Rational design and development of SARS-CoV-2 serological diagnostics by immunoaffinity proteomics

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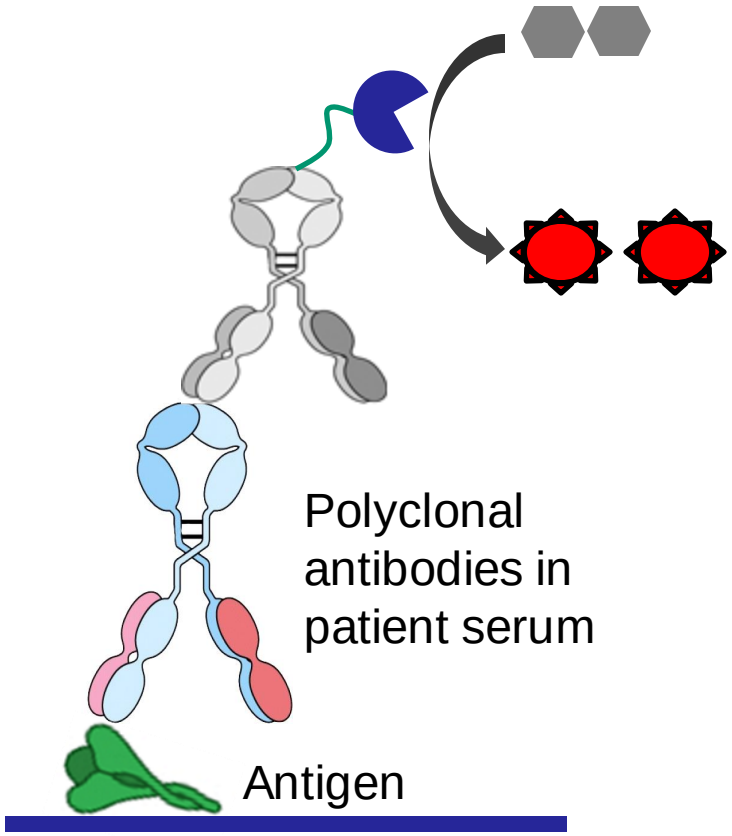
Conflict of Interest declaration

- No Conflicts

Limitations of Conventional Serological Diagnostics

Indirect ELISA



Limitations

- Non-specific binding
- Cross-reactivity
- Semi-quantitative (“titer” vs concentration)
- Challenges with inter-lab standardization
- No multiplexing (IgG1, IgG2, IgG3, IgG4)

Interpretation of results

- Diagnostic specificity of 60 FDA-authorized COVID-19 serological tests is ~99.3%
- This allows for testing populations with COVID-19 prevalence >6%
- To achieve 90% PPV during early stages of pandemic (~0.1% prevalence), serological tests require diagnostic specificity of **99.99%**

Serological Diagnostics by Proteomics

Mol. Cell. Proteomics, **2023**
Mol. Cell. Proteomics, **2021**, *20,* 100075
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Mol. Cell. Proteomics, **2012**, *11,* 422-434
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J. Proteome Res. **2018,** *17*, 2282-2292
J. Proteome Res. **2014,** *13*, 1077-1087
J. Proteome Res. **2012**, *11*, 3986-3995
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Analytical Chemistry ***2022,*** *94,* 12990–12999

Science Transl. Med. **2013**, *5*, 212ra160
Nature Rev. Urol. **2014**, *11*, 278–288
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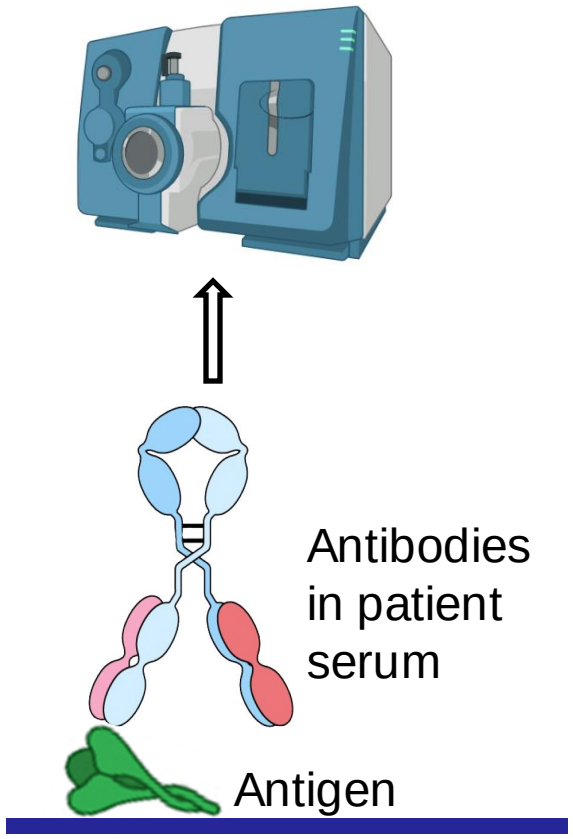
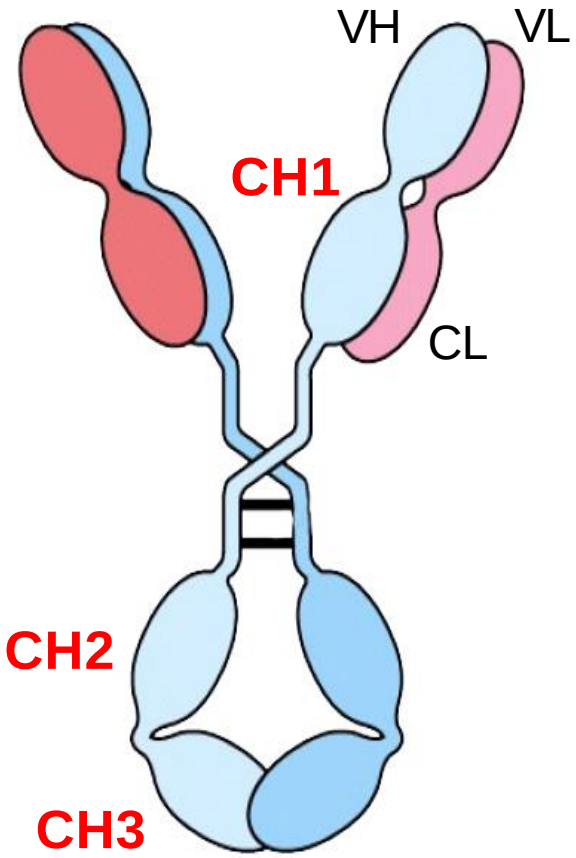


