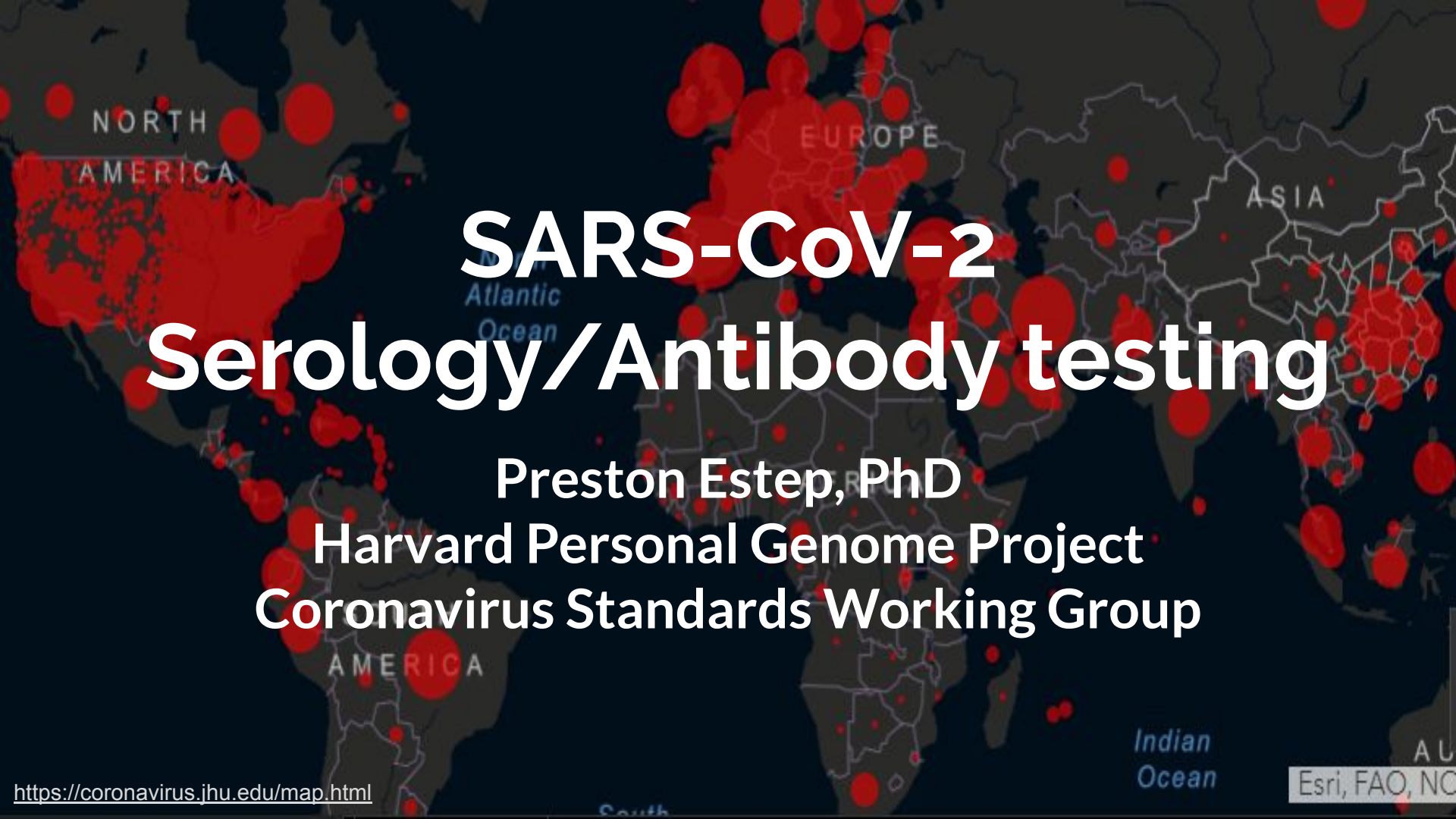
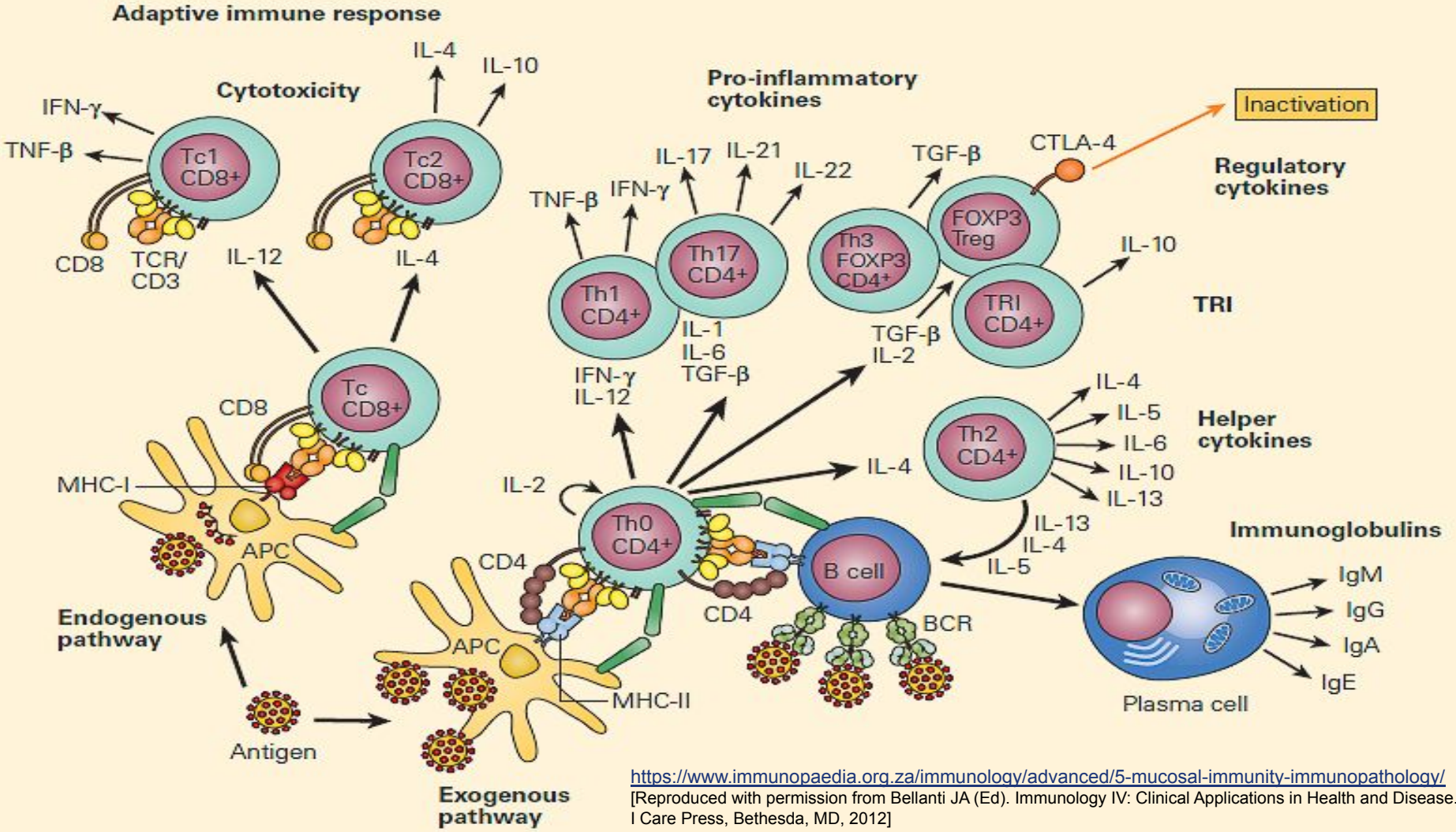


