

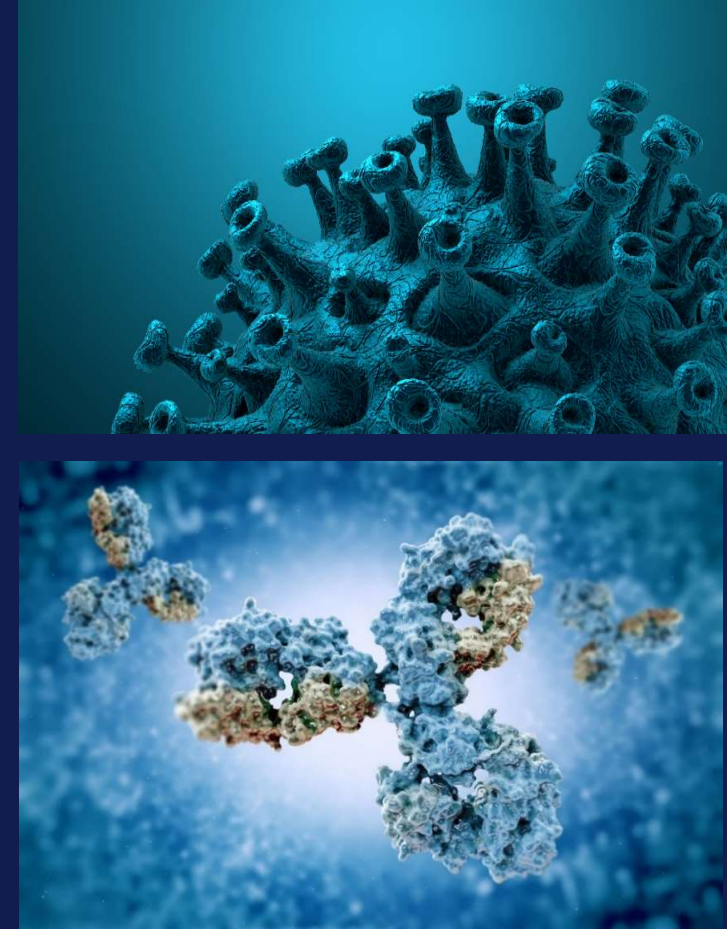
Results from a comprehensive, international external quality assessment for SARS-CoV-2-specific antibody assays - the EQALM-SARS-CoV-2 antibody study with 1704 participant laboratories from 65 countries

A cooperation of the following EQA Providers: AOU Careggi (Italy), AQALM (Ukraine), Biologie-prospective (France), Calilab (Romania), CROQUALM (Croatia), ESfEQA (international), IfQ Lübeck (Germany), INSTAND e.V. (Germany, international), Labquality (Finland, international), LGC (United Kingdom, international), ÖQUASTA (Austria), Qualicont (Hungary), RfB (Germany, international), SEKK (Czech Republic), SKML (The Netherlands), UK NEQAS (United Kingdom, international), WEQAS (Wales)

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Center for Virology

Medical University of Vienna





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The role of antibody assays in the diagnosis of SARS-CoV-2 infection

MAJOR:

- Diagnosis of past „natural“ infection
(antibodies against the Nucleocapsid-protein: infection vs. vaccination):
 - Sequelae of SARS-CoV-2 infection e.g. „Long-CoVID“, fulminant hepatitis in children
- Correlate for the strength of the humoral immune response against the Spike-protein

Reference: Neutralizing antibodies against the circulating variant (in-house LDT)!

BUT: No clear correlate of protection (against infection!)

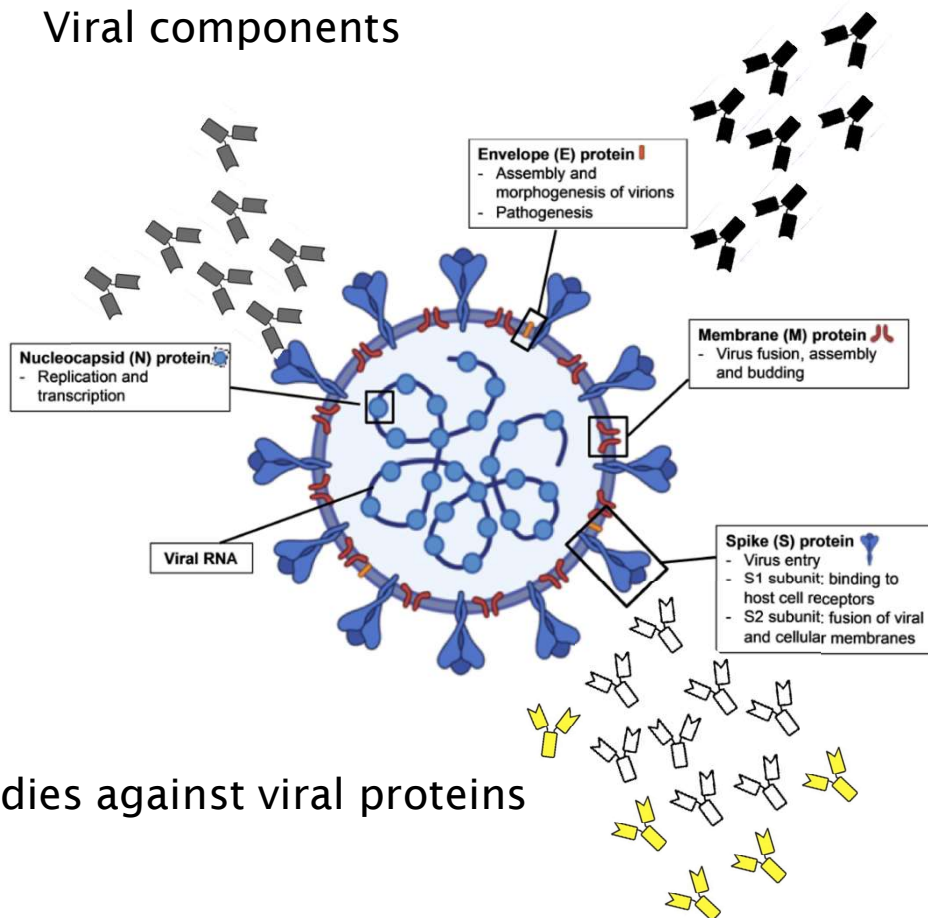
MINOR:

- « Staging » of the infection, re-infection vs. breakthrough
(immunglobulin classes, avidity, concentration)
- Diagnosis of acute SARS-CoV-2 infection in hospitalized patients
(SARS-CoV-2 PCR from the upper respiratory tract NEGATIVE!)