Advantages

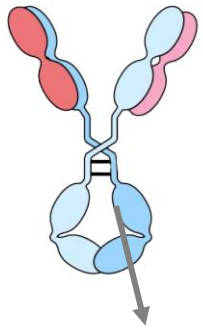
- Direct measurements (lower non-specific binding)
- High selectivity (low cross-reactivity)
- Absolute quantification (ng/mL)
- Standardization
- Multiplexing (IgG1, IgG2, IgG3, IgG4)

Human immunoglobulins

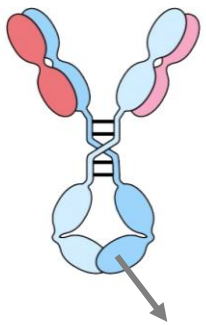


Allotypes and variants were excluded (gnomAD)

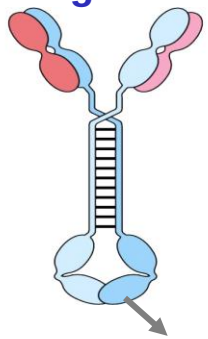
IgG


IgG1

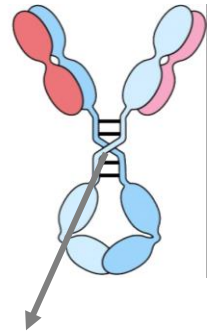
GPSVFPLAPSSK


IgG2

GLPAPIEK

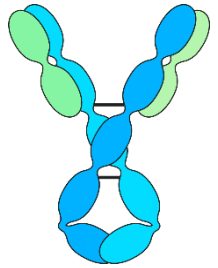

IgG3

WYVDGVEVHNAK

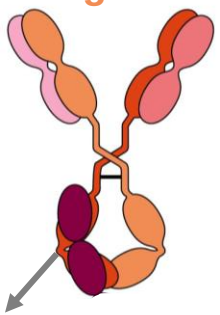

IgG4

TTPPVLDSDGSFFLYSR

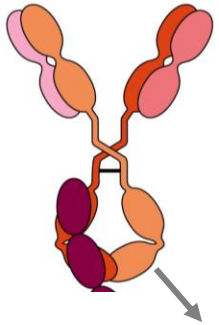
IgE


AVHEAASPSQTVQR

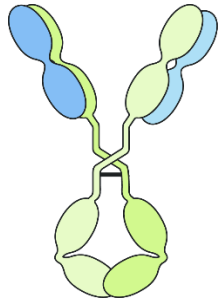
IgA


IgA1

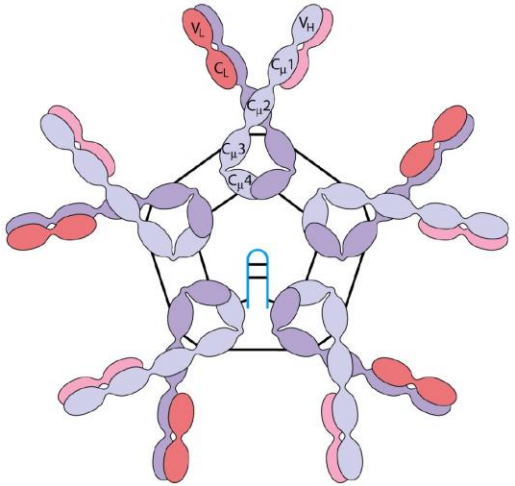