SARS-CoV-2 Serology/Antibody testing

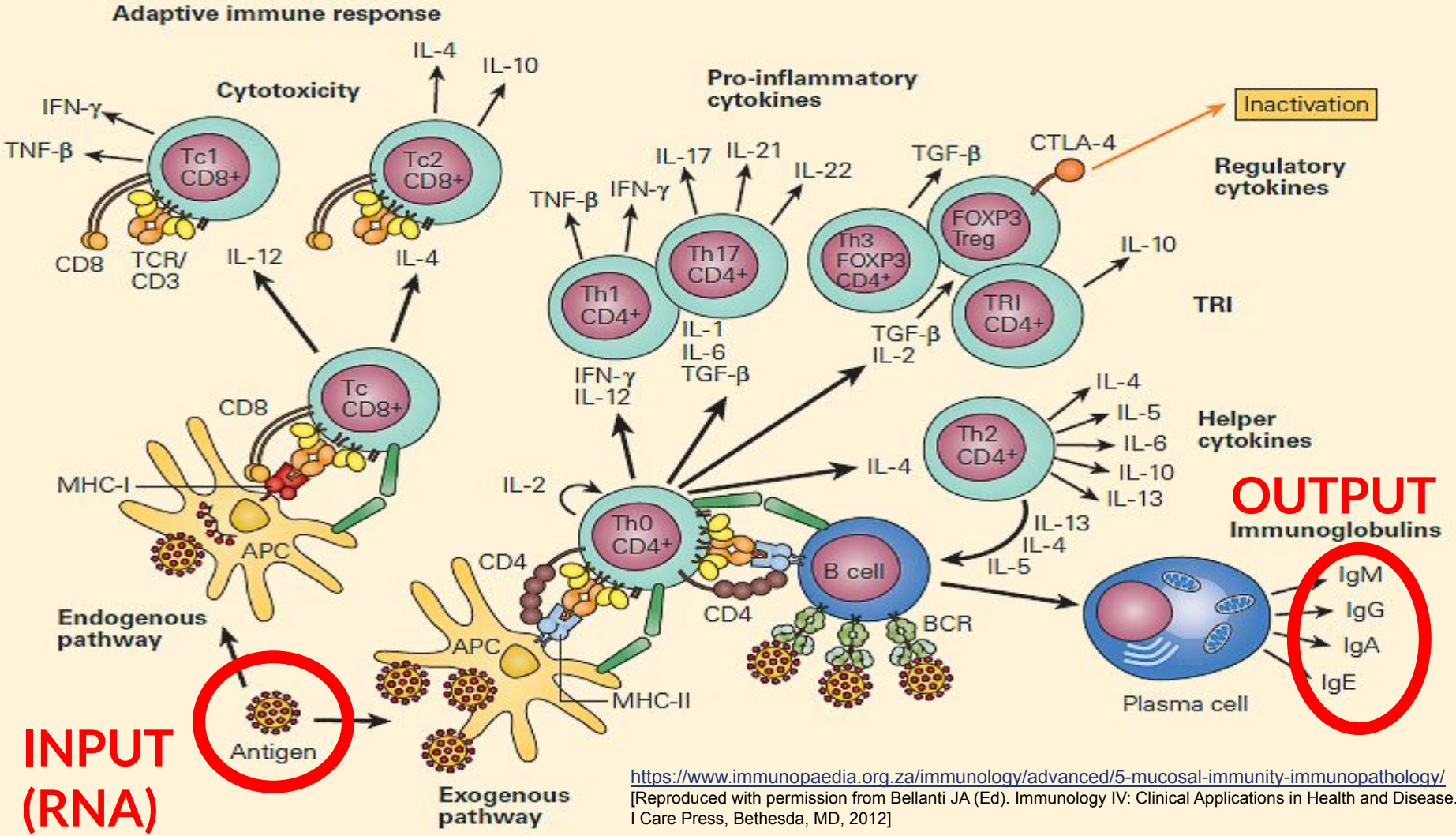
Preston Estep, PhD
Harvard Personal Genome Project
Coronavirus Standards Working Group

https://coronavirus.jhu.edu/map.html



https://www.immunopaedia.org.za/immunology/advanced/5-mucosal-immunity-immunopathology/