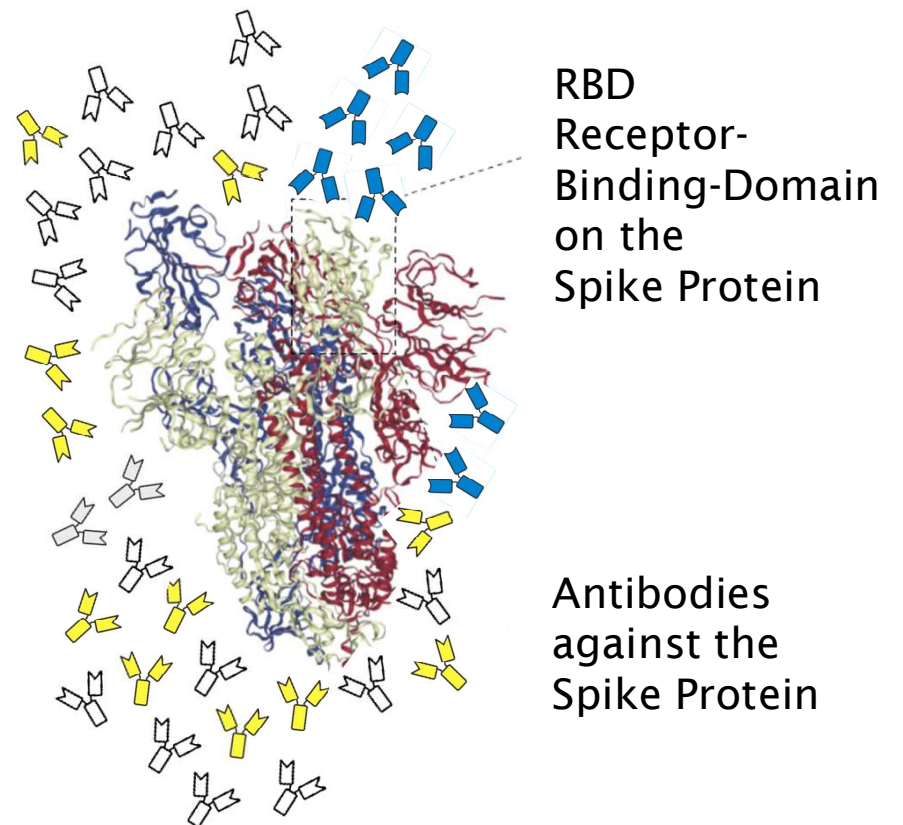


Antibodies against SARS-CoV-2

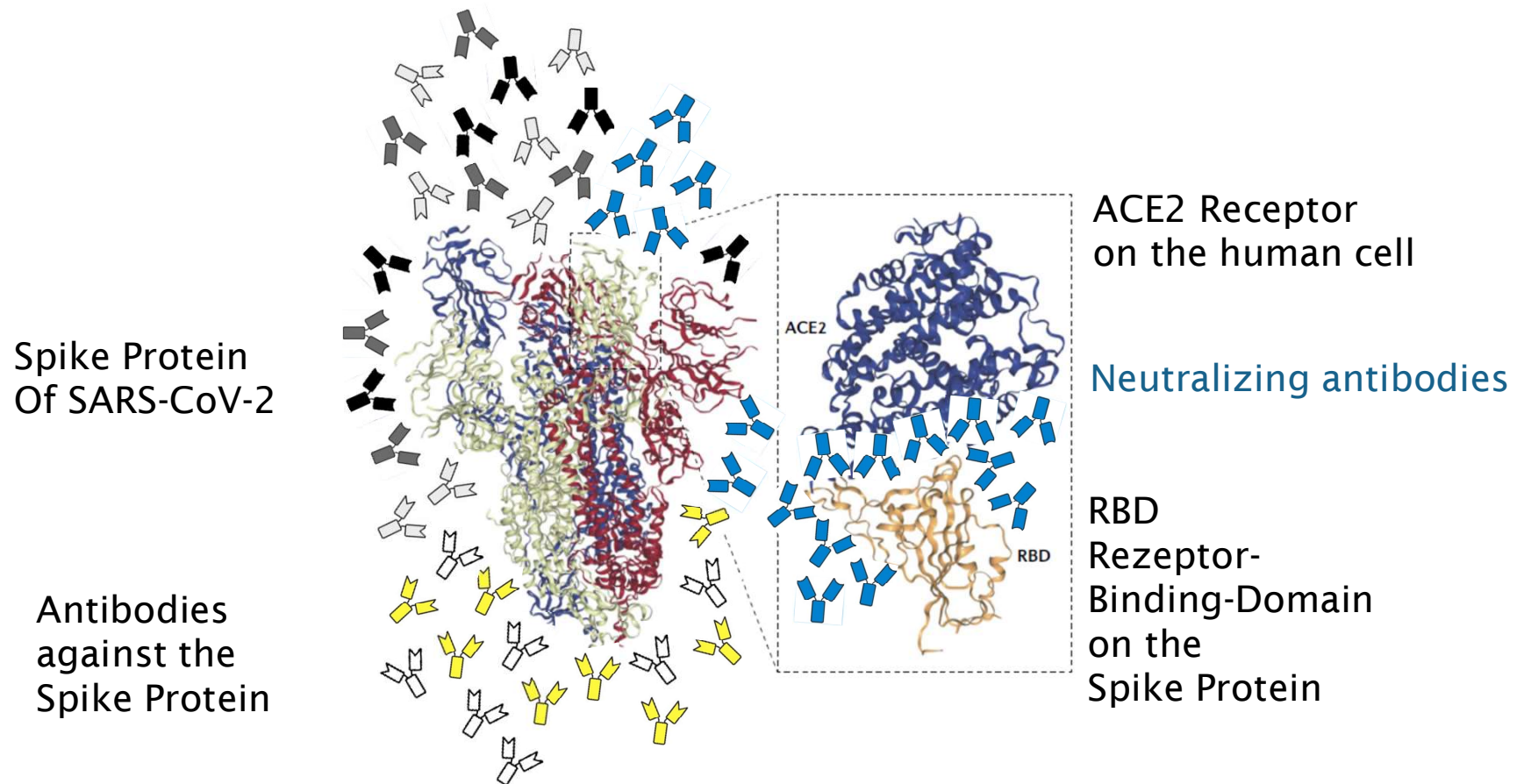
Viral components



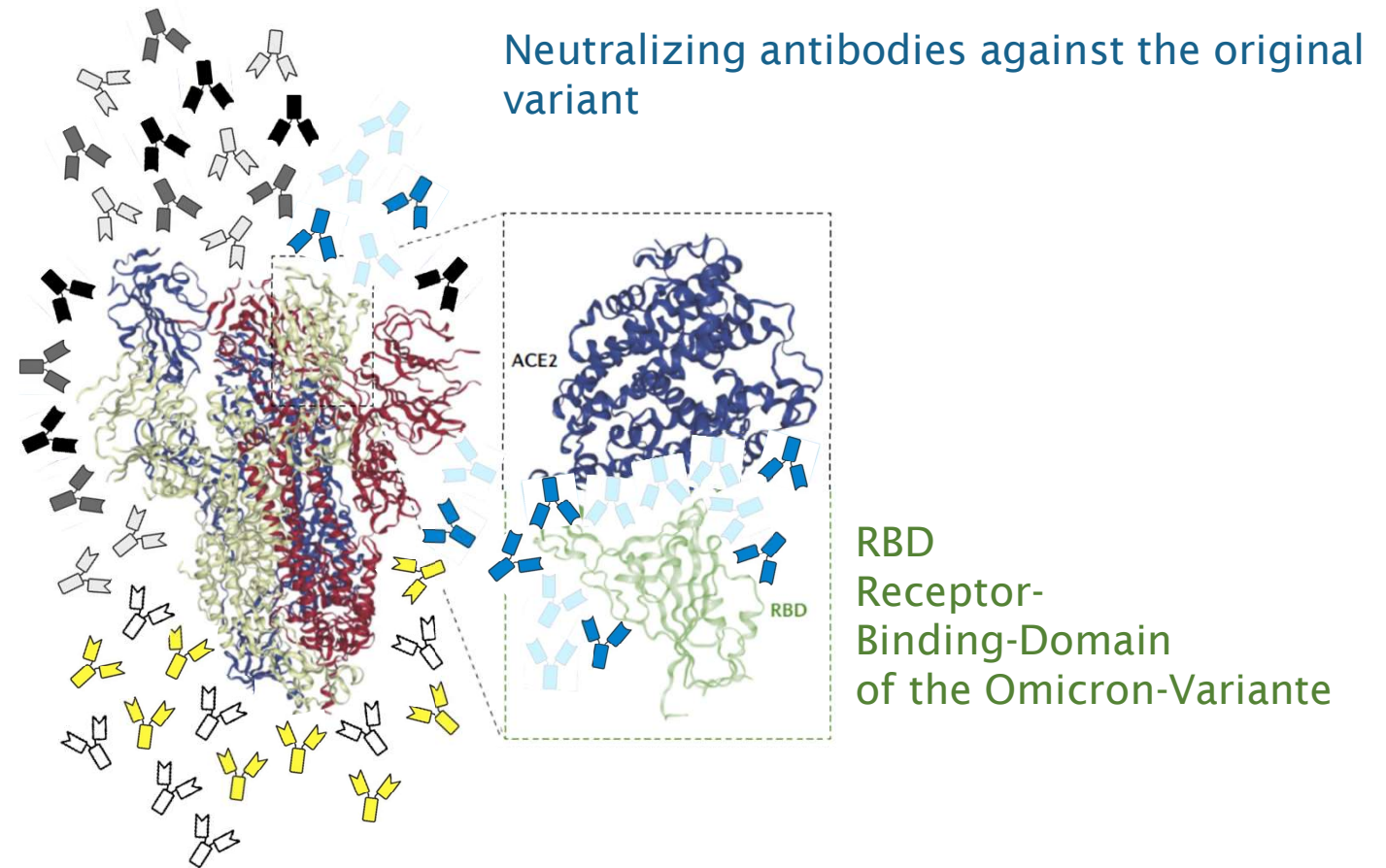
Spike Protein of SARS-CoV-2



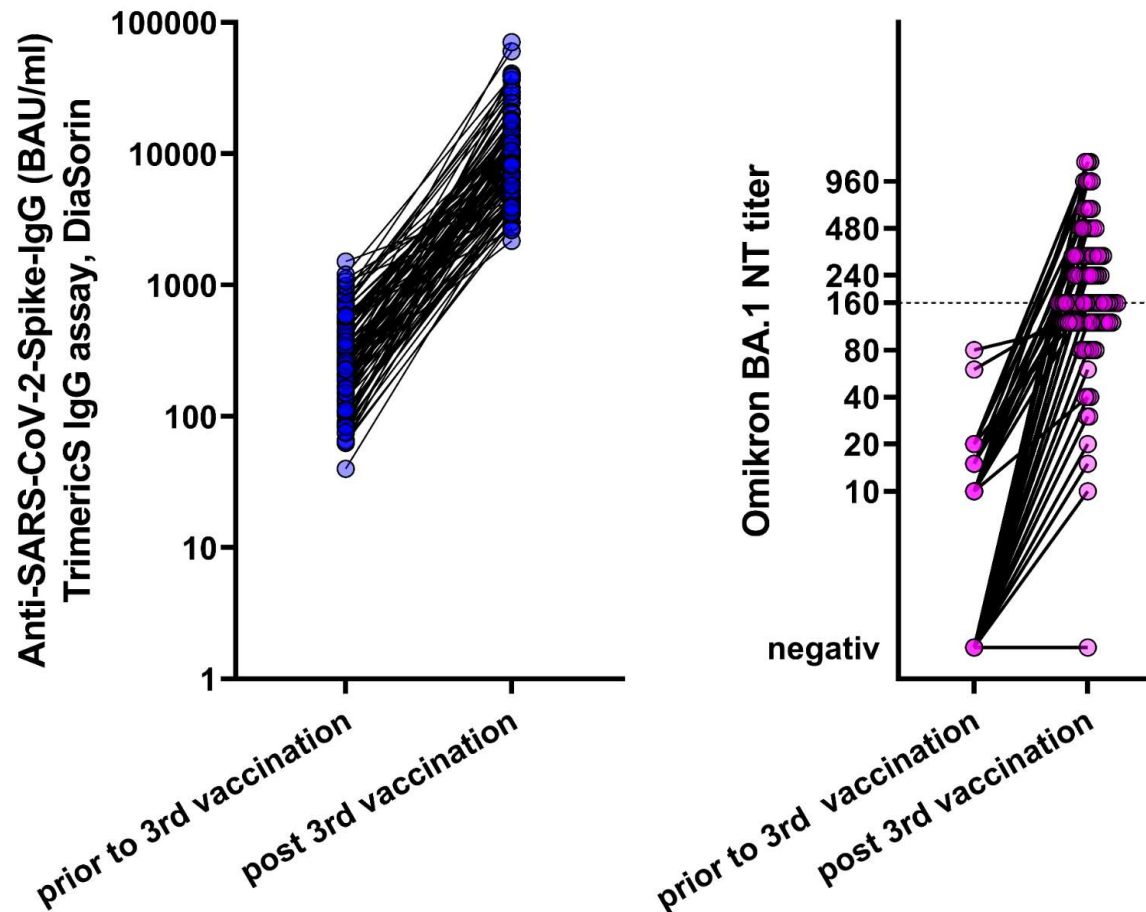