DASGVTFTWTPSSGK


IgA2

DASGATFTWTPSSGK


IgD

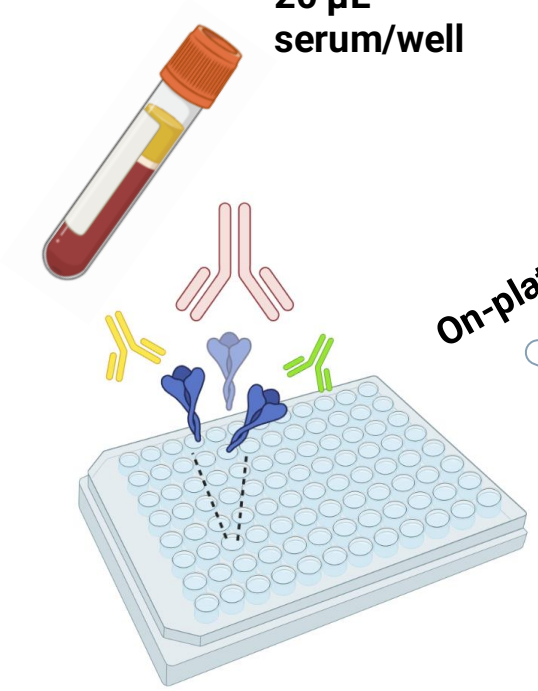
APDVFPIISGCR



IgM

YVTSAPMPEPQAPGR

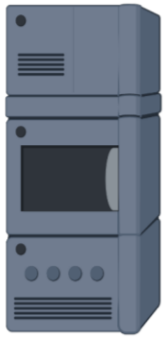
Assay design: Simplicity and Throughput



20 µL serum/well

~0.5 µg antigen/well

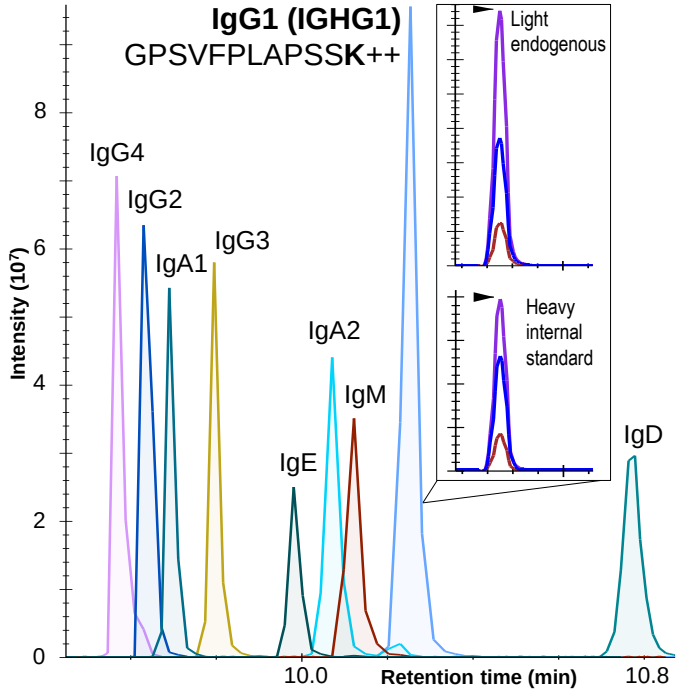
On-plate proteomic sample prep



QTRAP 6500+

12 min runs
~120 replicates/day

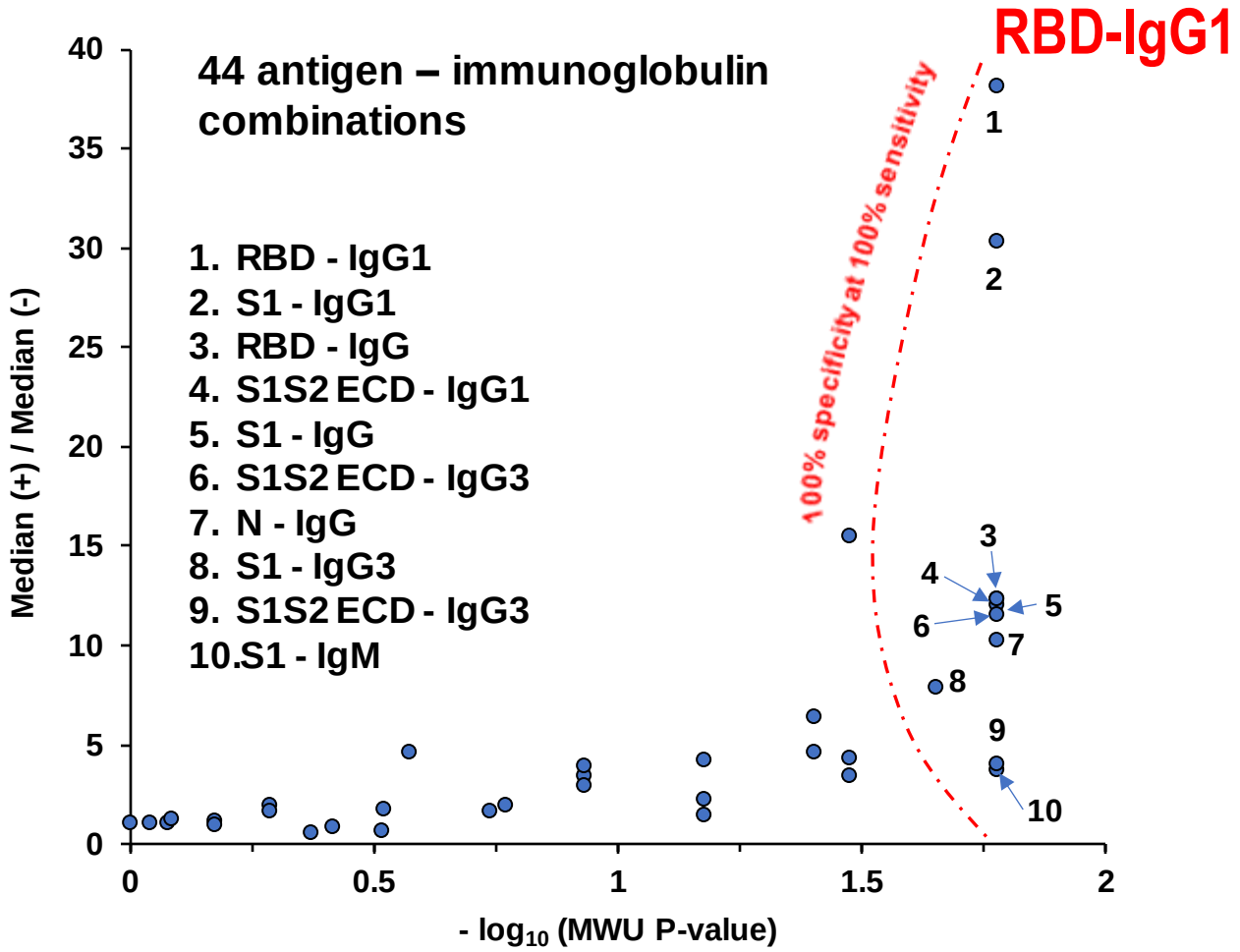
Isotype/Subclass	Internal Standard (fmol/µL)
IGHG1	GPSVFPLAPSS**K**-tql
IGHG2	GLPAPI**E**K-tql
IGHG3	TPLGDTTHT**C**P**R**-tql
IGHG4	GLPSSI**E**K-tql
IgG-Total	NQVSLT**C**LV**K**-tql
IGHA1	DASGVTFTWTPSSG**K**-tql
IGHA2	DASGATFTWTPSSG**K**-tql
IgA-Total	WLQGSQELP**R**-tql
IGHD	APDVFPIISG**C**R-tql
IGHE	AVHEAASPSQTVQ**R**-tql
IGHM	YVTSAPMPEPQAPG**R**-tql