[Reproduced with permission from Bellanti JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012]



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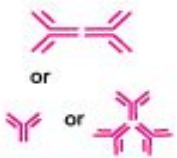
Separation of Systemic and Mucosal Immune Monitoring

Types and Characteristics of Antibodies

IgG		• ~75% of systemic antibody in circulatory system. • Minor fraction of Ab in mucosal tissue.
IgM		• Produced first upon antigen invasion. Increases transiently.
sIgA IgA		• 80+% of antibody in mucosal tissues. • Also expressed systemically at lower levels.

Main Location in the Body

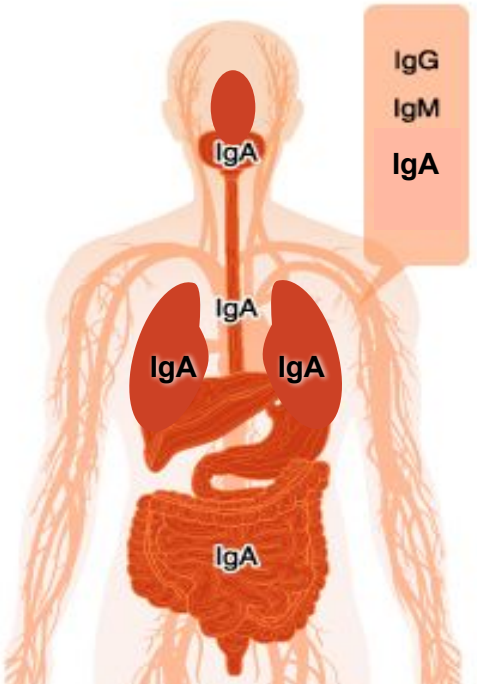
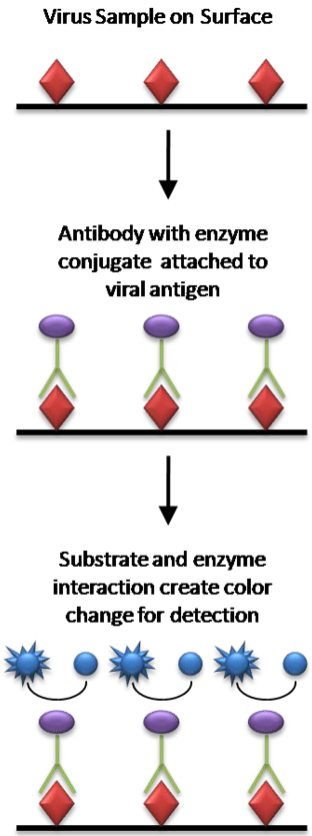
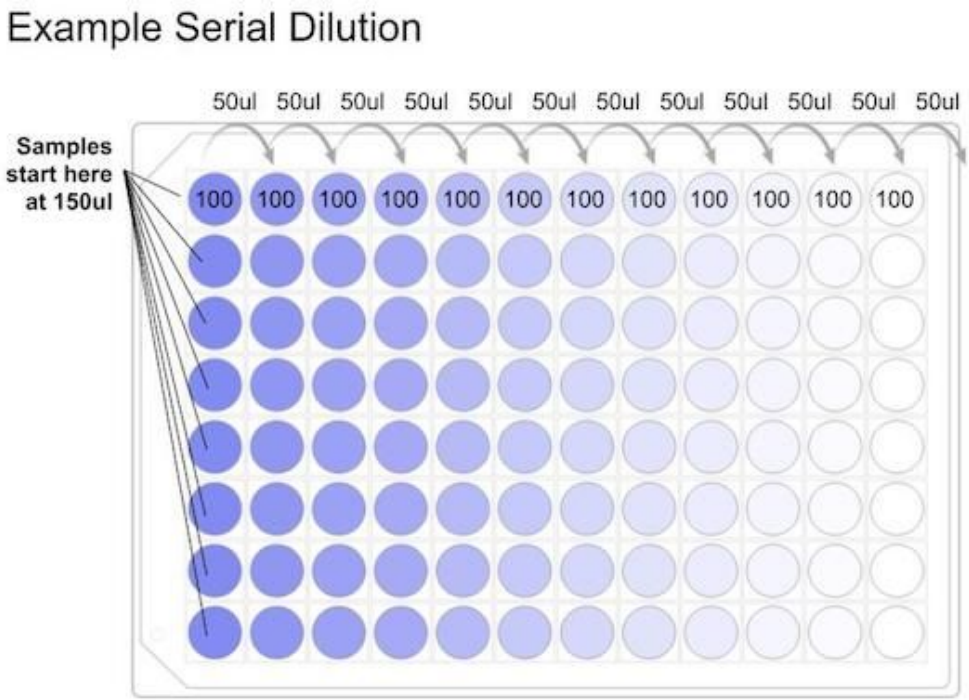


