A laboratory-developed test (LDT) for COVID-19 serology

- Acute need for COVID-19 antibody testing for patients and HCWs
- Shifting landscape of commercial tests
- Sensitivity/specificity not always clear
- Hard to ‘future-proof’ given high demand and limited supply
- Can we leverage a spike-based research ELISA to develop a clinical test?
Einstein–Montefiore collaboration

Jonathan Lai
Michael Prystowsky
Amy Fox
Louis Weiss
James Faix
Evan Cadoff
Liise-Anne Pirofski
Yitzchak Goldstein
‘COVID Crew’

Chandran Lab
Denise Haslwanter
Ethan Laudermilch
Max Fels
Ariel Wirchnianski
Catalina Florez
Euge Dieterle
Rob Bortz III
Rohit Jangra
Gorka Lasso
‘COVID Crew’

Lai Lab
Olivia Vergnolle
George Georgiev
Ryan Malonis
Margarette Mariano
Karen Tong
Gregory Quevedo
Jose Quiroz

Pirofksi Lab
Antonio Nakouzi
Johanna Rivera
Rachelle Babb

Daily Lab
Amanda Mengotto
Christine Shen
Reise Sample
Duncan Kimmel
Andrea Bae
Rosa Park
Johanna Rivera
Rachelle Babb

Pathology/MMC
Sean Campbell
Erika Orner
Wendy Szymczak
Producing SARS-CoV-2 spike protein at scale

PDB: 6VSB (McLellan Lab)

Turning ELISA curves into a single cut-off value for Dx

Serum IgG

R. Bortz, E. Laudermilch, A. Wirchnianski, C. Florez
Turning ELISA curves into a single cut-off value for Dx

Serum IgA

R. Bortz, E. Laudermilch, A. Wirchnianski, C. Florez
Screening COVID-19–convalescent and control cohorts

IgG (A_{450})

Convalescents (n=224)
Jan 2020 (n=45)
Pre-COVID (n=207)

Serum 1:1,000

Mean+4SD

R. Bortz, E. Laudermilch, A. Wirchnianski, C. Florez
Screening COVID-19–convalescent and control cohorts

Serum 1:1,000 IgG ($A_{450}$)

Convalescents ($n=118$)

Jan 2020 ($n=45$)

Pre-COVID ($n=207$)

Mean+4SD

R. Bortz, E. Laudermilch, A. Wirchnianski, C. Florez
### Performance characteristics of IgG and IgA tests

#### IgG

<table>
<thead>
<tr>
<th>Sample cohort</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>% Positive</th>
<th>% Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convalescent</td>
<td>197</td>
<td>27</td>
<td>224</td>
<td>87.9%</td>
<td>12.1%</td>
</tr>
<tr>
<td>Control (Jan 2020)</td>
<td>1</td>
<td>44</td>
<td>45</td>
<td>2.2%</td>
<td>97.8%</td>
</tr>
<tr>
<td>Control (Pre-COVID)</td>
<td>7</td>
<td>200</td>
<td>207</td>
<td>3.4%</td>
<td>96.6%</td>
</tr>
<tr>
<td>All controls</td>
<td>8</td>
<td>244</td>
<td>252</td>
<td>3.2%</td>
<td>96.8%</td>
</tr>
</tbody>
</table>

#### IgA

<table>
<thead>
<tr>
<th>Sample cohort</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>% Positive</th>
<th>% Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convalescent</td>
<td>75</td>
<td>43</td>
<td>118</td>
<td>63.6%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Control (Jan 2020)</td>
<td>1</td>
<td>44</td>
<td>45</td>
<td>2.2%</td>
<td>97.8%</td>
</tr>
<tr>
<td>Control (Pre-COVID)</td>
<td>2</td>
<td>72</td>
<td>74</td>
<td>3.4%</td>
<td>96.6%</td>
</tr>
<tr>
<td>All controls</td>
<td>8</td>
<td>244</td>
<td>252</td>
<td>3.2%</td>
<td>96.8%</td>
</tr>
</tbody>
</table>