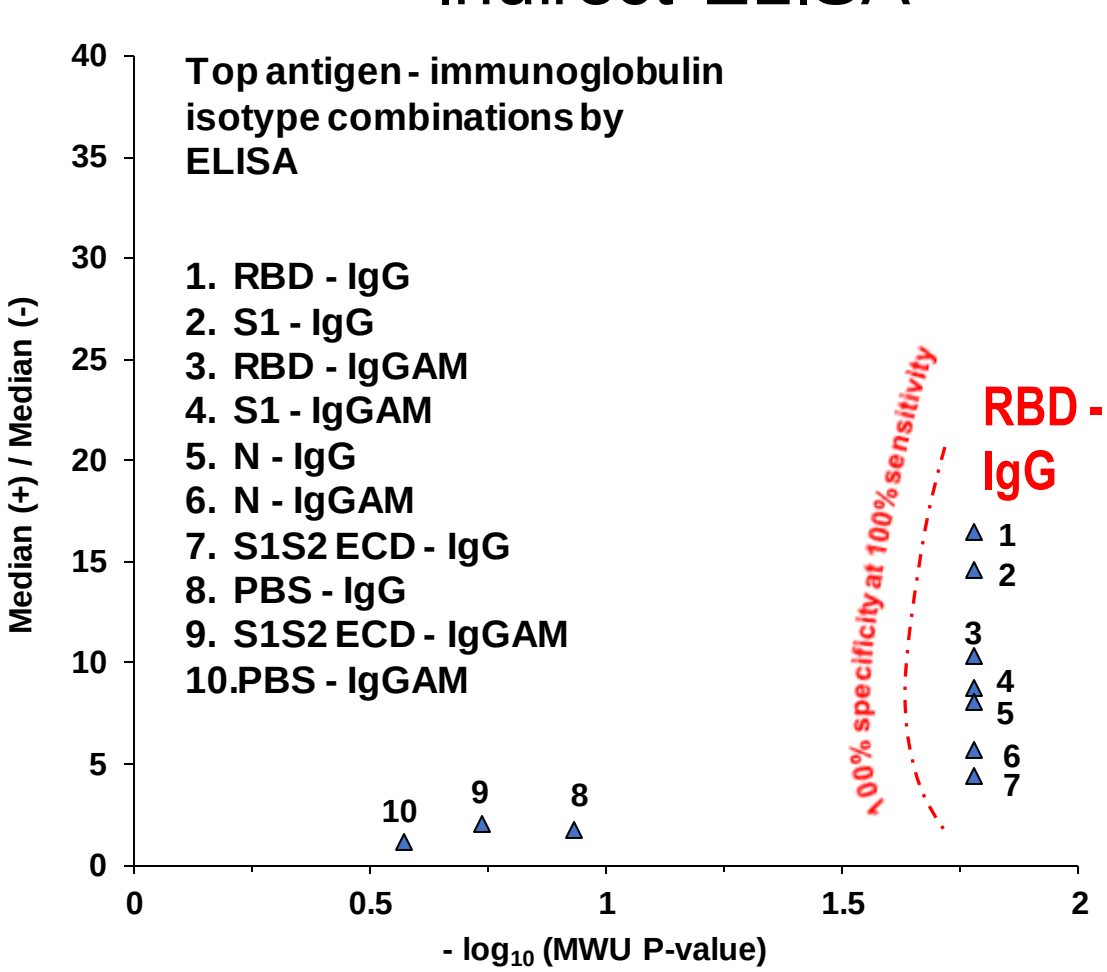
Fu, Z.; Rais, Y.; Dara, D.; Jackson, D; Drabovich, A.P. *Analytical Chemistry* 2022, 94, 12990–12999