Figure modified from MBL

ELISA, Enzyme-Linked Immunosorbent Assay





User Inputs:

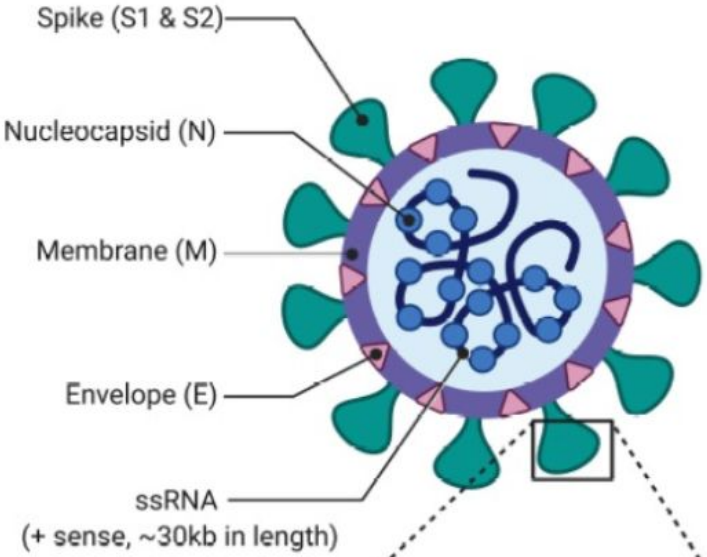
Total Mixing Volume = 150

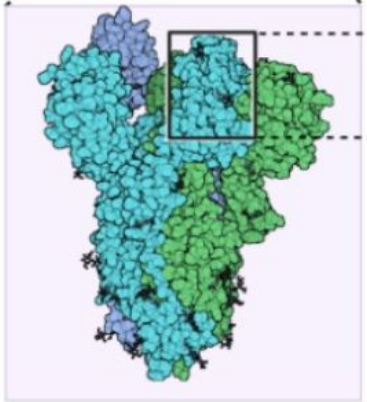
Number of Dilutions = 11

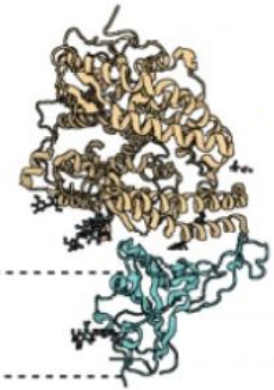
Dilution Factor = 3

[Opentrons example protocol]

SARS-CoV-2 Structure and Proteome







Angiotensin converting enzyme 2 (ACE2)

Receptor binding domain (RBD)

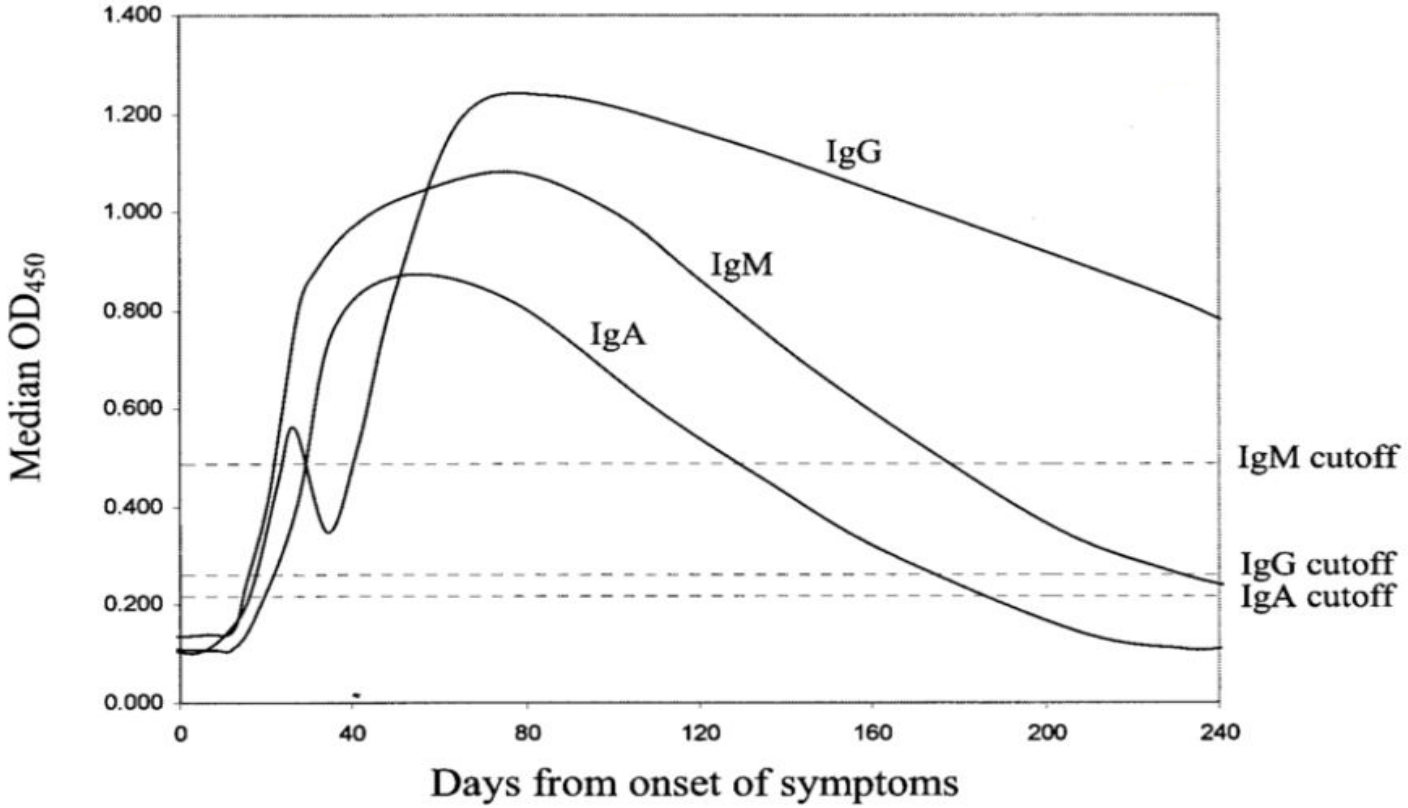
Predicted ORFs in SARS-CoV-2 Genome (~29.8 kb)