Spike-specific antibodies, RBD, neutralizing antibodies



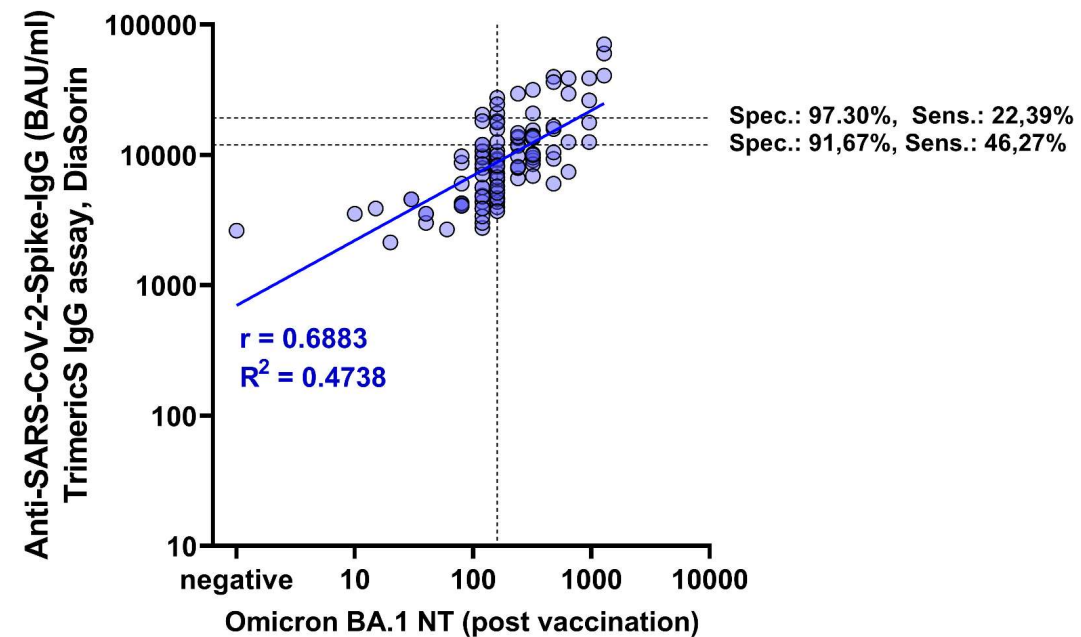
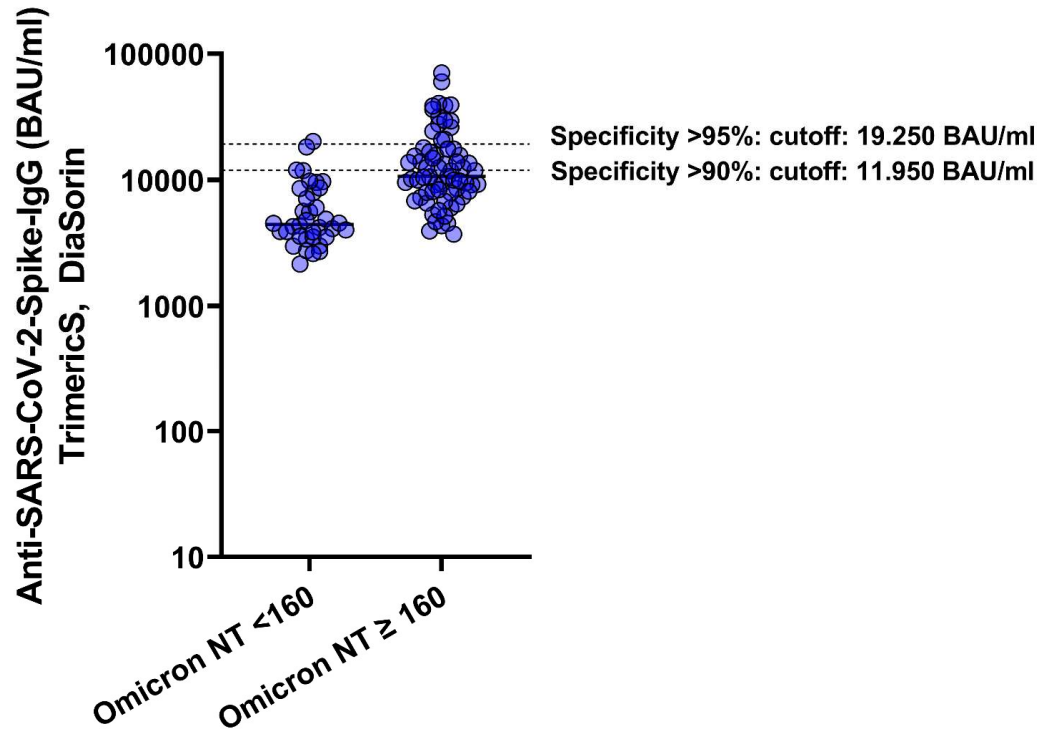
Pre-existing antibodies display reduced binding against the Omicron variant