Rational Design: RBD-IgG1 is a top combination

IA-Mass Spectrometry



Indirect ELISA



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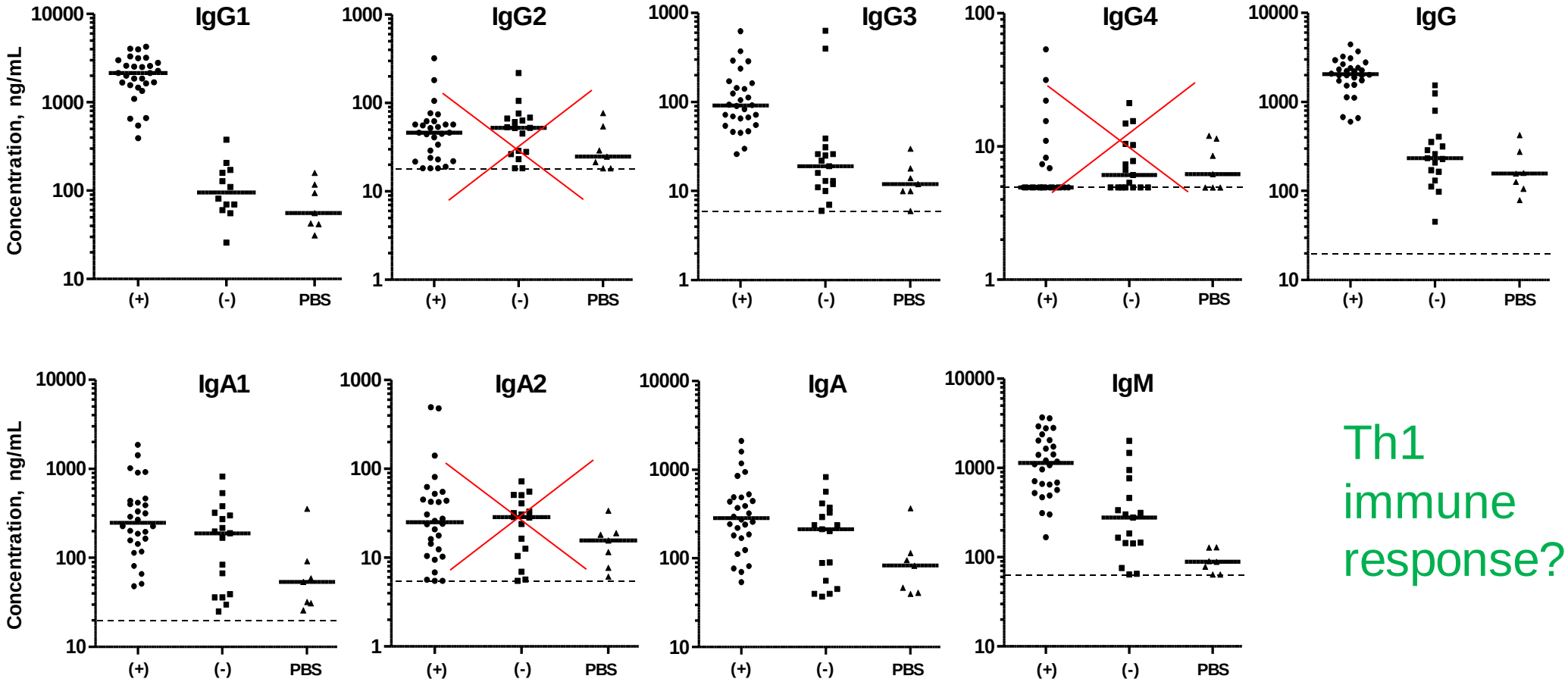
Diagnostic performance: IgG1, IgG3, IgA1, IgM



Th1 immune response?

Positive convalescent (N=29), negative pre-2019 (N=12) and PBS controls (no RBD antigen). Analytical CVs ~3%. Dash lines represent limits of detection.

