and IgA (CE marked)], and two rapid lateral flow (immunocromatographic) tests [Acro Biotech 2019-nCoV IgG/IgM (CE marked) and Xiamen Biotime Biotechnology SARS-COV-2 IgG/IgM (CE marked)] with a microneutralisation test (MNT). Two specimen panels from serum samples sent to Helsinki University Hospital Laboratory (HUSLAB) were compiled: the patient panel (N = 70) included sera from PCR confirmed COVID-19 patients, and the negative panel (N = 81) included sera sent for screening of autoimmune diseases and respiratory virus antibodies in 2018 and 2019. The MNT was carried out for all COVID-19 samples (70 serum samples, 62 individuals) and for 53 samples from the negative panel. Forty-one out of

62 COVID-19 patients showed neutralising antibodies.The specificity and sensitivity values of the commercial tests against MNT, respectively, were as follows: 95.1 %/80.5 % (Abbott Architect SARS-CoV-2 IgG), 94.9 %/43.8 % (Diasorin Liaison SARS-CoV-2 IgG; RUO), 68.3 %/87.8 % (Euroimmun SARS-CoV-2 IgA), 86.6 %/70.7 % (Euroimmun SARS-CoV-2 IgG), 74.4 %/56.1 % (Acro 2019-nCoV IgG), 69.5 %/46.3 % (Acro 2019-nCoV IgM), 97.5 %/71.9 % (Xiamen Biotime SARS-CoV-2 IgG), and 88.8 %/81.3 % (Xiamen Biotime SARS-CoV-2 IgM). This study shows variable performance values. Laboratories should carefully consider their testing process, such as a two-tier approach, in order to optimize the overall performance of SARS- CoV-2 serodiagnostics.

1. Introduction

Serosurveys are considered essential for creating timely snapshots for global and regional public health management of the ongoing COVID-19 pandemic [1]. Thus, there is an urgent need for the development of high-throughput serological assays, which enable population screening, as well as other epidemiological investigations.

Setting up a serological assay for a completely novel pathogen is challenging in many respects. At present, there is inadequate knowledge as to when and what kind of immune response follows SARS-CoV-2 infection [2]. We are also yet to learn about factors that may disturb reliable serology, such as potential cross reaction from seasonal coronaviruses.

The aim of this study was to compare the performance of four automated immunoassays [Abbott SARS-COV-2 IgG (chemiluminescent microparticle immunoassay (CMIA); CE marked), Diasorin Liaison® SARS-CoV-2 S1/S2 IgG (chemiluminescent assay (CLIA); research use only, RUO), Euroimmun SARS-CoV-2 IgG (enzyme linked immunoassay (ELISA); CE marked), and Euroimmun SARS-CoV-2 IgA (enzyme linked immunoassay (ELISA); CE marked)], and two rapid lateral flow (immunocromatographic) tests [Acro Biotech 2019-nCoV IgG/IgM (CE marked) and Xiamen Biotime Biotechnology SARS-CoV-2 IgG/IgM (CE

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https://doi.org/10.1016/j.jcv.2020.104512
Received 7 June 2020; Accepted 14 June 2020

Table 1
Negative serum sample panel consisting of samples collected retrospectively during years 2018-2019, prior the SARS-CoV-2 epidemic.