Spike-specific antibodies in HCWs after the third RNA vaccination (n=103)

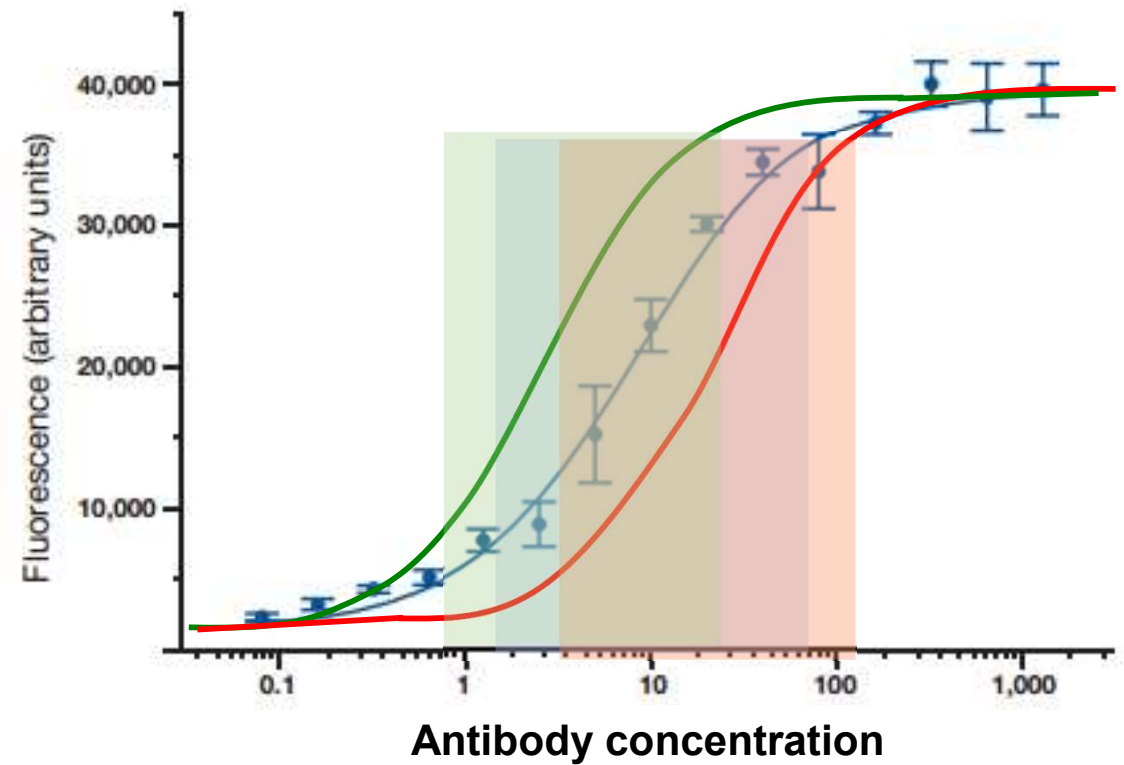
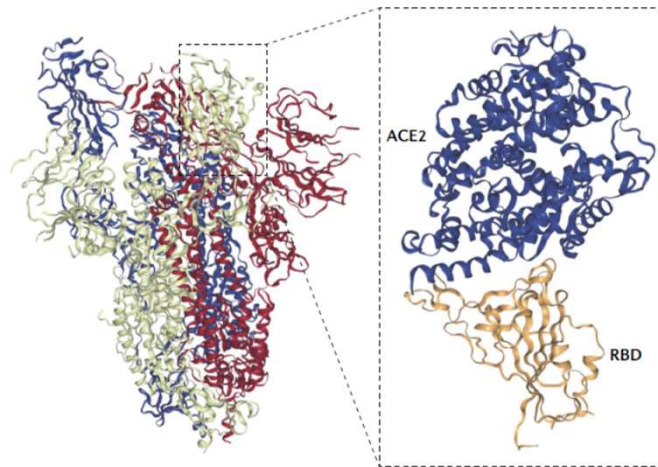


Spike-specific antibodies in HCWs after the third RNA vaccination (n=103)



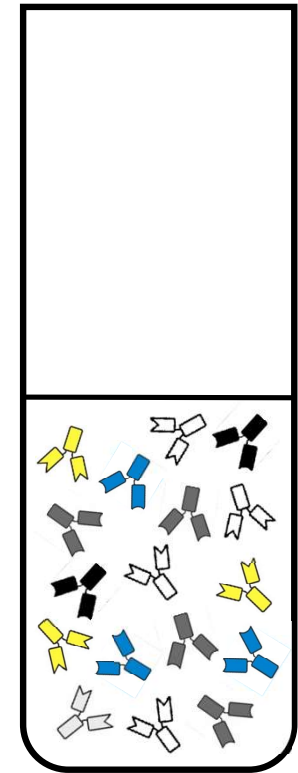
Data obtained by in-house LDTs are the (only) functional basis for using IVDR-CE-marked commercial tests!

Functional background of the variation in antibody assays



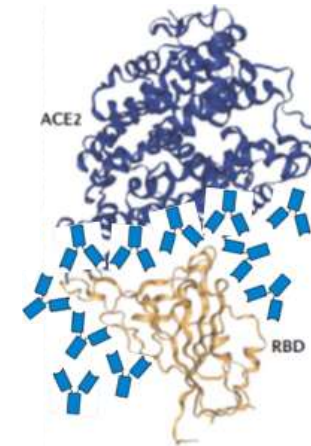
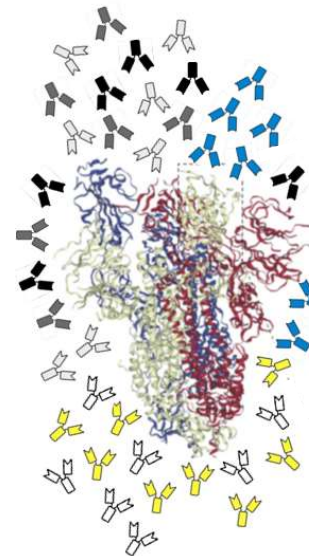
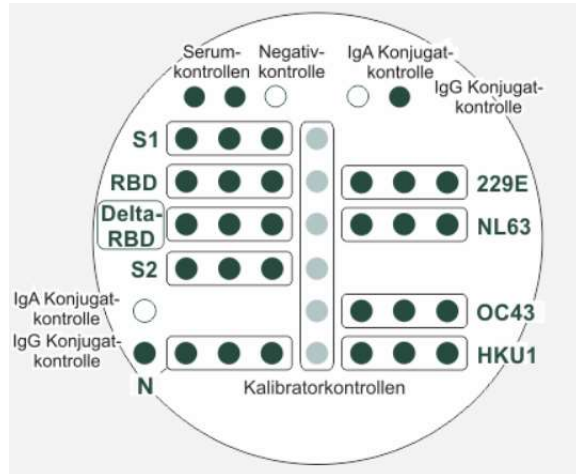
Characterization of samples

- Sera from 7 donors
- (6 convalescents after SARS-CoV-2 infection \pm vaccination,
1 donor pre-pandemic (negative control sample))
- Pooled based on the concentration of SARS-CoV-2-specific antibodies:
Four reference samples (S1-S4)
- **S1: Long past SARS-CoV-2 infection \pm SARS-CoV-2 vaccination**
- **S2: Recently past SARS-CoV-2 infection \pm SARS-CoV-2 vaccination**
- **S3: Acute SARS-CoV-2 infection/vaccination
(RBD-specific IgM antibodies detectable)**
- **S4: no evidence for SARS-CoV-2 infection/vaccination**



Characterization of samples

- SARS-CoV-2 ViraChip IgM, IgA, IgG Microarray, Viramed, Planegg, Germany,
- LIAISON® SARS-CoV-2 TrimericS IgG assay, CLIA, DiaSorin S.p.A, Saluggia, Italy,
- Anti-SARS-CoV-2-QuantiVac-ELISA (IgG), Euroimmun, Lübeck, Germany
- cPass, SARS-CoV-2 Neutralization Antibody Detection Kit