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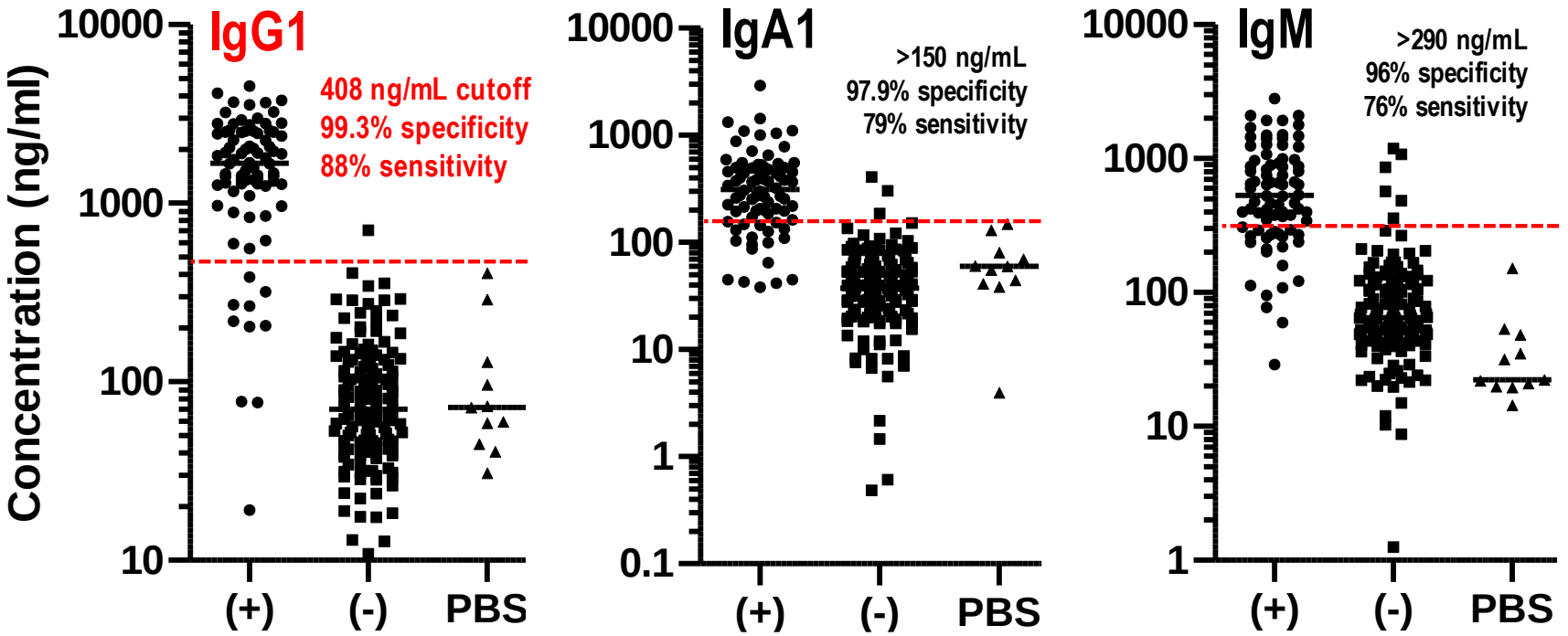
Validation of diagnostic performance by IA-MS

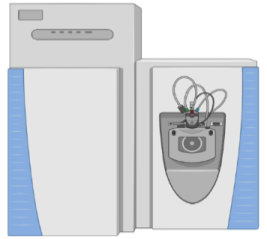


Independent set of positive convalescent plasma (N=82), pre-pandemic plasma/serum (N=149), and assay controls (no-antigen PBS)

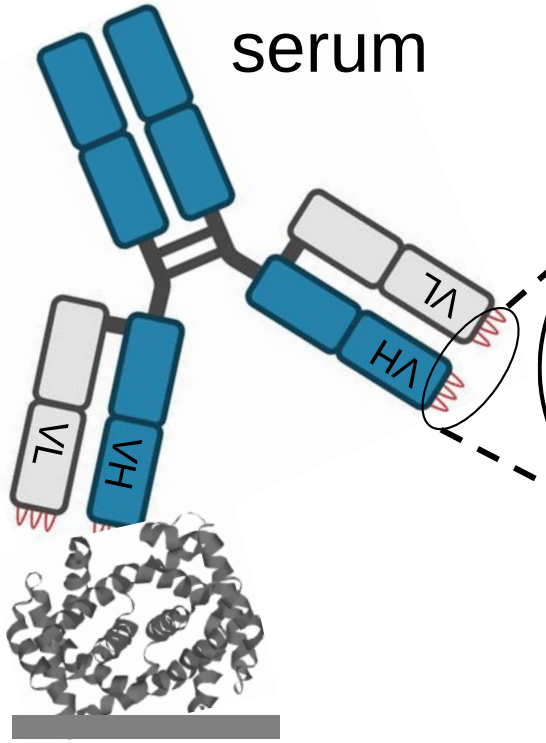
	ng/ml	Sensitivity	Specificity
IgG1	408	88%	99.3%
IgA1	150	79%	97.9%
IgM	290	76%	95.8%
IgG1+IgA1		95.1%	100%
IgG1+IgM		95.1%	100%
IgG1+IgA1+IgM		**96.3%**	**100%**