Number and type of samples (serum)	[a]Abbott, IgG, nucleoprotein antigen (INDEX)	[b]Euroimmun, IgA, S1 antigen (ratio)	[b]Euroimmun, IgG, S1 antigen (ratio)	[c]Liaison, IgG, S1/S2 antigen (AU/mL)	[d]Acro IgG/IgM (x/x), pos or neg	[e]Xiamen Biotime IgG/IgM (x/x), pos or neg	[f]MNT (titer)
Nuclear Ab, pattern (titer)[1] Rf (+/-)[1]							
1 homogeneous (1280), Rf (-)	NEG (0.03)	NEG (0.59)	NEG (0.35)	NEG (0.95)	pos/pos	neg/neg	< 40
2 homogeneous (1280,) Rf (-)	NEG (0.07)	NEG (0.20)	NEG (0.43)	NEG (2.38)	pos/pos	neg/neg	< 40
3 homogeneous (> 5000), Rf(-)	NEG (0.09)	INCONC.(1.05)	NEG (0.31)	INCONC.(13.2)	pos/pos	neg/neg	< 40
4 homogeneous (1280), Rf (-)	NEG (0.31)	NEG (0.54)	NEG (0.58)	NEG (3.02)	pos/neg	neg/neg	< 40
5 homogeneous (> 5000), Rf(-)	NEG (0.06)	NEG (0.15)	INCONC.(0.93)	NEG (5.25)	pos/neg	neg/neg	< 40
6 homogeneous (1280,) Rf (-)	NEG (0.04)	INCONC.(0.99)	NEG (0.44)	NEG (2.56)	neg/neg	neg/neg	< 40
7 homogeneous (1280), Rf (-)	NEG (0.03)	POS (2.45)	POS (1.13)	Invalid result	neg/neg	pos/pos	< 40
8 speckled (> 5000), Rf(-)	NEG (0.13)	NEG (0.39)	NEG (0.31)	NEG (2.25)	neg/neg	neg/neg	< 40
9 speckled (1280), Rf(-)	NEG (0.09)	NEG (0.55)	NEG (0.28)	NEG (3.38)	neg/neg	neg/neg	< 40
10 speckled (> 5000), Rf(-)	NEG (0.03)	POS (1.12)	NEG (0.41)	NEG (2.56)	neg/neg	neg/neg	< 40
11 speckled (1280), **Rf(+)**	NEG (0.06)	NEG (0.69)	NEG (0.61)	NEG (6.91)	neg/neg	neg/neg	< 40
12 speckled (1280), Rf(-)	POS (1.82)	NEG (0.21)	NEG (0.38)	NEG (2.28)	neg/neg	neg/neg	< 40
13 speckled (1280), Rf(-)	NEG (0.04)	INCONC.(0.96)	NEG (0.61)	NEG (3.01)	neg/neg	neg/neg	< 40
14 speckled (> 5000), **Rf(+)**	NEG (0.02)	NEG (0.31)	NEG (0.33)	NEG (4.30)	neg/neg	neg/neg	< 40
15 Centromere + AMA (1280), Rf(-)	NEG (0.02)	NEG (0.15)	NEG (0.29)	NEG (2.06)	neg/neg	neg/neg	< 40
16 centromere (1280), Rf(-)	NEG (0.07)	POS (9.42)	NEG (0.64)	NEG (1.50)	neg/neg	neg/neg	< 40
17 centromere (1280), Rf(-)	NEG (0.01)	INCONC.(1.01)	NEG (0.68)	NEG (3.12)	neg/neg	neg/neg	< 40
18 centromere (1280), Rf(-)	NEG (0.01)	NEG (0.16)	NEG (0.24)	NEG (3.22)	neg/neg	neg/neg	< 40
19 centromere (1280), Rf(-)	NEG (0.02)	NEG (0.07)	NEG (0.23)	POS (35.5)	neg/neg	neg/neg	< 40
20 nucleolar. (80), Rf(-)	NEG (0.02)	NEG (0.47)	NEG (0.28v	NEG (1.28)	pos/neg	neg/neg	< 40
21 speckled (5000) and nuclear dots (1280), Rf(-)	NEG (0.39)	NEG (0.20)	NEG (0.32)	NEG (4.31)	neg/pos	neg/neg	< 40
Phospolipase receptor 2A pos (titer)[1], Rf(+/-)[1]	*20/21 neg*	*14/21 neg*	*19/21 neg*	*18/21 neg*	*14/21 neg*	*20/21 neg*	
1 50, Rf(-)	NEG (0.04)	NEG (0.13)	NEG (0.20	NEG (1.49)	neg/neg	neg/neg	< 40
2 50, Rf(-)	NEG (0.01)	NEG (0.17)	NEG (0.19	NEG (2.43)	pos/pos	neg/neg	< 40
3 50, Rf(-)	NEG (0.06)	NEG (0.11)	NEG (0.22	NEG (0.96)	neg/neg	neg/neg	< 40
4 50, Rf(-)	NEG (0.03)	NEG (0.42)	NEG (0.21	NEG (2.11)	neg/pos	neg/pos	< 40
5 50, Rf(-)	NEG (0.03)	POS (2.06)	NEG (0.37	NEG (3.51)	pos/pos	neg/pos	< 40
6 50, Rf(-)	NEG (0.05)	NEG (0.46)	NEG (0.31	NEG (1.64)	neg/pos	neg/neg	< 40
7 250, Rf(-)	NEG (0.02)	NEG (0.21)	NEG (0.21	NEG (1.49)	neg/neg	neg/neg	< 40
8 50, Rf(-)	NEG (0.01)	NEG (0.30)	NEG (0.41	NEG (0.93)	pos/neg	neg/neg	< 40
9 50, Rf(-)	NEG (0.01)	NEG (0.11	NEG (0.16	NEG (0.27)	pos/neg	neg/neg	< 40
10 50, **Rf(+)**	NEG (0.15)	NEG (0.26	NEG (0.32	NEG (1.69)	neg/neg	neg/neg	< 40
GBM Ab pos (titer)[1], Rf(+/-)[1]	*10/10 neg*	*9/10 neg*	*10/10 neg*	*10/10 neg*	*4/10 neg*	*8/10 neg*	
1 250, **Rf(+)**	NEG (0.04)	NEG (0.18)	NEG (0.24)	NEG (3.02)	neg/neg	neg/neg	< 40
2 250, Rf(-)	NEG (0.04)	NEG (0.19)	NEG (0.32)	Invalid result	neg/neg	neg/neg	< 40
3 50, Rf(-)	NEG (0.14)	NEG (0.35)	NEG (0.23)	NEG (2.50)	neg/pos	neg/neg	< 40
ANCA Ab pos (titer)[1], Rf(+/-)[1]	*3/3 neg*	*3/3 neg*	*3/3 neg*	*2/3 neg*	*2/3 neg*	*3/3 neg*	
1 atypical C-ANCA (50), Rf (-)	NEG (0.10)	NEG (0.69)	NEG (0.45)	NEG (3.73)	neg/neg	neg/neg	< 40
2 C-ANCA (1280), Rf(-)	NEG (0.12)	NEG (0.44)	NEG (0.30)	NEG (4.34)	neg/neg	neg/neg	< 40
3 P-ANCA (200), Rf(-)	NEG (0.03)	NEG (0.28)	NEG (0.24)	Invalid result	neg/neg	neg/neg	< 40
4 C-ANCA (50), P-ANCA (1280), Rf(-)	NEG (0.07)	NEG (0.28)	NEG (0.31)	NEG (2.20)	neg/neg	neg/neg	< 40
5 P-ANCA (200), **Rf(+)**	NEG (0.07)	NEG (0.13)	NEG (0.23)	NEG (3.02)	pos/pos	neg/neg	< 40
Primary EBV infection (IgG, IgM, AVI)[2]	*5/5 neg*	*5/5 neg*	*5/5 neg*	*4/5 neg*	*4/5 neg*	*5/5 neg*	
1 POS, POS, LOW	NEG (0.07)	NEG (0.73)	INCONC.(1.09)	NEG (3.71)	pos/pos	neg/pos	< 40
2 POS, POS, LOW	NEG (0.02)	NEG (0.32)	NEG (0.33)	NEG (1.83)	neg/pos	neg/pos	< 40
3 POS, POS, LOW	NEG (0.09)	INCONC.(0.82)	INCONC.(0.96)	NEG (1.86)	neg/pos	neg/neg	< 40
HCoV samples[3]	*3/3 neg*	*2/3 neg*	*1/3 neg*	*3/3 neg*	*0/3 neg*	*1/3 neg*	
1 HCoV OC43	NEG (0.05)	INCONC.(0.97)	NEG (0.13)	NEG (3.97)	neg/neg	neg/neg	< 40
2 HCoV OC43	NEG (0.02)	NEG (0.09)	NEG (013)	NEG (2.33)	pos/neg	neg/neg	< 40
3 HCoV OC43	NEG (0.05)	NEG (0.25)	NEG (0.17)	NEG (2.39)	neg/neg	neg/neg	< 40
4 HCoV OC43	NEG (0.08)	POS (1.22)	POS (2.54)	NEG (2.20)	neg/neg	neg/neg	< 40
Samples from year 2019[4]	*4/4 neg*	*2/4 neg*	*3/4 neg*	*4/4 neg*	*4/4 neg*	*4/4 neg*	

(*continued on next page*)

Table 1 (*continued*)

Number and type of samples (serum)	[a]Abbott, IgG, nucleoprotein antigen (INDEX)	[b]Euroimmun, IgA, S1 antigen (ratio)	[b]Euroimmun, IgG, S1 antigen (ratio)	[c]Liaison, IgG, S1/S2 antigen (AU/mL)	[d]Acro IgG/IgM (x/x), pos or neg	[e]Xiamen Biotime IgG/IgM (x/x), pos or neg	[f]MNT (titer)
Nuclear Ab, pattern (titer)[1] Rf (+/-)[1]							
1	NEG (0.04)	POS (5.12)	INCONC.(1.07)	NEG (2.19)	neg/neg	neg/neg	< 40
2	NEG (0.02)	NEG (0.23)	NEG (0.16)	POS (17.8)	neg/neg	neg/neg	< 40
3	NEG (0.15)	NEG (0.43)	NEG (0.30)	POS (16.0)	neg/pos	neg/neg	< 40
4	NEG (0.07)	INCONC.(1.07)	INCONC.(0.96)	NEG (3.27)	neg/neg	neg/neg	< 40
5	NEG (0.02)	POS (1.73)	POS (5.71)	NEG (2.44)	neg/pos	neg/pos	< 40
6	NEG (0.03)	POS (1.25)	POS (2.42)	NEG (2.47)	pos/neg	nd/nd	< 40
7	NEG (0.02)	POS (4.51)	POS (1.70)	NEG (1.70)	pos/pos	nd/nd	< 40
8	NEG (0.04)	POS (1.52)	NEG (0.28)	NEG (1.89)	nd/nd	nd/nd	nd
9	NEG (0.11)	NEG (0.23)	NEG (0.35)	NEG (3.73)	nd/nd	nd/nd	nd
10	NEG (0.07)	NEG (0.28)	NEG (0.29)	NEG (3.25)	nd/nd	nd/nd	nd
11	NEG (0.03)	NEG (0.48)	NEG (0.76)	NEG (5.76)	nd/nd	nd/nd	nd
12	NEG (0.01)	NEG (0.25)	NEG (0.29)	NEG (2.11)	nd/nd	nd/nd	nd
13	NEG (0.04)	NEG (0.18)	NEG (0.14)	NEG (1.59)	nd/nd	nd/nd	nd
14	NEG (0.11)	POS (7.96)	NEG (0.60)	NEG (3.02)	nd/nd	nd/nd	nd
15	NEG (0.03)	INCONC.(1.02)	NEG (0.31)	NEG (2.72)	nd/nd	nd/nd	nd
16	NEG (0.02)	NEG (0.09)	NEG (0.25)	NEG (5.60)	nd/nd	nd/nd	nd
17	NEG (0.02)	NEG (0.42)	NEG (0.27)	NEG (1.48)	nd/nd	nd/nd	nd
18	NEG (0.02)	NEG (0.17)	NEG (0.22)	NEG (1.32)	nd/nd	nd/nd	nd
19	NEG (0.02)	NEG (0.56)	NEG (0.23)	NEG (2.13)	nd/nd	nd/nd	nd
20	NEG (0.02)	NEG (0.39)	NEG (0.20)	NEG (2.86)	nd/nd	nd/nd	nd
21	NEG (0.02)	NEG (0.26)	NEG (0.18)	NEG (1.15)	nd/nd	nd/nd	nd
22	NEG (0.02)	NEG (0.77)	NEG (0.18)	NEG (1.00)	nd/nd	nd/nd	nd
23	NEG (0.01)	NEG (0.73)	NEG (0.13)	NEG (1.04)	nd/nd	nd/nd	nd
24	NEG (0.03)	NEG (0.43)	NEG (0.32)	NEG (2.36)	nd/nd	nd/nd	nd
25	POS (2.09)	NEG (0.35)	NEG (0.21)	NEG (1.64)	nd/nd	nd/nd	nd
26	NEG (0.01)	NEG (0.21)	NEG (0.15)	NEG (2.04)	nd/nd	nd/nd	nd
27	NEG (0.10)	POS (6.82)	NEG (0.44)	NEG (1.54)	nd/nd	nd/nd	nd
28	NEG (0.02)	POS (1.52)	NEG (0.71)	NEG (5.75)	nd/nd	nd/nd	nd
29	NEG (0.04)	POS (1.80)	NEG (0.21)	NEG (5.41)	nd/nd	nd/nd	nd
30	NEG (0.05)	NEG (0.61)	NEG (0.30)	NEG (2.69)	nd/nd	nd/nd	nd
31	NEG (0.01)	NEG (0.75)	NEG (0.30)	NEG (2.26)	nd/nd	nd/nd	nd
32	NEG (0.01)	NEG (0.16)	NEG (0.21)	NEG (1.28)	nd/nd	nd/nd	nd
33	NEG (0.03)	NEG (0.77)	NEG (0.29)	NEG (2.87)	nd/nd	nd/nd	nd
34	NEG (0.01)	NEG (0.08)	NEG (0.14)	NEG (0.73)	nd/nd	nd/nd	nd
35	NEG (0.04)	NEG (0.23)	NEG (0.29)	NEG (1.70)	nd/nd	nd/nd	nd
	34/35 neg	*24/35 neg*	*30/35 neg*	*33/35 neg*	*3/7 neg*	*4/5 neg*	
Specificity %	97.5 %	75.3 %	87.7 %	91.4 %	30/53 neg	45/51 neg	
Assay process successful % of samples	100 %	100 %	100 %	96.3 %			