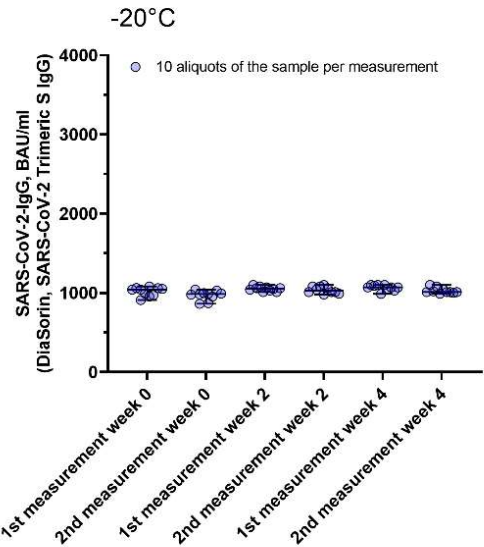


Characterization of samples

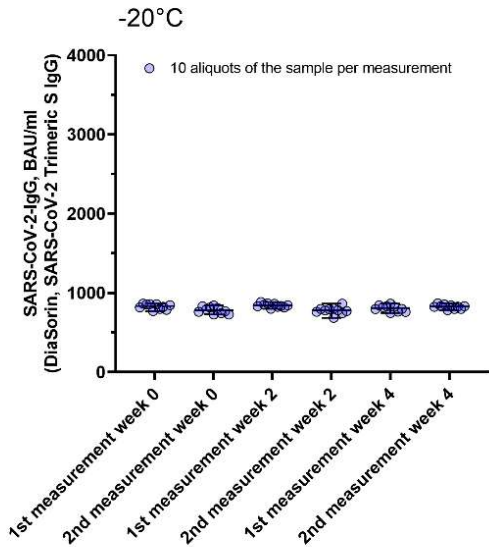
Anti-SARS-CoV-2 antibodies	S1	S2	S3	S4
S1-IgG qualitative (DiaSorin)	positive	positive	positive	negative
S1-IgG quantitative (BAU/ml) (DiaSorin)	993	804	3172	<4,81
S1-IgG qualitative (Euroimmun)	positive	positive	positive	negative
S1-IgG quantitative (BAU/ml) (Euroimmun)	1020	341	1822	<7,8
RBD-IgG qualitative	positive	positive	positive	negative
RBD-IgG quantitative (BAU/ml)	>250	>250	>250	<5
S2-IgG qualitative	positive	positive	positive	negative
S2-IgG quantitative (BAU/ml)	>400	>400	>400	<25
NCP-IgG qualitative	positive	weakly positive	positive	negative
NCP-IgG quantitative (BAU/ml)	127	95	114	0
S1-IgM qualitative	negative	negative	borderline	negative
S1-IgM quantitative (Virachip Units, cutoff: 100)	0	0	70	0
RBD-IgM qualitative	negative	weakly positive	positive	negative
RBD-IgM quantitative (Virachip Units, cutoff:100)	9	90	188	0
S2-IgM qualitative	negative	negative	negative	negative
S2-IgM quantitative (Virachip Units, cutoff: 100)	0	0	0	0
NCP-IgM qualitative	borderline	negative	borderline	negative
NCP-IgM quantitative (Virachip Units, cutoff:100)	89	69	86	0
S1-IgA qualitative	positive	positive	positive	negative
S1-IgA quantitative (Virachip Units, cutoff: 100)	208	224	251	0
RBD-IgA qualitative	positive	positive	positive	negative
RBD-IgA quantitative (Virachip Units, cutoff: 100)	159	182	190	0
S2-IgA qualitative	negative	positive	positive	negative
S2-IgA quantitative (Virachip Units, cutoff: 100)	66	108	236	0
NCP-IgA qualitative	negative	negative	negative	negative
NCP-IgA quantitative (Virachip Units, cutoff: 100)	17	41	28	0
Surrogate neutralizing antibodies qualitative	positive	positive	positive	negative
Surrogate neutralizing antibodies quantitative (% ACE2 RBD Binding, cutoff: 30)	87,2	77,06	93,56	5,94

Homogeneity and stability of SARS-CoV-2 antibodies in the samples (-20°C)