nsp1	nsp6	nsp12
nsp2	nsp7	nsp13
nsp3	nsp8	nsp14
nsp4	nsp9	nsp15
nsp5	nsp10	nsp16

27 proteins

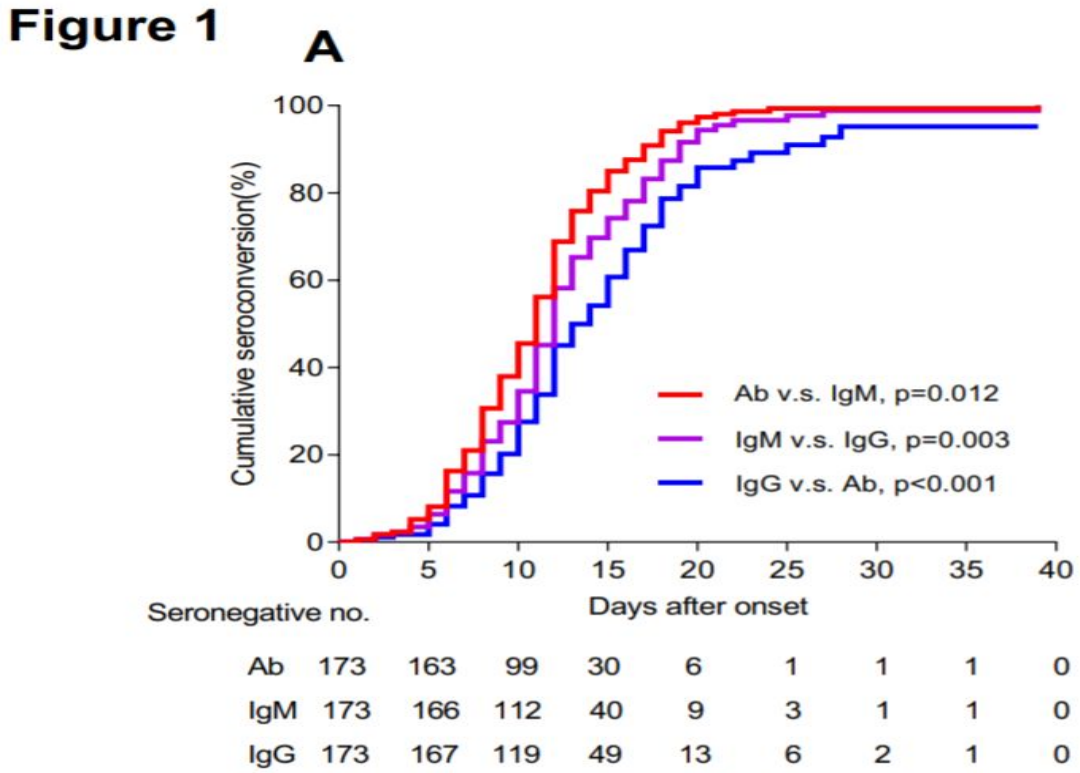
- 4 structure proteins
- 8 accessory proteins
- 15 nonstructural proteins (nsp)

SARS-CoV Antibody Response



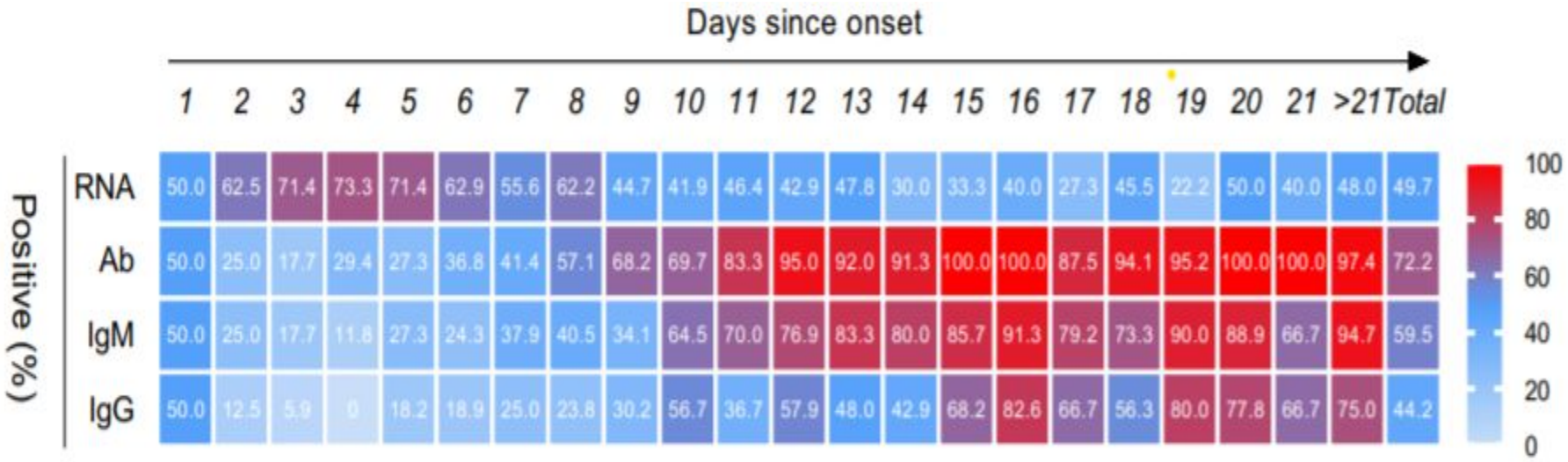
Longitudinal profile of IgG, IgM, and IgA antibodies to SARS-CoV nucleocapsid protein in patients with pneumonia due to SARS-CoV.