Neg, negative; pos, positive; inconc., inconclusive; nd, not determined; Rf (-), rheumatoid factor negative; Rf(+), rheumatoid factor positive; PLA2R, phospolipase A2 receptor; GBM, glomerular basement membrane; ANCA, antineutrophil cytoplasmic antibodies; EBV, Epstein-Barr virus; MNT, microneutralisation assay.

[1] Nuclear, phospholipase A2 receptor (PLA2R), glomerular basement membrane (GBM), antineutrophil cytoplasmic (ANCA) antibodies were detected using immunofluorescence assays of NOVA Lite® DAPI ANA Kit (Inova Diagnostics, California, USA), Anti-Phospholipase A2 Receptor IIFT (IgG) (Euroimmun, Lübeck, Germany), EUROPLUS kidney (monkey) and GBM antigen IIFT (Euroimmun, Lübeck, Germany) and NOVA Lite® ANCA IFA Kit (Inova Diagnostics, California, USA), respectively. Rheumatoid factors (Rf) were determined using RapiTex® RF (Siemens Healthcare Diagnostics, Erlangen, Germany).

[2] EBV IgG and IgM were determined using Enzygnost Anti-EBV/IgG and Anti-EBV/IgM II (Siemens Healthcare Diagnostics, Erlangen, Germany).

[3] HCoV were detected using xTAG® Respiratory Viral panel kit (Luminex Corporation, Texas, USA) from nasopharyngeal samples and corresponding serum samples taken from the patient were used for testing antibodies.

[4] All serum samples were sent for antibody testing of influenza A, B, respiratory syncytial virus, parainfluenza virus, enterovirus IgG antibodies (HUSLAB, Finland) in 2019.

[a] Architect SARS-CoV-2 IgG Assay (Abbott, Illinois, USA).

[b] Anti-SARS-CoV-2 IgA and IgG EIA (Euroimmun, Lübeck, Germany).

[c] LIAISON® SARS-CoV-2 IgG (DiaSorin, Saluggia, Italy).

[d] 2019-nCoV IgG/IgM Rapid Test Cassette (Acro Biotech, California, USA).

[e] SARS-CoV-2 IgG/IgM Rapid Test (Xiamen Biotime, Fujian, China).

[f] Microneutralisation assay were carried out according protocol described by Haveri et al. (2020).

marked)] with a SARS-CoV-2 microneutralisation test (MNT) by using clinical serum specimens.

2. Materials and methods

The patient samples consisted of serum specimens sent to the Department of Virology and Immunology, Helsinki University Hospital Laboratory, Finland for diagnostic purposes. A subset of these specimens has been included in a previous publication evaluating the Euroimmun SARS-CoV-2 IgG and IgA assays, and are included here for comparison [3].

3. Serum samples comprising the negative panel

The negative panel consisted of 81 serum samples (from 81 individuals) (median age 64 years, range 2–89 years; 33 males, 48 females) (Table 1). All of these samples originated from 2018 – 2019, i.e. before the circulation of SARS-CoV-2 in Europe.

Thirty-nine out of 81 samples contained autoantibodies: 21 had anti-nuclear antibodies in Hep-2 cell IFA analysis [NOVA Lite® DAPI

Table 2
a and b. Days after onset of symptoms and results from PCR-confirmed COVID-19 patients using microneutralisation test and Abbott SARS-CoV-2 IgG, Euroimmun SARS-CoV-2 IgA, Euroimmun SARS-CoV-2 IgG and LiaisonSARS-CoV-2 IgG (Diasorin) automated immunoassays (a) and two lateral flow rapid tests of Acro and Xiamen Biotime aimed for detection of IgG and IgM antibodies (b).