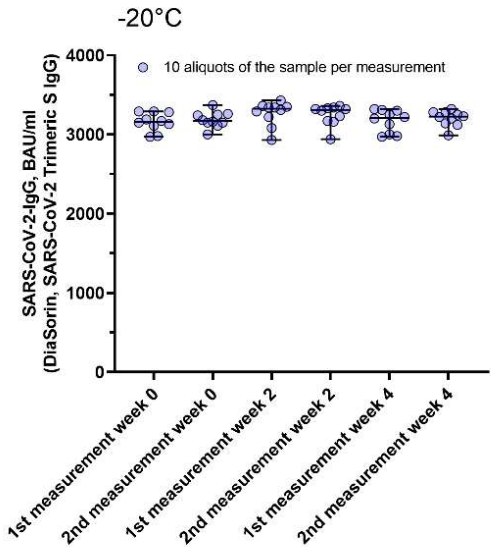
S1



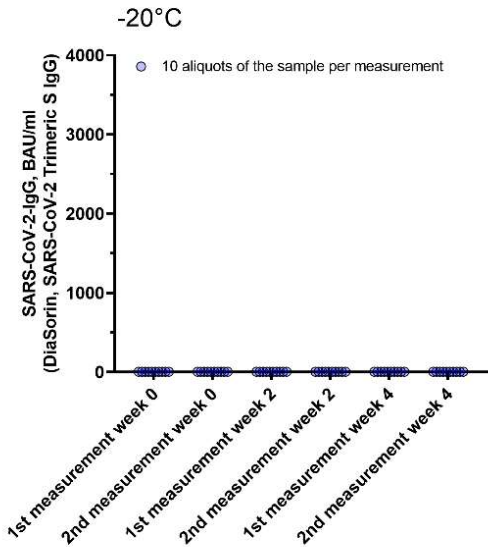
S2



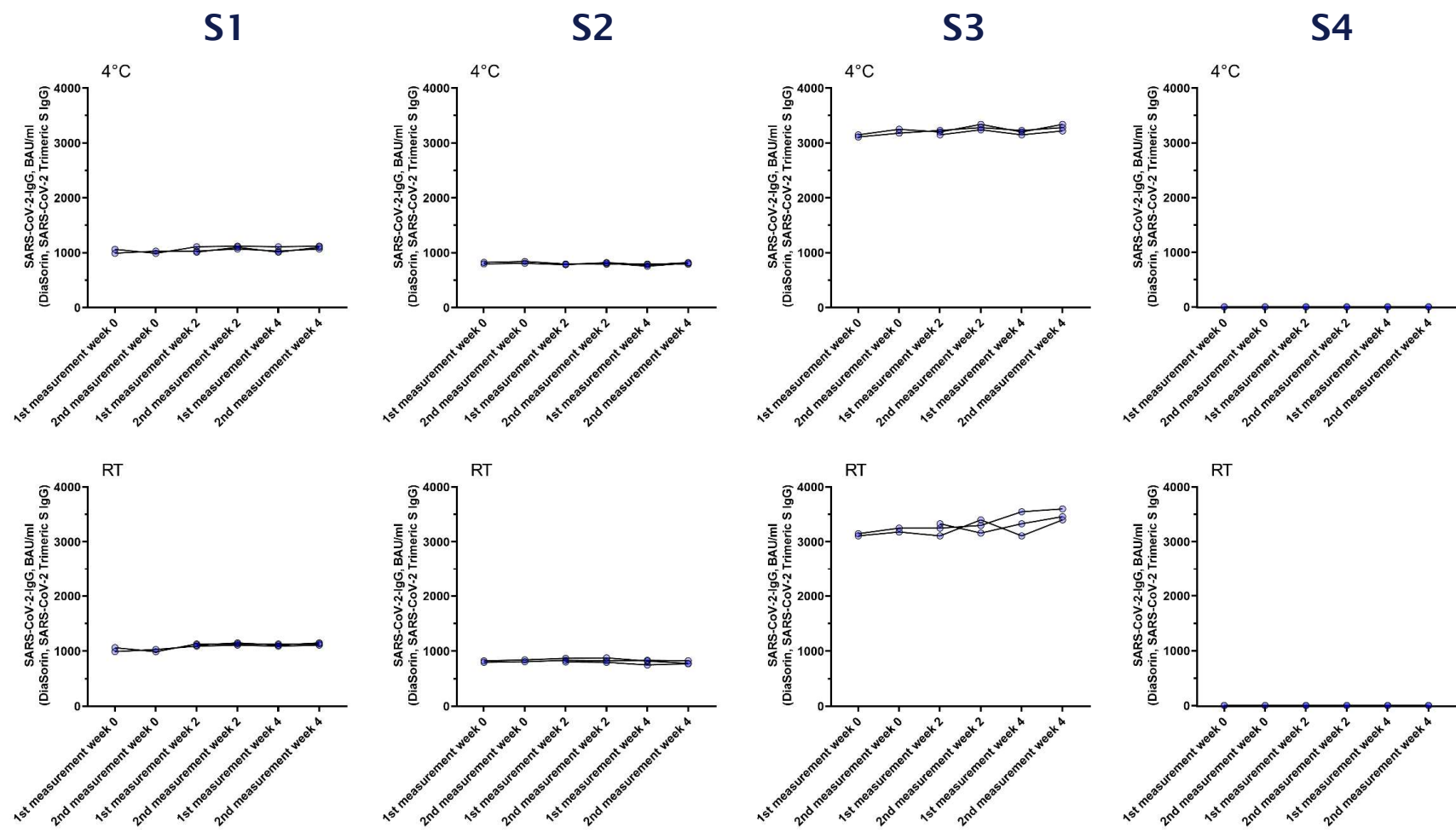
S3



S4



Stability of SARS-CoV-2 antibodies in the samples (4°C, Room temperature)



Participating laboratories

- **Total number of laboratories: n= 1704**
- EQA Providers: AOU Careggi (Italy), AQALM (Ukraine), Biologie-prospective (France), Calilab (Romania), CROQUALM (Croatia), ESfEQA (international), IfQ Lübeck (Germany), INSTAND e.V. (Germany, international), Labquality (Finland, international), LGC (United Kingdom, international), ÖQUASTA (Austria), Qualicont (Hungary), RfB (Germany, international), SEKK (Czech Republic), SKML (The Netherlands), UK NEQAS (United Kingdom, international), WEQAS (Wales)
- **Participating countries: n=65**
- Argentina (n=1), Australia (n=1), Austria (n=65), Belgium (n=3), Bulgaria (n=12), Canada (n=5), Chile (n=1), Colombia (n=10), Croatia (n=49), Cyprus (n=2), Czech Republic (n=87), Denmark (n=8), Egypt (n=1), Estonia (n=9), Finland (n=8), France (n=589), French Guyana (n=3), French Polynesia (n=3), Georgia (n=2), Germany (n=170), Greece (n=4), Guadeloupe (n=5), Hong Kong (n=2), Hungary (n=22), Iceland (n=1), India (n=1), Ireland (n=10), Israel (n=17), Italy (n=107), Kenya (n=1), Kuwait (n=1), La Reunion (n=5), Latvia (n=3), Lithuania (n=8), Luxembourg (n=7), Malta (n=1), Martinique (n=2), Mauritius (n=1), Mayotte (n=2), Moldavia (n=2), Morocco (n=5), New Caledonia (n=2), North Macedonia (n=3), Norway (n=7), Pakistan (n=1), Poland (n=37), Portugal (n=18), Romania (n=10), Saint Marteen (n=1), Saint Pierre et Miquelon (n=2), Senegal (n=2), Singapore (n=2), Slovakia (n=17), Slovenia (n=6), South Africa (n=6), Spain (n=43), Sweden (n=13), Switzerland (n=18), The Netherlands (n=41), Turkey (n=1), Ukraine (n=53), United Arab Emirates (n=6), United Kingdom (n=177), USA (n=1)

Immunoassays used by the participants

- **Antibody assay manufacturers (n=90):**

AAZ diagnostics, Abbott Diagnostics, Acro Bio, Aesku Diagnostics, ANSHLABS, Artron, AST Biomedical, Beckman-Coulter, Best Diagnostics LLC, Biodiagnostics, Biomerieux, Bio-Rad, Biosynex, Biozek, Boditech, BTNX, Cellex, CMD, Demeditec, Diagnostics Biochem Canada, DiaLab, DIAPROF-MED JSC, DiaSorin, DiaSys, DiESSE, DRG Instruments, EKVITESTLAB LLC, Epitope Diagnostics, Euroimmun, Eurospital, Fortress Diagnostics, Genscript, Getein Biotech, Hangzhou Biotech, IDK, IDVET, ImmunoDiagnostik, ImmunoTech, Innobiochips, Institut Virion/Serion GmbH, KHB, Luminex, LumiraDx, Mediagnost, Mikrogen, Mindray, Nal Von Minden, Novatec, Opti Medical Systems, Ortho Clinical Diagnostics, PANBIO, Roche, SD Biosensor, Sentinel, Seramun, Shanghai Kehua Bio-Engineering, Shenzhen Yhlo Biotech, Siemens, Snibe Diagnostic, Sure Screen, Tecan, Technogenetics, Teco Medical Group, Test-it, TestLine, Theradiag, Thermo Fisher, TOB Xema, Viramed, Vircell, Vitrotest Bioreagent LLC, Wantai, Wondfo, Wuhan UNscience Biotechnolog, ZHEJIANG ORIENT GENE BIOTECH

Immunoassays used by the participants

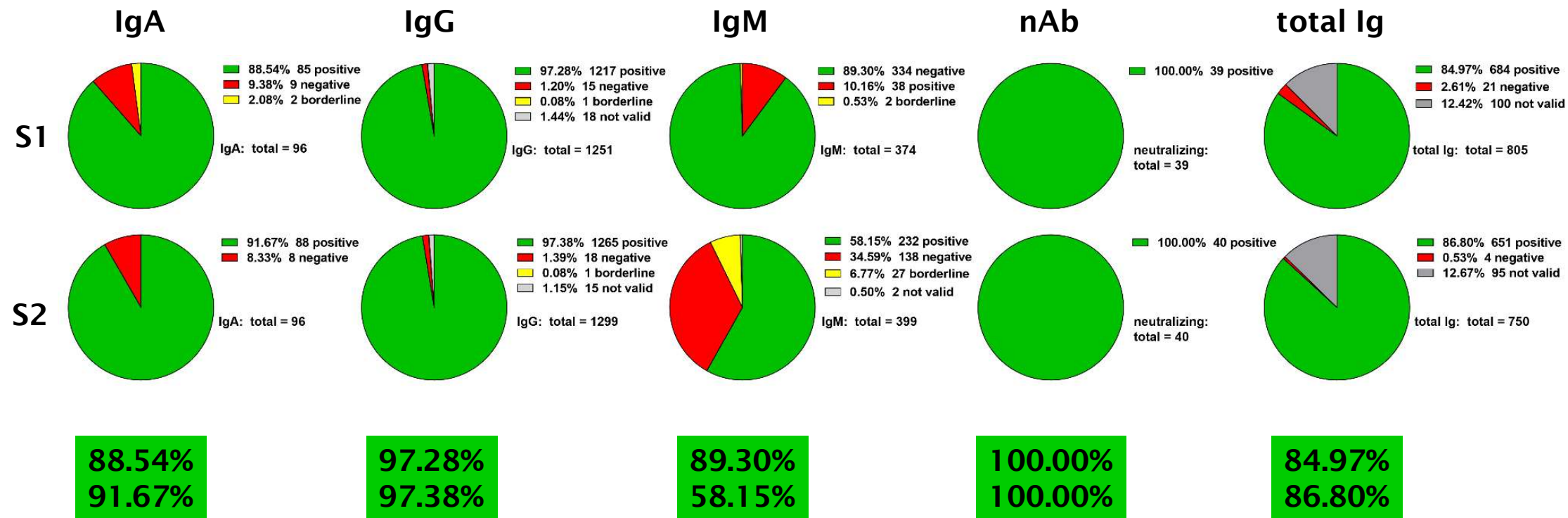
- Antibody assays (n=176)
- Immunoglobulin classes: IgM, IgA, IgG, total Ig
- Functional assays: surrogate neutralization, avidity
- Principle: ELISAs, CLIAs, ECLIAs, rapid tests (POC tests), Immunoblots