SARS-CoV-2 Antibody Response



Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Zhao J et al.

Time Courses of RNA and Antibody Tests

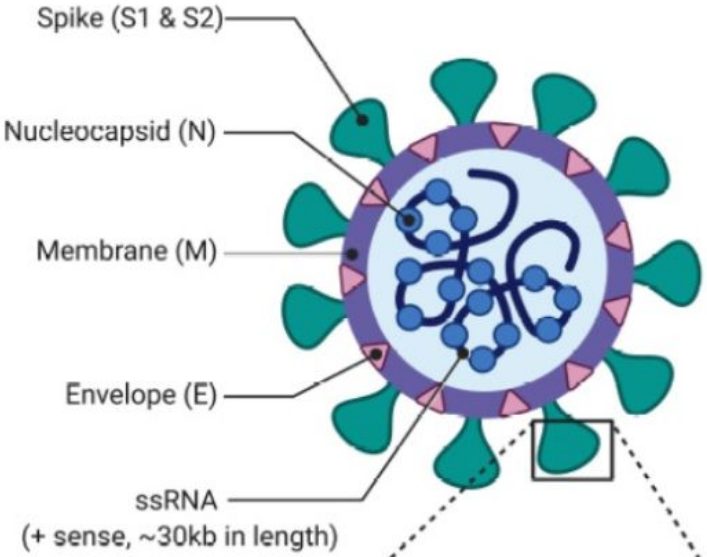


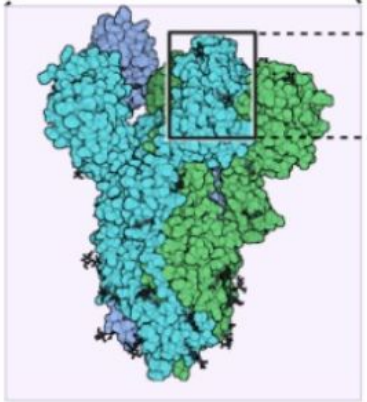
Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Zhao J et al.
Additional info: **Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients,** Yu et al

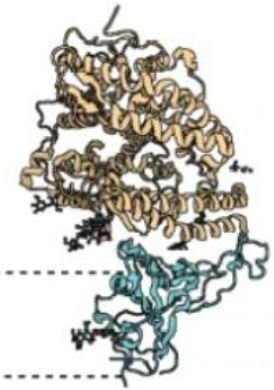
Different Times and Uses for Different Tests

	RNA	Antibody
Initial	Early, at symptom onset, variable False Negative rate	Mid to long term, day 7+, high early False Negative rate. Low but variable FN rate by day 15
Duration	Tapers off, reduced signal by day 10	IgM and IgA, moderate duration IgG, longer term ??
Blood	Ideal, lowest FP, FN	Ideal, lowest FP, FN
Saliva	Moderate to good, higher FN	Poor to Moderate, higher FN
Nasal	Moderate to good, higher FN	Poor to Moderate, higher FN

SARS-CoV-2 Structure and Proteome







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nsp4	nsp9	nsp15
nsp5	nsp10	nsp16