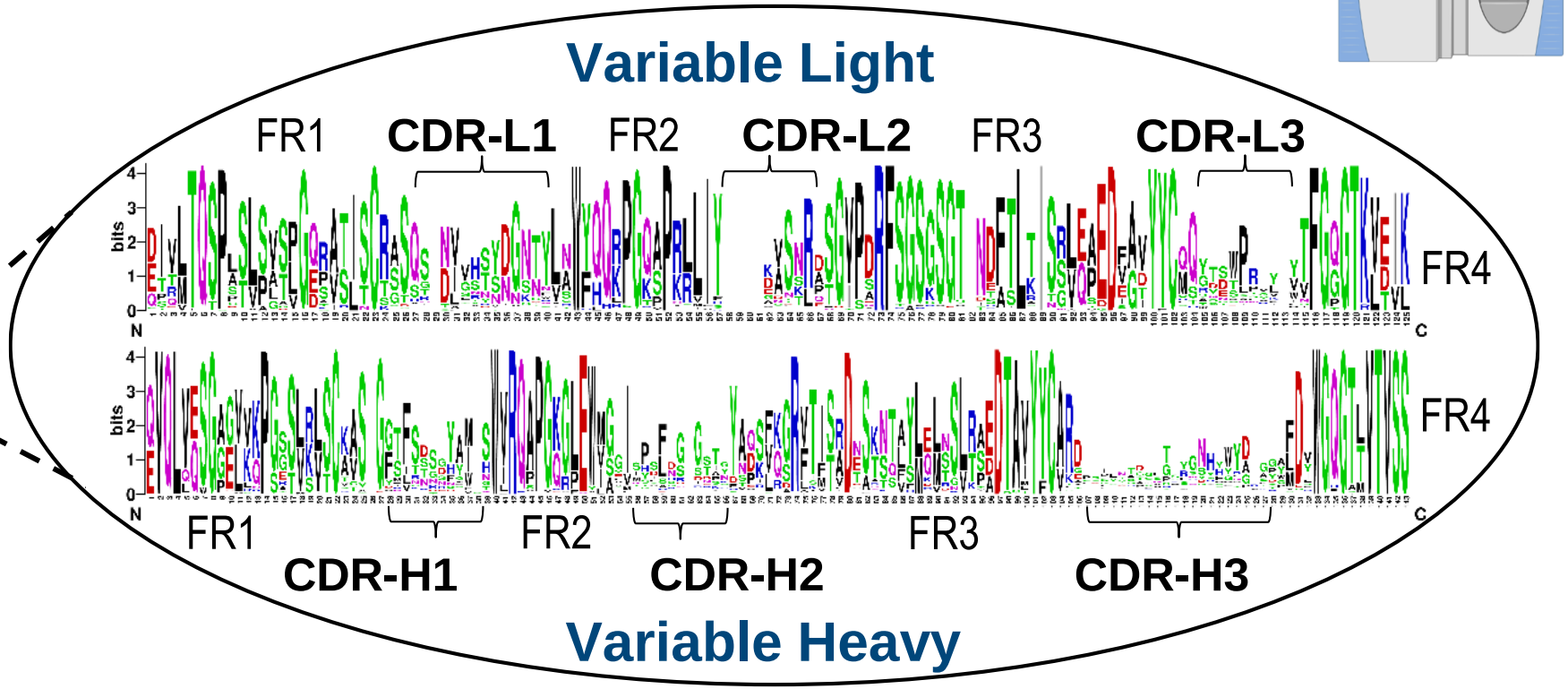
Fu, Z.; Rais, Y.; Dara, D.; Jackson, D; Drabovich, A.P. *Analytical Chemistry* 2022, 94, 12990–12999