a

Days after onset of symptoms	ID No	Severity	MNT (titer)	Abbott IgG CMIA (N ag; index)	EIM IgA ELISA (S1 ag; ratio)	EIM IgG ELISA (S1 ag; ratio)	Liaison IgG CLIA (S1/S2 ag; AU/mL)
1 - 5	1	Mild	< 40	neg (0.02)	neg (0.54)	neg (0.26)	neg (5.15)
	2	Mild	< 40	neg (0.03)	neg (0.36)	neg (0.34)	neg (2.16)
	4	Mild	< 40	neg (0.02)	neg (0.43)	neg (0.35)	neg (1.91)
	48	Mild	< 40	neg (0.05)	neg (0.3)	neg (0.31)	neg (1.56)
	6	Mild	< 40	neg (0.04)	neg (0.41)	neg (0.35)	neg (2.4)
	46	Severe	< 40	neg (0.02)	neg (0.73)	neg (0.19)	neg (1.75)
	3 − 1	Moderate	< 40	neg (0.02)	eq (0.8)	neg (0.26)	neg (1.95)
	51	Moderate	< 40	neg (0.02)	eq (0.82)	neg (0.47)	neg (2.94)
	7 − 1	Moderate	< 40	neg (0.06)	eq (0.98)	neg (0.3)	neg (2.42)
	39	Severe	< 40	neg (0.02)	eq (0.19)	neg (0.16)	neg (11.7)
	36	Severe	80	pos (5.63)	pos (4.43)	pos (2.89)	neg (11.6)
	50	Moderate	160	pos (5.27)	pos (3.96)	pos (6.22)	pos (22.8)
6 -14	10 − 1	Moderate	< 40	neg (0.03)	neg (0.52)	neg (0.40)	neg (1.28)
	26	Severe	< 40	neg (0.01)	neg (0.39)	neg (0.16)	neg (1.43)
	24	Severe	< 40	neg (0.05)	neg (0.19)	neg (0.19)	neg (1.83)
	38	Severe	< 40	neg (0.07)	neg (0.78)	neg (0.25)	neg (2.95)
	43	Severe	< 40	neg (0.04)	neg (0.62)	neg (0.22)	neg (3.56)
	32	Severe	< 40	neg (0.07)	neg (0.39)	neg (0.16)	neg (5.08)
	3 − 2	Moderate	< 40	neg (0.02)	eq (0.92)	neg (0.21)	neg (1.61)
	20	Mild	< 40	neg (0.02)	pos (4.00)	neg (0.79)	neg (1.18)
	8 − 1	Moderate	< 40	neg (0.09)	pos (2.29)	neg (0.56)	neg (3.27)
	44 − 1	Moderate	< 40	neg (0.22)	pos (1.80)	neg (0.32)	neg (4.65)
	7 − 2	Moderate	< 40	neg (0.07)	pos (1.24)	neg (0.26)	neg (2.63)
	13	Severe	< 40	neg (0.03)	pos (2.24)	neg (0.25)	neg (7.59)
	42	Moderate	< 40	pos (1.93)	pos (1.56)	neg (0.33)	neg (2.23)
	29	Moderate	40	neg (0.06)	neg (0.17)	neg (0.28)	neg (7.78)
	40	Moderate	40	neg (0.72)	neg (0.75)	eq (1.06)	neg (2.60)
	25 − 1	Moderate	40	neg (1.22)	pos (1.31)	neg (0.49)	neg (5.72)
	28	Severe	40	neg (1.11)	pos (1.74)	neg (0.32)	neg (10)
	14	Moderate	40	neg (0.45)	pos (4.02)	neg (0.46)	neg (7.59)
	15	Severe	80	pos (1.64)	neg (0.67)	neg (0.34)	neg (3.17)
	49	Severe	80	pos (2.48)	pos (3.85)	neg (0.78)	neg (1.97)
	22	Moderate	80	pos (7.48)	pos (1.88)	neg (0.64)	neg (3.43)
	62	Mild	80	pos (6.48)	pos (1.47)	neg (0.50)	ND
	47	Severe	80	pos (2.23)	pos (4.14)	pos (1.33)	neg (8.60)
	16	Moderate	80	pos (5.69)	pos (4.86)	pos (1.50)	neg (3.76)
	8 − 2	Moderate	80	pos (3.12)	pos (6.90)	pos (2.01)	eq (13.1)
	30	Severe	160	neg (0.24)	neg (0.53)	neg (0.21)	neg (2.61)
	10 − 2	Moderate	160	pos (2.04)	pos (3.42)	neg (0.55)	eq (12.4)
	18	Severe	160	pos (1.52)	pos (8.66)	eq (0.84)	neg (5.17)
	23	Severe	160	pos (5.05)	pos (3.11)	pos (1.62)	neg (11.8)
	21	Moderate	160	pos (5.39)	pos (3.50)	pos (1.54)	neg (5.34)
	35	Severe	160	pos (4.15)	pos (10.51)	pos (5.96)	eq (14.8)
	34	Moderate	160	pos (7.91)	pos (4.19)	pos (1.68)	pos (6.18)
	44 − 2	Moderate	160	pos (4.75)	pos (30.12)	pos (1.90)	pos (43.1)
	31	Severe	160	pos (7.27)	pos (5.39)	pos (1.3)	pos (20.0)
	53	Moderate	320	pos (8.57)	pos (28.17)	pos (10.84)	pos (51.4)
	25 − 2	Moderate	320	pos (6.03)	pos (5.73)	pos (5.24)	pos (22.8)
	12	Moderate	640	neg (0.97)	pos (6.37)	neg (0.44)	neg (4.29)
	27	Mild	> 2560	pos (4.74)	pos (10.9)	pos (1.84)	pos (40.2)
15 - 21	52	Moderate	< 40	neg (0.17)	pos (1.43)	neg (0.42)	neg (2.92)
	5 − 1	Mild	< 40	pos (1.74)	pos (4.26)	neg (0.75)	neg (9.91)
	5 − 2	Mild	< 40	pos (2.39)	pos (3.19)	neg (0.60)	neg (5.28)
	37 − 1	Moderate	320	pos (4.00)	pos (31.11)	pos (2.42)	pos (45.3)
	19	Mild	1280	pos (2.57)	pos (31.11)	pos (10.09)	pos (42.0)
	33	Severe	> 2560	pos (7.92)	pos (31.11)	pos (13.41)	pos (52.4)
> 21	57	Mild	80	pos (4.01)	pos (8.95)	pos (6.60)	ND
	58	Mild	80	pos (3.38)	pos (5.48)	pos (8.08)	ND
	61	Mild	160	pos (9.27)	pos (2.93)	pos (12.49)	ND
	37 − 2	Moderate	320	pos (6.13)	pos (31.11)	pos (9.22)	pos (78.2)
	59	Mild	320	pos (4.68)	pos (4.93)	pos (11.44)	ND
	60	Severe	640	pos (6.49)	pos (30.22)	pos (14.58)	ND
	56	Mild	1280	pos (8.26)	pos (30.22)	pos (15.07)	ND

(*continued on next page*)

Table 2 (*continued*)

a

Days after onset of symptoms	ID No	Severity	MNT (titer)	Abbott IgG CMIA (N ag; index)	EIM IgA ELISA (S1 ag; ratio)	EIM IgG ELISA (S1 ag; ratio)	Liaison IgG CLIA (S1/S2 ag; AU/mL)
NA	45	Moderate	< 40	neg (0.01)	neg (0.67)	neg (0.15)	neg (1.79)
	17	NA	< 40	neg (0.02)	neg (0.19)	neg (0.19)	neg (2.04)
	9	NA	< 40	neg (0.06)	neg (0.51)	eq (0.85)	neg (2.54)
	41	NA	80	pos (2.69)	pos (6.27)	eq (0.97)	pos (27.3)
	55	NA	80	pos (2.07)	pos (6.59)	pos (4.46)	ND
	11	NA	160	neg (0.81)	pos (2.49)	neg (0.47)	neg (1.97)
	54	Mild	640	pos (8.10)	pos (5.95)	pos (14.45)	ND