27 proteins

- 4 structure proteins
- 8 accessory proteins
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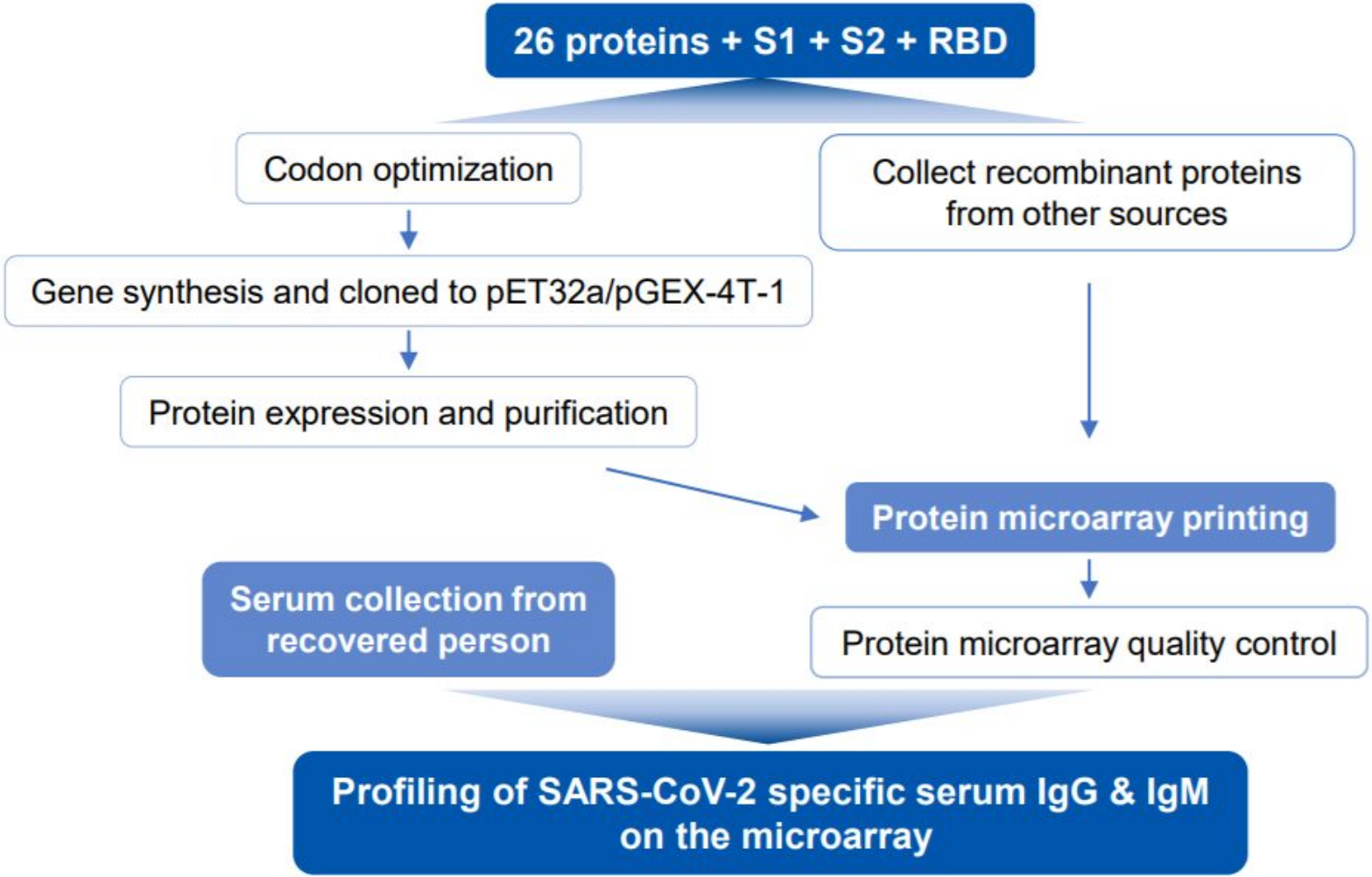
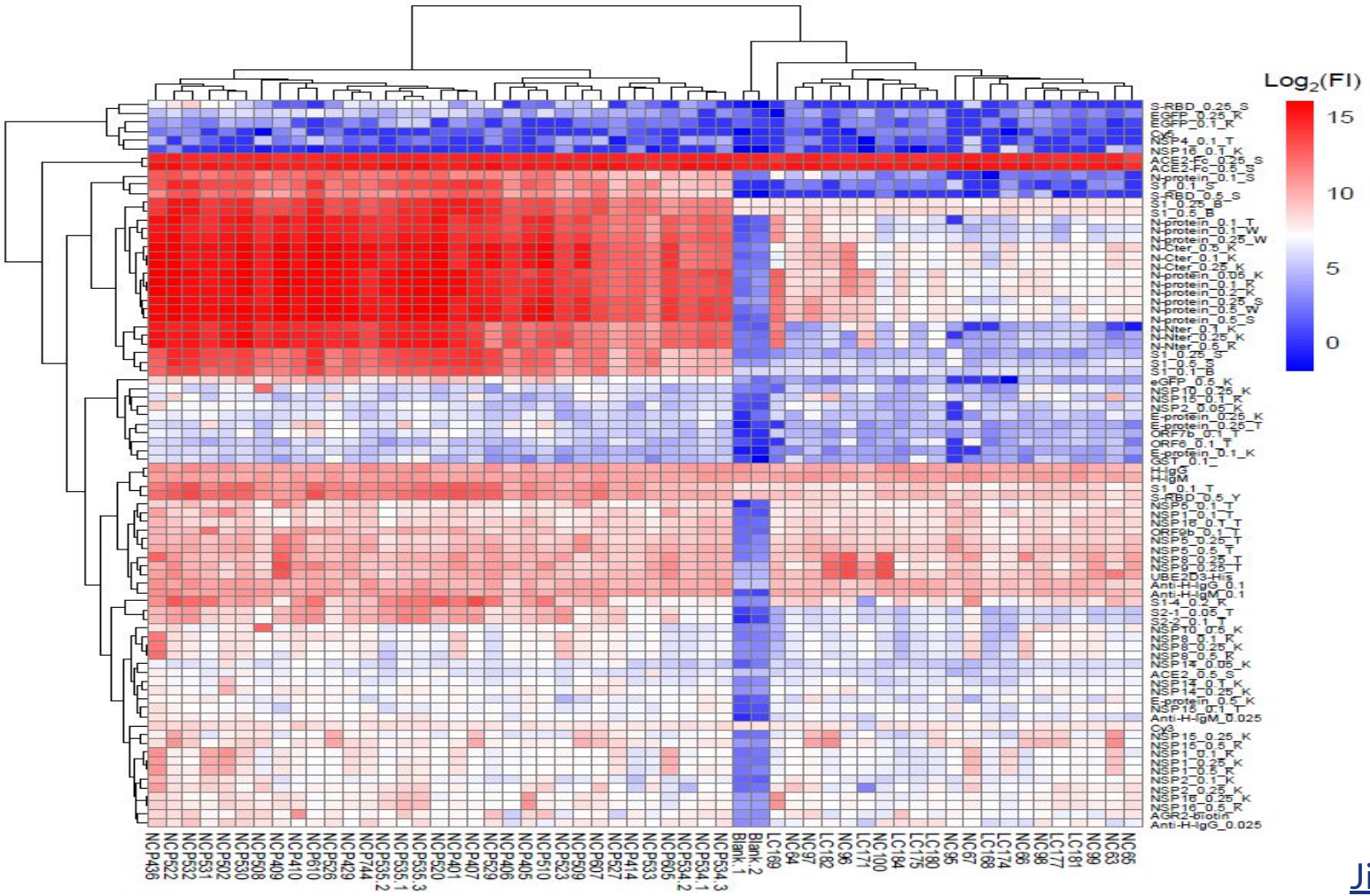
Developing Antibody/Serology Standards - Core