Next Steps: Antibody Sequencing by MS



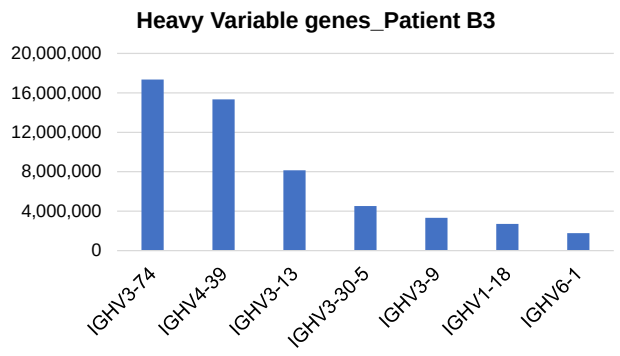
Patient serum

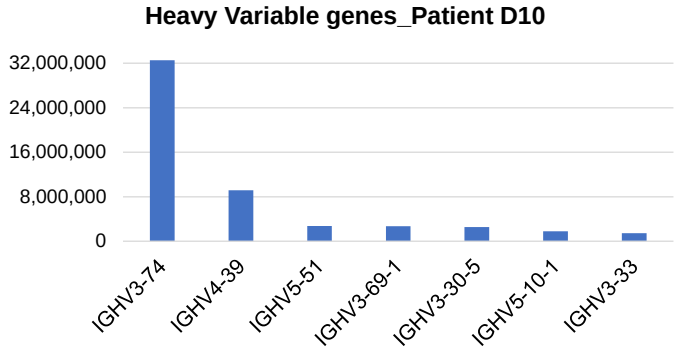


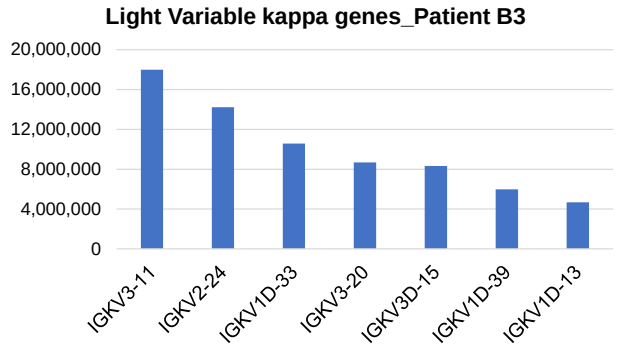


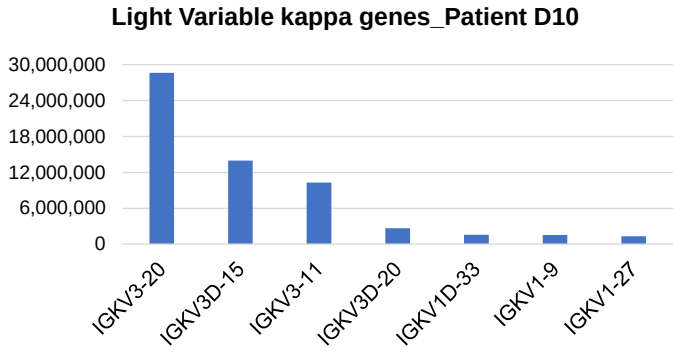
Spike_SARS2 RBD antigen

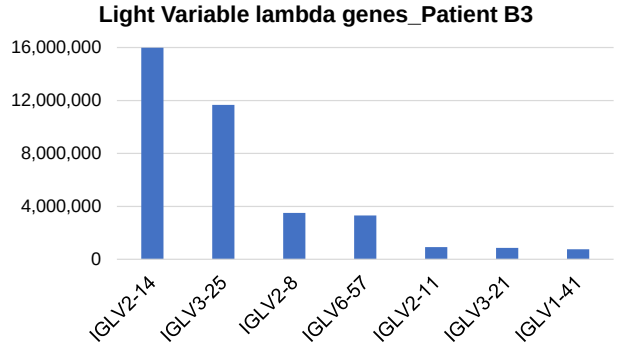
Patient-specific V(D)J engagement

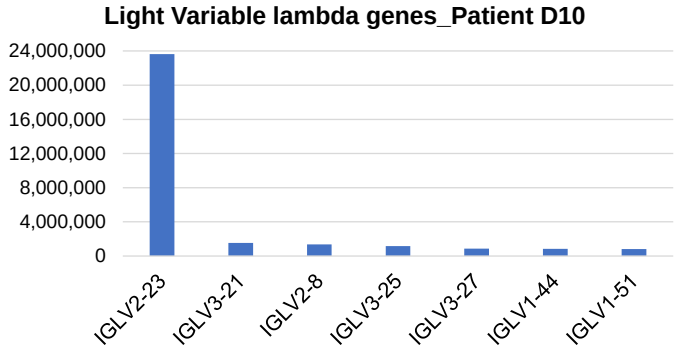


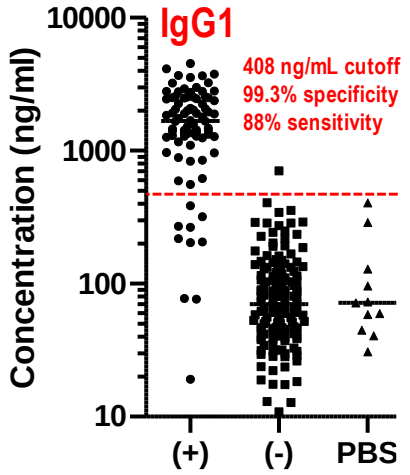


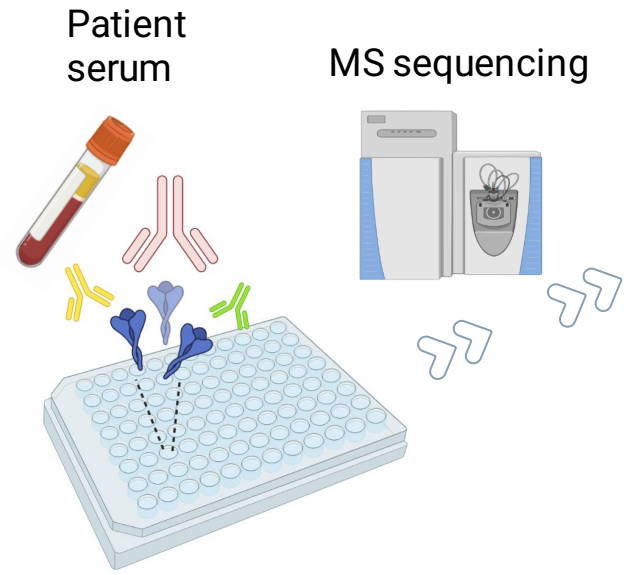












Generation of monoclonal Abs directly from patient sera

Rais, Y.; Drabovich, A.P. *unpublished*

Lessons Learned

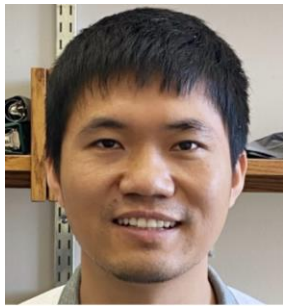
- RBD-IgG1 is a combination with the highest diagnostic performance
- 400 ng/mL IgG1 cut-off provides 99.3% specificity at 88% sensitivity
- IgG1/IgM/IgA1 multiplexing provides ~100% specificity at 96.3% sensitivity
- IgG1 levels in COVID-19 convalescent plasma may reach ~5,000 ng/mL
- "Gold standard" assay based on MS could enable inter-hospital standardization (ng/mL) using stable quantifiable synthetic peptide standard
- Throughput is ~700 samples/week
- Comprehensive investigation of immune response (IgG1, IgG3, IgA1, IgM)
- MS sequencing: engagement of V(D)J genes (precision immunology)

Bottlenecks

- High cost of samples available for research (200$ per 1 mL sera)

Acknowledgments

Dr. Zhiqiang Fu
Yasmine Rais
Delaram Dara



COVID-19 IMMUNITY TASK FORCE
GROUPE DE TRAVAIL SUR L'IMMUNITÉ FACE À LA COVID-19

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