b

Days after onset of symptoms	ID No	Severity	MNT (titer)	Acro IgG	Acro IgM	Xiamen Biotime IgG	Xiamen Biotime IgM
1 - 5	1	Mild	< 40	neg	neg	neg	neg
	2	Mild	< 40	neg	neg	neg	neg
	4	Mild	< 40	pos	neg	neg	neg
	48	Mild	< 40	neg	neg	neg	neg
	6	Mild	< 40	neg	neg	neg	neg
	46	Severe	< 40	neg	pos	neg	neg
	3 − 1*	Moderate	< 40	neg	neg	neg	neg
	51	Moderate	< 40	neg	neg	neg	neg
	7 − 1*	Moderate	< 40	neg	neg	neg	neg
	39	Severe	< 40	neg	neg	neg	neg
	36	Severe	80	pos	pos	pos	pos
	50	Moderate	160	neg	neg	pos	pos
6 -14	10 − 1*	Moderate	< 40	neg	neg	neg	neg
	26	Severe	< 40	neg	pos	neg	neg
	24	Severe	< 40	neg	neg	neg	neg
	38	Severe	< 40	neg	pos	neg	neg
	43	Severe	< 40	neg	pos	neg	neg
	32	Severe	< 40	neg	neg	neg	neg
	3 − 2*	Moderate	< 40	neg	pos	neg	neg
	20	Mild	< 40	pos	pos	neg	pos
	8 − 1*	Moderate	< 40	neg	neg	neg	neg
	44 − 1*	Moderate	< 40	pos	pos	pos	pos
	7 − 2*	Moderate	< 40	neg	neg	neg	neg
	13	Severe	< 40	neg	pos	neg	neg
	42	Moderate	< 40	pos	neg	pos	pos
	29	Moderate	40	neg	neg	neg	neg
	40	Moderate	40	neg	neg	neg	neg
	25 − 1*	Moderate	40	pos	pos	neg	pos
	28	Severe	40	neg	neg	neg	pos
	14	Moderate	40	neg	pos	neg	pos
	15	Severe	80	pos	pos	pos	pos
	49	Severe	80	pos	pos	pos	pos
	22	Moderate	80	pos	neg	pos	pos
	62	Mild	80	neg	neg	ND	ND
	47	Severe	80	pos	pos	pos	pos
	16	Moderate	80	pos	pos	pos	pos
	8 − 2*	Moderate	80	pos	neg	neg	neg
	30	Severe	160	neg	neg	neg	neg
	10 − 2*	Moderate	160	pos	pos	pos	pos
	18	Severe	160	pos	pos	neg	pos
	23	Severe	160	neg	pos	pos	pos
	21	Moderate	160	pos	pos	pos	pos
	35	Severe	160	pos	pos	pos	pos
	34	Moderate	160	neg	neg	pos	pos
	44 − 2*	Moderate	160	pos	pos	pos	pos
	31	Severe	160	pos	pos	pos	pos
	53	Moderate	320	neg	neg	pos	neg
	25 − 2*	Moderate	320	pos	pos	pos	pos
	12	Moderate	640	pos	neg	neg	neg
	27	Mild	> 2560	neg	pos	pos	pos
15 - 21	52	Moderate	< 40	neg	neg	neg	neg
	5 − 1*	Mild	< 40	pos	pos	neg	pos
	5 − 2*	Mild	< 40	pos	pos	neg	neg
	37 − 1*	Moderate	320	neg	neg	pos	pos
	19	Mild	1280	neg	pos	pos	pos
	33	Severe	> 2560	pos	pos	pos	pos

(*continued on next page*)

Table 2 (*continued*)

b

Days after onset of symptoms	ID No	Severity	MNT (titer)	Acro IgG	Acro IgM	Xiamen Biotime IgG	Xiamen Biotime IgM
> 21	57	Mild	80	pos	neg	ND	ND
	58	Mild	80	pos	neg	ND	ND
	61	Mild	160	neg	neg	ND	ND
	37 – 2*	Moderate	320	neg	neg	pos	pos
	59	Mild	320	neg	neg	ND	ND
	60	Severe	640	pos	neg	ND	ND
	56	Mild	1280	neg	neg	ND	ND
NA	45	Moderate	< 40	neg	neg	neg	neg
	17	NA	< 40	neg	neg	neg	neg
	9	NA	< 40	pos	neg	neg	neg
	41	NA	80	pos	neg	pos	pos
	55	NA	80	pos	neg	ND	ND
	11	NA	160	pos	pos	pos	pos
	54	Mild	640	neg	neg	ND	ND

ND, not determined due to the limitation of tests available; NA, not available; MNT, microneutralisation test; eq, equivocal; pos, positive; neg, negative.
a) All results were determined according to manufacturers´ instructions. In this study, the Liaison SARS-CoV-2 IgG chemiluminescent assay (CLIA; Diasorin) was research use only (RUO) kit. Abbott SARS-CoV-2 IgG chemiluminescent microparticle immunoassay (CMIA), Euroimmun (EIM) SARS-CoV-2 IgA and IgG enzyme linked immunoassay (ELISA) were all CE marked kits.
b) All results were determined according to manufacturers´ instructions. Acro IgG/IgM rapid lateral flow test and Xiamen Biotime IgG/IgM rapid lateral flow test are both CE marked kits.
* Two separate samples were available from the patients. First number is the identification code for patient, second for the sample; examples, 3 – 1 and 3 – 2, 5 – 1 and 5 – 2.

ANA Kit, Inova Diagnostics, California, USA; staining patterns: homogenous, 7/21; speckled 7/21; centromere 4/21; centromere + antimitochondrial antibodies 1/21; nucleolar 1/21; speckled and nuclear dots 1/21], 10 were positive for phospholipase receptor A2 (PLA2R) antibodies (Anti-Phospholipase A2 Receptor IIFT (IgG) (Euroimmun, Lübeck, Germany), three for glomerular basement membrane (GBM) antibodies (EUROPLUS kidney (monkey) and GBM antigen IIFT, Euroimmun, Lübeck, Germany), and five for antineutrophil cytoplasmic (ANCA) antibodies (NOVA Lite® ANCA IFA Kit (Inova Diagnostics, California, USA) (Table 1). Presence of rheumatoid factor was tested for these 39 samples using RapiTex® RF (Siemens Healthcare Diagnostics, Erlangen, Germany) agglutination assay.

Three serum samples were from patients with primary Epstein-Barr virus infection (mononucleosis; EBV IgG and IgM were determined using Enzygnost Anti-EBV/IgG and Anti-EBV/IgM II kits (Siemens Healthcare Diagnostics, Erlangen, Germany)), four were from patients who had an ongoing Human coronavirus (HCoV) OC43 infection. HCoVs were detected using xTAG® Respiratory Viral panel kit (Luminex Corporation, Texas, USA) from nasopharyngeal samples and corresponding serum samples were collected and used for testing antibodies. In addition, 35 were serum samples originally sent for testing of respiratory virus antibodies (Helsinki University Hospital Laboratory, HUSLAB, Helsinki, Finland; Table 1).

4. Serum samples comprising the COVID-19 patient panel

The patient panel consisted of serum samples from coronavirus 19 disease (COVID-19) patients, who had been diagnosed by PCR-based methods from nasopharyngeal samples in our laboratory (Table 2). For molecular testing, three different methods were used: cobas® SARS-CoV-2 test on the Cobas® 6800 system (Roche Diagnostics, Basel, Switzerland), Amplidiag® COVID-19 test (Mobidiag, Espoo, Finland) and a protocol based on Corman et al. [2020; [4]].

In total, 70 serum samples from 62 individuals (median age 54 years, range 24–86 years; 28 males, 34 females; Table 2) were available for this study. Data were collected and samples treated according to permit HUS/32/2018 (Helsinki University Hospital, Finland).

5. Automated immunoassays for anti-SARS-CoV-2 IgG or IgA detection

The analysis of SARS-COV-2 IgG or IgA antibodies were carried out using the Architect Plus i2000sr Analyzer (Abbott, Illinois, USA) and SARS-COV-2 IgG CMIA kit (nucleoprotein based antigen; Abbott; CE marked), EUROLabworkstation (Euroimmun, Lübeck, Germany) and SARS-COV-2 IgG and IgA ELISA kits (S1-based antigen; Euroimmun) and Diasorin Liaison® XL (DiaSorin, Saluggia, Italy) and SARS-CoV-2 S1/S2 IgG CLIA kit (S1/S2 based antigen; DiaSorin; RUO) according to the manufacturers´ instructions. All samples from the negative panel (N = 81) and the patient panel (N = 70) were tested with Abbott SARS-CoV-2 IgG, and Euroimmun SARS-CoV-2 IgA and IgG. All samples from the negative panel (N = 81) and (due to limited kit supply) 61/70 samples (53/62 individuals) from the COVID-19 patient panel were tested with DiaSorin SARS-CoV-2 S1/S2 IgG.