- Current targets (proven for SARS-CoV)
  - Spike protein (S protein)*
  - Spike receptor binding domain (RBD)*
  - Other portions of Spike: S1, S2
  - Nucleocapsid protein (N protein)
    - Most sensitive, but noisier than Spike (SARS, Jiang et al)
- Current discordance, ELISA/IgG
  - Zhao et al showed 100% of patients with later-stage samples showed IgG+IgM seroconversion (Spike-RBD)
  - But Wu et al showed that ~30% of convalescent patients have very low/no titers of neutralizing IgG antibody (Spike RBD, S1, S2)

Amanat et al. A serological assay to detect SARS-CoV-2 seroconversion in humans


26 proteins + S1 + S2 + RBD
Codon optimization
Collect recombinant proteins from other sources
Gene synthesis and cloned to pET32a/pGEX-4T-1
Protein expression and purification
Protein microarray printing
Serum collection from recovered person
Protein microarray quality control
Profiling of SARS-CoV-2 specific serum IgG & IgM on the microarray

Jiang et al


Log2(FI)
15
10
5
0
S-RBD_0.25_S
EGFP_0.25_K
EGFP_0.1_K
CoV3
NSP4_0.1_T
NSP15_0.1_K
ACE2-Fc_0.25_S
ACE2-Fc_0.5_S
N-protein_0.1_S
S1_0.1_S
S-RBD_0.5_S
S1_0.25_B
S1_0.5_B
N-protein_0.1_T
N-protein_0.1_W
N-protein_0.25_W
N-Cter_0.5_K
N-Cter_0.1_K
N-Cter_0.25_K
N-protein_0.05_K
N-protein_0.1_K
N-protein_0.2_K
N-protein_0.25_S
N-protein_0.5_W
N-protein_0.5_S
N-Nter_0.1_K
N-Nter_0.25_K
N-Nter_0.5_K
S1_0.25_S
S1_0.5_S
S1_0.1_B
eGFP_0.5_K
NSP10_0.25_K
NSP15_0.1_K
NSP2_0.05_K
E-protein_0.25_K
E-protein_0.25_T
ORF7b_0.1_T
ORF8_0.1_T
E-protein_0.1_K
GST_0.1_
H-IgG
H-IgM
S1_0.1_T
S-RBD_0.5_Y
NSP5_0.1_T
NSP10_0.1_T
NSP15_0.1_T
ORF9b_0.1_T
NSP5_0.25_T
NSP5_0.5_T
NSP8_0.25_T
NSP9_0.25_T
UBE2D3-His
Anti-H-IgG_0.1
Anti-H-IgM_0.1
S1-1_0.5_K
S2-1_0.05_T
S2-2_0.1_T
NSP10_0.5_K
NSP8_0.1_K
NSP8_0.25_K
NSP8_0.5_K
NSP14_0.05_K
ACE2_0.5_S
NSP14_0.1_K
NSP14_0.25_K
E-protein_0.5_K
NSP15_0.1_T
Anti-H-IgM_0.025
CoV3
NSP15_0.25_K
NSP15_0.5_K
NSP1_0.1_K
NSP1_0.25_K
NSP1_0.5_K
NSP2_0.1_K
NSP2_0.5_K
NSP16_0.25_K
NSP16_0.5_K
AGR2-biotin
Anti-H-IgG_0.025
NCP436
NCP522
NCP532
NCP531
NCP502
NCP530
NCP508
NCP409
NCP410
NCP610
NCP526
NCP429
NCP744
NCP535.2
NCP535.1
NCP535.3
NCP520
NCP401
NCP407
NCP529
NCP406
NCP405
NCP510
NCP523
NCP509
NCP807
NCP527
NCP414
NCP533
NCP805
NCP534.2
NCP534.1
NCP534.3
Blank.1
Blank.2
LC169
NC64
NC97
LC182
NC96
LC171
NC100
LC184
LC175
LC180
NC95
NC67
LC168
LC174
NC66
NC98
LC177
LC181
NC99
NC63
NC65

Jiang et al

Developing Antibody/Serology Standards - more ...

- Epitope mapping
  - Proteome - proteins, low resolution
    - Cross-reactivity to other viruses/pre-existing patient antibodies
  - Proteome/epitopes - Hi resolution; peptide libraries/arrays
    - Convalescent protective antibody epitope mapping
    - Linear epitope testing/target validation (e.g. Spike-RBD epitopes not yet found in convalescent sera; possibly structural rather than linear epitopes)
    - Vaccine epitope selection
- And more… ?

Summary

- RNA-based test is best to detect early infection
- Antibody based tests are essential as people recover
  - Immunity passports
- Technologies:
  - ELISA
  - Lateral flow assays (Cellex) including testing at home
  - Protein and peptide arrays
  - Immunome and PBMC transcriptomics + machine learning
- Open research questions
  - Best protein or peptide targets? S, S1, S2, N, portions?
    - Sensitivity and specificity
    - False positive and negative rates
  - Preexisting coronavirus antibody cross reactivity
- Aspirations: Standards for high quality antibody test of easily collected sample (saliva, nasal or oral swab, etc.)


Thanks and be safe!