Parameter	Result	Unit	Equipment manufacturer	equipment	Reagents manufacturer	Reagents	Interpretation
SARS-CoV-IgG	777,89	BAU/mL	-----	Analyzer	-----	Euroimmun	positive
Anti-SARS-CoV-2	1,83	Ratio	-----	ELISA	Euroimmun	Nucleocapsid	-----
SARS-CoV-2 antibodies (total)	777,89	Arbitrary concentration	Euroimmun	-----	-----	QuantiVac	positive
Anti-SARS-CoV-2-IgG	777,89	-----	-----	Euroimmun QuantiVac (BAU/mL)	-----	-----	positive

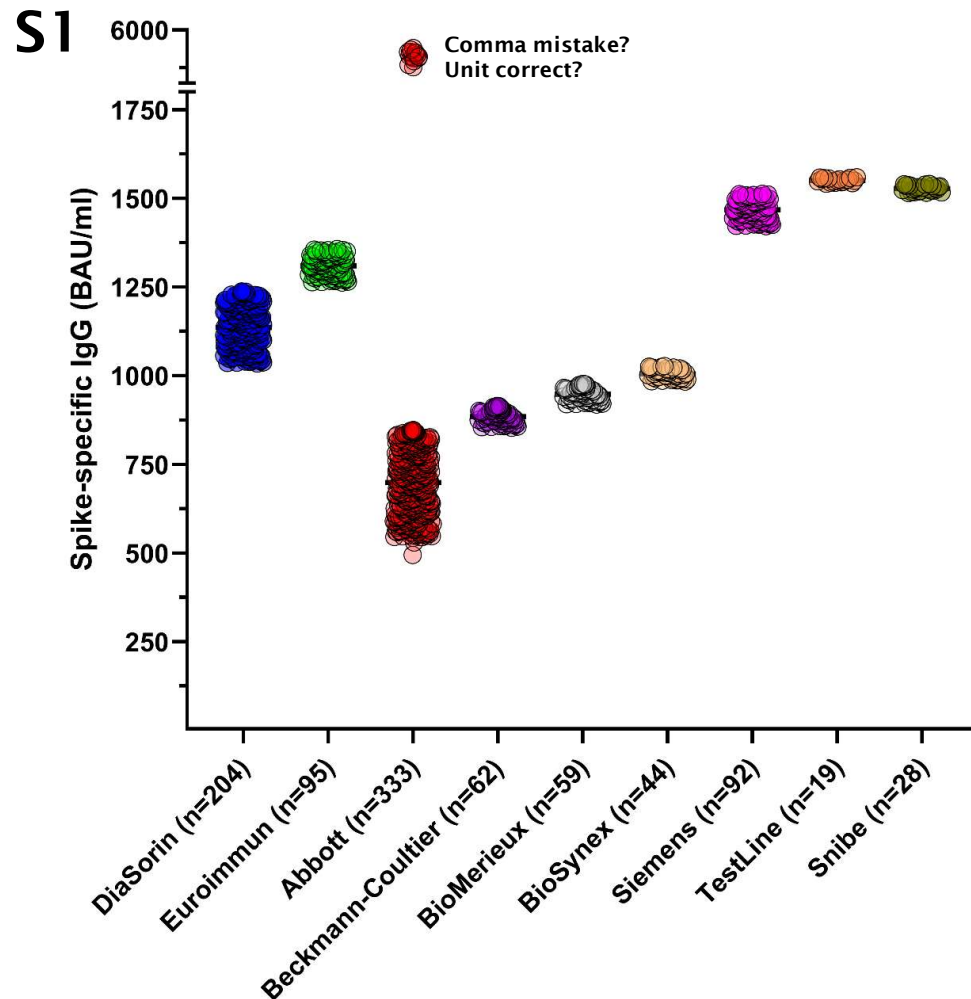
Result (1): all kinds of variation in reporting!!

Results (2): Qualitative Results for S1, S2

- Results reported:
- S1, S2: n=2567 (antibody assay not identifiable: n=67)

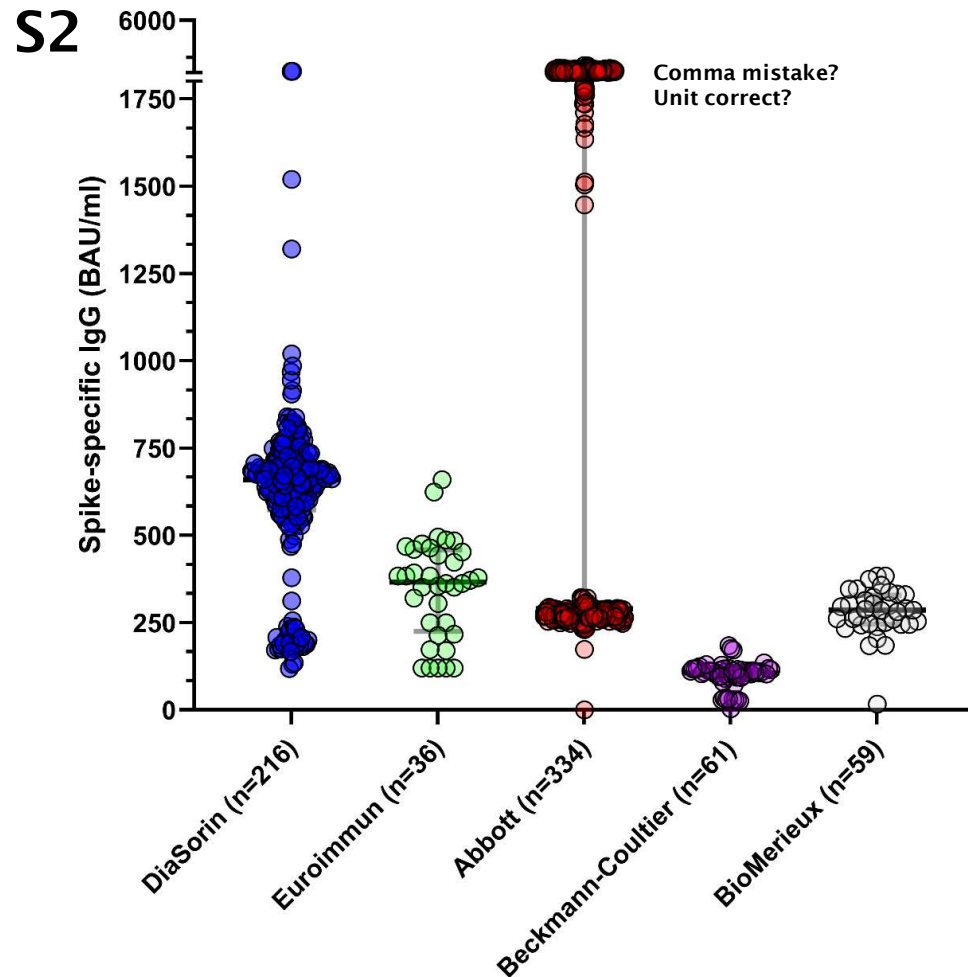


Results (3) Quantitative Results for S1, S-specific IgG



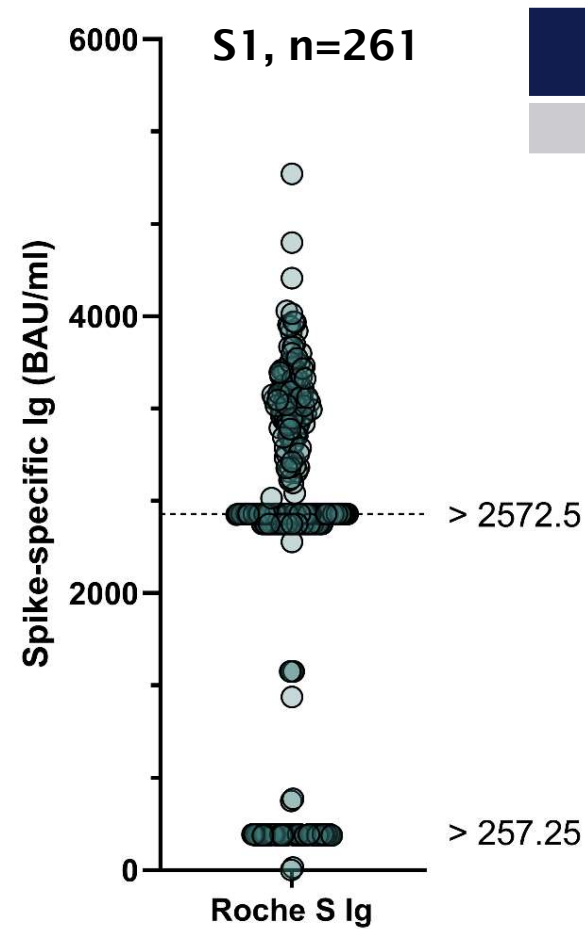
Assay used (manufacturer)	Coefficient of Variation (intra-assay, inter-laboratory)
DiaSorin	5,19%
Euroimmun	2,10%
Abbott	86,61%
Beckmann-Coulter	2,04%
Bio-Merieux	1,81%
BioSynex	1,28%
Siemens	1,82%
TestLine	0,36%
Snibe	0,52%