6. Rapid lateral flow tests

2019-nCoV IgG/IgM (Acro Biotech, California, USA; CE marked)] and SARS-CoV-2 IgG/IgM (Xiamen Biotime Biotechnology, Fujian, China; CE marked) rapid lateral flow (immunocromatographic) tests were evaluated. Altogether, 53/81 samples from the negative panel and all 70 specimens from the patient panel were tested with Acro Biotech, and 51/81 samples from the negative panel and 61/70 from the patient panel were tested with Xiamen Biotime.

7. Microneutralisation test

MNT was conducted for 53/81 of the negative panel and all of the 70 specimens from the COVID-19 patient panel (Tables 1 and 2). Microneutralisation assays were carried out for 39 (39/53) samples positive for autoantibodies, three (3/53) samples from patients with primary EBV infection, four (4/53) samples from patients with acute HCoV OC43 infection, and seven (7/53) samples which had been sent for testing of respiratory virus antibodies.

MNT was performed in a BSL-3 laboratory as described previously [5] with modifications. Briefly, SARS-CoV-2/Finland/1/2020 was passaged five times in Vero E6 cells in MEM supplemented with 2% of

Table 3
Specificity and sensitivity of the six commercial immunoassays compared with MNT. MNT titer ≥40 was considered as positive. Equivocal results of the commercial assays were regarded as reactive in this analysis. The total number of specimens tested with MNT, and each of the commercial immunoassays, with their respective results are presented in Tables 1–2.

Immunoassay, platform, antigen used, RUO/CE marked	Immunoassay qualitative test result	Number of specimens		Specificity (compared with MNT)	Sensitivity (compared with MNT)
		MNT titer < 40	MNT titer ≥40		
Abbott SARS-CoV-2 IgG CMIA Based on nucleoprotein antigen CE marked	pos	4	33	95.1 %	80.5 %
	neg	78	8		
	Total	*82*	*41*		
Euroimmun SARS-CoV-2 IgA ELISA Based on S1 antigen CE marked	pos	18	36	68.3 %	87.8 %
	eq	8	0		
	neg	56	5		
	Total	*82*	*41*		
Euroimmun SARS-CoV-2 IgG ELISA Based on S1 antigen CE marked	pos	5	26	86.6 %	70.7 %
	eq	6	3		
	neg	71	12		
	Total	*82*	*41*		
Liaison SARS-CoV-2 IgG CLIA Based on S1/S2 antigen Research Use Only (RUO)	pos	3	11	94.9 %	43.8 %
	eq	1	3		
	neg	75	18		
	*Total**	*79*	*32*		
Acro Biotech, 2019-nCoV IgG, lateral flow rapid test No antigen information provided CE marked	pos	21	23	74.4 %	56.1 %
	neg	61	18		
	Total	*82*	*41*		
Acro Biotech, 2019-nCoV IgM, lateral flow rapid test No antigen information provided CE marked	pos	25	19	69.5 %	46.3 %
	neg	57	22		
	Total	*82*	*41*		
Xiamen Biotime, SARS-CoV-2 IgG, lateral flow rapid test No antigen information provided CE marked	pos	2	23	97.5 %	71.9 %
	neg	78	9		
	*Total**	*80*	*32*		
Xiamen Biotime, SARS-CoV-2 IgM, lateral flow rapid test No antigen information provided CE marked	pos	9	26	88.8 %	81.3 %
	neg	71	6		
	*Total**	*80*	*32*		

Pos, positive; neg, negative; eq, equivocal; RUO, research use only; MNT, microneutralisation test.
* Due to limited kit supply, not all specimens tested with MNT could be analysed with these commercial tests.

heat-inactivated FBS, L-glutamine, penicillin and streptomycin. The infectious virus titer was determined by plaque assay in Vero E6 cells. For MNT, Vero E6 cells (50 000/well) were plated the previous day on 96-well plate in MEM with 10 % FBS. Inactivated serum samples were 2-fold serially diluted in triplicates starting from 1:40 dilution in MEM with 2% FBS. Fifty plaque forming units (PFU) of SARS-CoV-2 were added to serum dilutions and incubated for 1 h at 37 °C. The growth medium was removed and the virus–serum mixture was added to the cells and incubated for 4 days at 37 °C with 5% CO2, after which the cells were stained with crystal violet to detect cytopathic effect (CPE). The neutralisation endpoint titer was determined as the endpoint serum dilution that inhibited the SARS-CoV-2 induced CPE in at least 2 out 3 parallel wells. The MNT titer ≥40 was considered as positive.

8. Results

For 55 COVID-19 patients out of 62, the date of disease onset was available, and disease severity could be rated (mild, moderate or severe; based on Siddiqi et al. [2020; [6]]) (Table 2, Fig. 1). In the COVID-19 patients included in this study, the earliest time point for the MNT to become positive was 3 days from onset of illness (patient ID 50), while the furthest time point for a negative MNT was 16 days from onset (patient ID 5) (Fig. 1E; Table 2). Disease severity did not appear to be reflected in the MNT titers of the patients, however, the number of patients in each category was too low to assess significance (Table 2).

Numeric results of Abbott Architect SARS-CoV-2 IgG, Euroimmun SARS-CoV-2 IgA, Euroimmun SARS-CoV-2 IgG and Diasorin Liaison SARS-CoV-2 IgG (RUO) were plotted against the MNT titer values (Table 2, Fig. 1). The geometric mean of the patient panel specimens exceeded the test cut-off at the following MNT titers: Abbott IgG (test cut-off 1.4 index exceeded with geometric mean 3.50 index at MNT titer 80); Euroimmun IgA (test cut-off 1.1 ratio exceeded with geometric mean 3.79 ratio at MNT titer 80), Euroimmun IgG (test cut-off 1.1 ratio exceeded with geometric mean 1.57 ratio at MNT titer 80), and Liaison IgG (test cut-off 15 AU/mL exceeded with geometric mean 45.1 AU/mL at MNT titer 320) (Fig. 1). However, the geometric mean in the Euroimmun IgG assay lingered in close proximity (geometric mean 1.57 ratio) of the cut-off (1.1 ratio) still at MNT titer of 160.

Negative and positive agreements (specificity and sensitivity) for immunoassays were calculated in comparison with MNT, in which MNT titer < 40 was considered negative and ≥40 positive (Tables 1–3). Equivocal results (eq in Tables 1–3) of the commercial assays were regarded as reactive in the performance calculations. Altogether, 53 samples from the negative panel and all of the 70 samples from the COVID-19 patient panel were tested with MNT (Tables 1–3). The negative and positive agreement (specificity and sensitivity) values, respectively, were as follows: 95.1 %/80.5 % (Abbott Architect SARS-CoV-2 IgG), 94.9 %/43.8 % (Diasorin Liaison SARS-CoV-2 IgG; RUO), 68.3 %/87.8 % (Euroimmun SARS-CoV-2 IgA), 86.6 %/70.7 % (Euroimmun SARS-CoV-2 IgG), 74.4 %/56.1 % (Acro Biotech 2019-nCoV IgG), 69.5 %/46.3 % (Acro Biotech 2019-nCoV IgM), 97.5 %/71.9 % (Xiamen Biotime SARS-CoV-2 IgG), and 88.8 %/81.3 % (Xiamen Biotime SARS-CoV-2 IgM). Test results from the automated immunoassays plotted against each other are shown in Fig. 2. By using the cut-offs provided by the manufacturers, a trend was observed in which Abbott IgG yielded positive signals in specimens still negative in Euroimmun IgG and Liaison (RUO) IgG (Fig. 2). Rheumatoid factor was detected in five of negative panel specimens (Table 1). More detailed results are provided in Tables 1–3.

All of the six immunoassays gave reactive results to a varying degree for the negative panel specimens (Table 1). Particularly the Acro Biotech rapid test and Euroimmun IgA assay reacted in samples retrieved

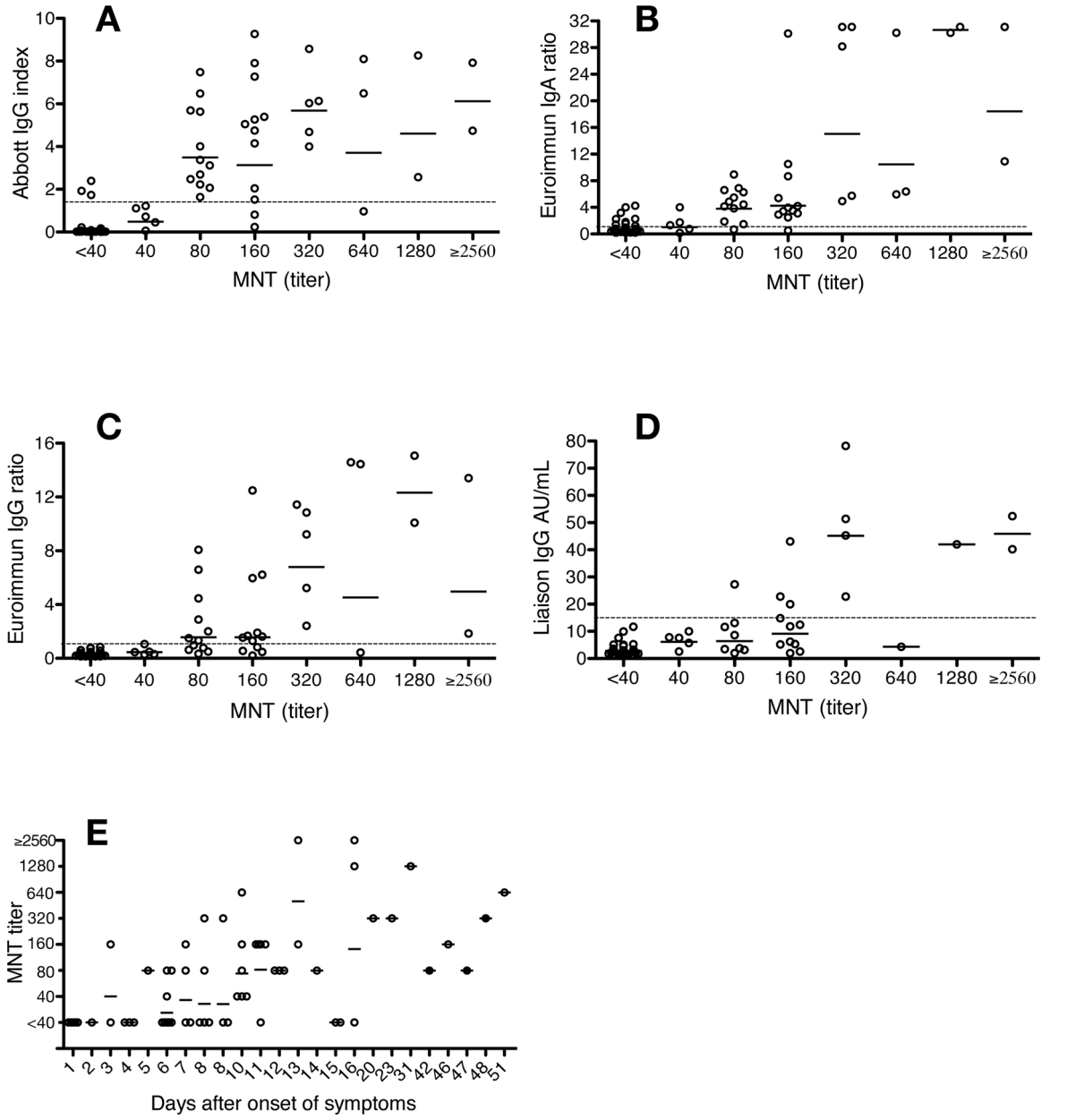

Fig. 1. Comparison of microneutralisation test (MNT) and immunoassay results, and onset of illness. A) Abbott SARS-CoV-2 IgG assay (index); n = 70. B) Euroimmun SARS-CoV-2 IgA (ratio), n = 70. C) Euroimmun SARS-CoV-2 IgG (ratio), n = 70, D) Liaison SARS-CoV-2 IgG (RUO) (AU/mL), n = 62. E) Microneutralisation titers for 63 serum samples collected from 55 COVID-19 patients, organized according to the time lapse between the onset of symptoms and the sample collection. The geometric mean is marked for each titer with a solid line and immunoassay cut-off values are indicated with a dotted line.

from patients with autoantibodies.

As the sensitivity of the Acro Biotech rapid test was lower than the other immunoassays tested, we randomly chose an MNT positive specimen (ID 61), conducted a dilution series of 1:2 for it, and tested the specimen again with the Acro Biotech test. An evident prozone effect was detected, and the originally negative test turned IgG positive at serum dilution 1:4 up until dilution of 1:16.

9. Discussion

As serological assays for SARS-CoV-2 are now becoming available in the market in abundance [7], assessment of their analytical performance by using clinical specimens is of critical importance. In this study, we assessed the specificity and sensitivity of six commercial immunoassays for the detection of SARS-CoV-2 antibodies, including two rapid lateral flow tests, in comparison with a neutralisation test. While neutralisation assays are considered to be the gold standard in terms of specificity, they also provide evidence as to development of immunity.

Eighty-one of the specimens were retrieved in 2018 and 2019 in Finland, rendering these specimens as ascertained negative for SARS-CoV-2 antibodies, and subsequently verifying the very high specificity of the neutralisation test we used (100 % were negative in MNT). We chose serum dilution 1/40 as the limit of detection for the MNT. Failure to detect very low antibody concentrations in this setup is possible. However, four of the 62 PCR-positive individuals showed neutralising antibodies without reactivity in any of the IgG tests used, suggesting a reasonable level of sensitivity in our neutralisation assay.

RF, which is an autoantibody against the Fc portion of IgG, and a common cause of cross-reactivity in immunoassays [8], was analysed in the specimens collected in 2018 and 2019. Five out of 39 of these specimens were positive for RF; 4/5 were negative in all SARS-CoV-2