Results (4): Quantitative Results for S2, S-specific IgG

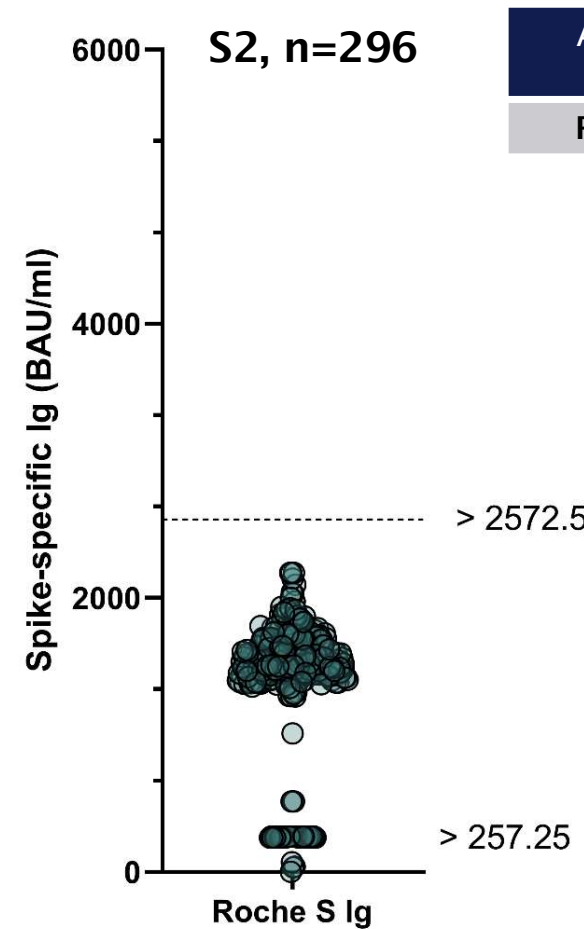


Assay	Coefficient of Variation
DiaSorin	39,01%
Euroimmun	40,75%
Abbott	83,76%
Beckmann-Coulter	35,93%
Bio-Merieux	24,46%

Results (5): Quantitative Results for S1, S2, S-specific total Ig

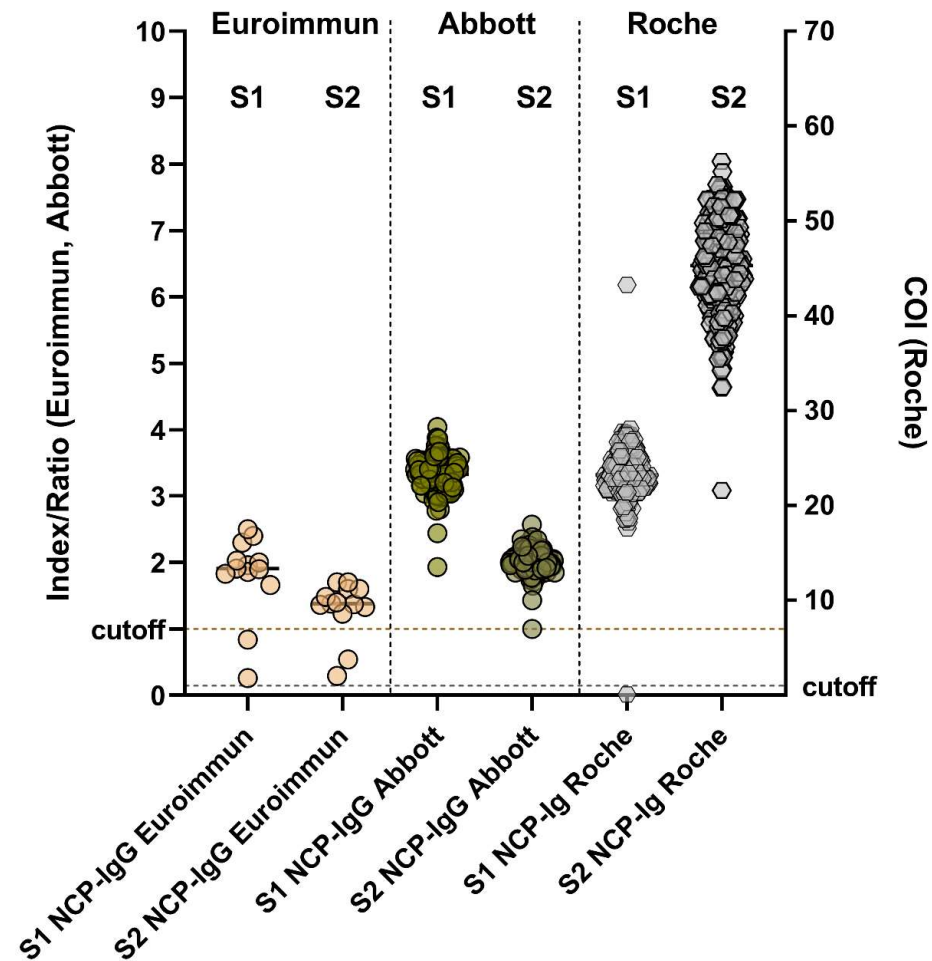


Assay	Coefficient of Variation
Roche	48,13%



Assay	Coefficient of Variation
Roche	35,91%

Results (6): Quantitative Results for S1, S2, NCP-specific IgG and total Ig



Results (7): Qualitative Results for S3, S4

- Results reported:
- S3: n=183 (IgA: n=5, IgG: n=78, IgM: n=28, nAbs: n=11, total Ig: n=68; assay n.i.: n=23)

IgA	100%	IgG	100%	IgM	96,43%	nAb	100%	total Ig	98,41%
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- S4: n=172 (IgA: n=4, IgG: n=73, IgM: n=26, nAbs: n=10, total Ig: n=55; assay n.i.: n=4)

IgA	100%	IgG	100%	IgM	100%	nAb	100%	total Ig	100%
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Summary of the results

- High grade of **variation** in **reporting results**

(name of the assay, name of the platform, target antigen, measuring unit, immunoglobulin class,...)

- **2567** reported **results** for **S1** and **S2**, number too low for S3 and S4
- S1, S2: 176 antibody assays:

Majority of participants reported **correct qualitative results**

(for IgA, IgG, nAbs, total Ig antibodies)

- **Lower concentration of IgM antibodies** in S2: rate of correct qualitative results: **58%**
- Quantitative results (in the WHO standardized-measuring unit BAU/ml)
 - S1 (high Ab concentration):
inter-laboratory, intra-assay variation: low; inter-assay variation: high
 - S2 (medium Ab concentration):
inter-laboratory, intra-assay variation: moderate; inter-assay variation: high (ratios not stable)
- Total Ig: Samples **often not diluted**, no exact quantification of antibody levels
- **Detection range of NCP-specific antibody assays** variable

Implications of the EQA results

Aims of diagnostic testing of SARS-CoV-2-specific antibodies



- **Correlate for humoral immune response against the Spike-protein**

Reference: Neutralizing antibodies against the circulating variant!

Correlation with the total concentration of Spike-specific antibodies!

- **Diagnosis of past „natural“ infection (antibodies against the Nucleocapsid-protein), infection vs. vaccination**





Thank you! Wim Coucke

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Netherlands), UK NEQAS (United
Kingdom, international), WEQAS
(Wales)



Spike-specific antibodies in HCWs after 2xRNA vaccinations (n=103)

in-house LDT! ★★★★★