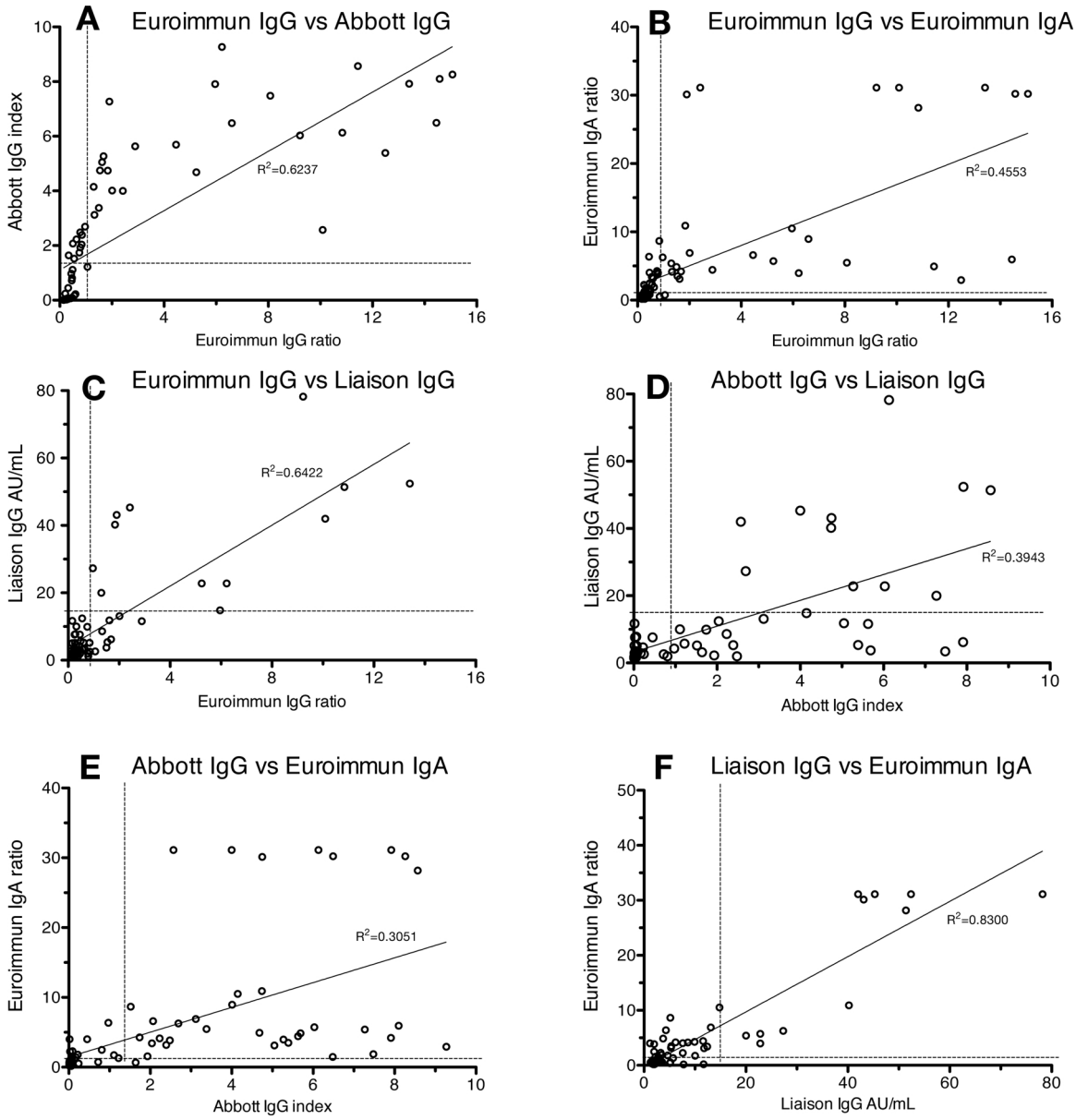

Fig. 2. Test results from the automated immunoassays plotted against each other. A) Euroimmun IgG vs Abbott IgG, B) Euroimmun IgG vs. Euroimmun IgA, C) Euroimmun IgG vs. Liaison IgG (RUO), D) Abbott IgG vs Liaison IgG (RUO), E) Abbott IgG vs Euroimmun IgA, F) Liaison IgG (RUO) vs Euroimmun IgA. The immunoassay cut-off values (dotted line) and trendlines are provided.

immunoassays, and 1/5 gave a positive reaction in the Acro IgG and IgM test. We conclude that the majority of positive test reactions in the six different immunoassays by using the negative serum panel from 2018 – 2019 were not due to RF. Of note, we observed a prozone phenomenon [9] by diluting specimen ID 61 for the Acro lateral flow assay. While we did not investigate prozone phenomenon extensively in this study, we do consider it may be an important cause for false negative test results. The prozone phenomenon has been reported for other lateral flow assays previously [10].

Of the automated assays included in this study, and by using the cut-off values set by the manufacturers, the best specificity values were observed with Abbott IgG (95.1 %). A previous report from the United States reported a 99.9 % specificity [11]. In our study, Liaison IgG (RUO) assay (94.9 %) also showed a good specificity. Euroimmun SARS-CoV-2 IgA assay had the best positive agreement (sensitivity) (87.8 %), while the positive agreement of the Liaison IgG (RUO) assay was the lowest (43.8 %). The CE marked Diasorin Liaison SARS-CoV-2 IgG assay was not available for this evaluation.

The automated assays from the three manufacturers were all based on different antigen components (S1, S2, nucleocapsid). This is noteworthy, as antibody responses against each of these antigens may develop with varying kinetics, which remains a subject for further investigation. In addition, the immunoassays may detect nonneutralizing antibodies, not detected by neutralization assays. However, the topic of interest in our study was specifically on comparability of the assays with neutralising antibodies.

When interpreting sensitivity values, the time from onset of illness in COVID-19 patients needs to be accounted for. By using the Abbott IgG assay, SARS-CoV-2 IgG seroconversion was previously reported in all patients by the day 17 post onset of illness [11]. Previous reports suggest a median seroconversion time for SARS-CoV-2 from 11 days [12] to 13 days [13]. The present study also suggests a relatively long period required for serological response to take place (Table 2, Fig. 1E). Even though extensive conclusions cannot be made from our data, Liaison IgG (RUO) appears to turn positive at a later point in time from onset of illness in comparison with the other immunoassays evaluated

in our study (Table 2). Perkmann et al. ([14]; 2020) have also reported this phenomenon, and it should be investigated more thoroughly whether antibodies against SARS-CoV-2 S1/S2 antigen, in general, are detected in later time point.

Of the two rapid lateral flow assays, the Xiamen IgG/IgM showed a good specificity (97.5 % / 88.8 %) with a modest positive agreement (sensitivity) (71.9 % / 81.3 %). In line with a previous report [15], the performance of the Acro Biotech IgG/IgM rapid test appears not to be adequate for clinical use, with specificity of 74.4 % / 69.5 % and positive agreement (sensitivity) of 56.1 % / 46.3 %.

The currently very low seroprevalence of SARS-CoV-2 in most regions globally render low positive predictive values in the serological testing of individual patients. This can be somewhat improved by good targeting of groups tested. The analytical test performance can be optimised by placing several consecutive assays, with varying antigenic features, in the test workflow, ideally emphasizing sensitivity in the screening and specificity in the second-line testing. The very variable performance values observed in this study highlights the need for laboratories to carefully consider their testing process in order to optimize the overall performance of SARS-CoV-2 serodiagnostics.

Funding

Funded by Helsinki University Hospital, HUSLAB, Helsinki, Finland (KLIMIK).

Contribution

AJJ carried out the collection of retrospective samples together with HKK and MA. AJJ carried out the immunoassays (EIAs and rapid tests) and analysed the resulting data. SK carried out the microneutralisation assays. ELKE collected the data from patient records and carried out part of the rapid tests. AJJ and HJ carried out RF tests. HKK, RL, SKU and ML took part in the PCRs evaluation and setting up PCR assays in the laboratory. AJJ, SK, SKU, HJ and ML reviewed the data. HJ helped in formulating the Tables and Figures. SKU wrote the manuscript together with AJJ. AJ, SK, HKK, OV, HJ, ELKE, MA, RL, SKU and ML reviewed and modified the manuscript and approved its final version.

Declaration of Competing Interest

None of the authors have any conflict of interest.

Acknowledgements

We would like to thank Pamela Österlund (Finnish Institute for Health and Welfare, Helsinki, Finland) for providing the virus strain. We would also like to thank (in alphabetical order) Anu Jääskeläinen (Helsinki University Hospital), Pia Jokela (Helsinki University Hospital), Laura Mannonen (Helsinki University Hospital), Tarja Sironen (University of Helsinki), Satu Suuronen (Helsinki University Hospital), Anne Toivonen (Helsinki University Hospital), and Mira Utriainen (University of Helsinki).

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