- IgG→IgA testing identifies more positives in convalescent cohort
Performance characteristics in hospitalized cohort

- IgG can be reliably detected 7–9 days post Sx
- Almost all patients develop serological evidence of infection

C. Florez, E. Orner
• IgA can be reliably detected 7–9 days post Sx

• Almost all patients develop serological evidence of infection
Spike-based Dx test distinguishes SARS2 from hCoVs

Confirmed COVID

Confirmed hCoV

SARS2

MERS

HKU1

E. Laudermilch, C. Florez, R. Bortz, A. Wirchnianski
SARS2 specificity with larger Swedish cohort of pre-COVID swab-confirmed hCoV cases

E. Laudermilch, C. Florez, R. Bortz, A. Wirchnianski. Umeå Univ: Julia Wigren, Mattias Forsell
Where do things stand?

- Validated against Wadsworth NYS test (N antigen/Luminex)
- Validated against Columbia LDT (S and N antigens/ELISA)
- Larger cross-validation study at MMC almost complete (S. Forest)
- Application for NYS emergency use authorization (EUA) under review (Weiss, Fox, Faix)
- Assay has been ported to automated format in MMC Pathology CLIA-certified lab and cross-validated (Campbell, Faix)
- Deployment is expected in the near future
Convalescent plasma appears to have value in improving outcomes in moderate-severely ill COVID-19 patients.

Antibody therapeutics shown to be effective against other viral diseases.

Can the same approach be applied to COVID-19?
Prometheus: Developing human mAb therapeutics and prophylactics against WHO Blueprint pathogens

Adimab, LLC
Laura Walker
Anna Wec

Ben-Gurion University
Leslie Lobel

Mapp Biopharmaceutical
Zachary Bornholdt
Larry Zeitlin

Washington State Univ
Bronwyn Gunn

Wistar Institute
David Weiner
Ami Patel

Institut Pasteur
Félix Rey

Umeå University
Mattias Forsell
Clas Ahlm

University of New Mexico
Steven Bradfute

University of Texas-Austin
Jason McLellan

US Army Medical Institute of Infectious Diseases
John Dye
Andrew Herbert

Universidad del Desarrollo
Cecilia Vial
Pablo Vial

University of Helsinki
Tomas Strandin

Einstein
Kartik Chandran
Eva Mittler
Rohit Jangra
Jonathan Lai
Johanna Daily
Human convalescent donor

FACS isolation of single viral Ag-positive B cells

Viral Ag-specific memory B cells

VH/VL PCR products from single B cells

Yeast recombinational cloning

Fluorescently-labeled soluble GP (viral Ag) or rVSV-GP

Viral binding & neutralization screens

mAb:Ag affinity by high-throughput biolayer interferometry

IgG secretion by yeast & high-throughput protein A affinity purification

Sequencing of good expressors & computational analysis of VH/VL sequences
Recombinant soluble spike antigen for B-cell sorting

Jason McLellan, U Texas at Austin
PBMC from convalescent donors, Bronx March–April 2020

![Graph showing ELISA titers and SARS-CoV-2-specific IgG B cell percentages for different donors labeled EMC9 to EMC8.](image)
Characterization of SARS2 spike-specific mAbs

SHM of SARS-CoV-2 binders

Clonal lineages across donors

Note: Clones in gray belong to unique clonal lineages

Adimab: A. Wec, L. Walker
Characterization of SARS2 spike-specific mAbs

SHM of SARS-CoV-2 binders

VH germline gene usage of SARS2 binders

*Briney et al., Nature (2019)

Adimab: A. Wec, L. Walker
BSL-2 assay needed to rapidly screen for neutralizing antibodies
Recombinant VSVs expressing Ebola virus GP

Ebola virus

rVSV-GP
Recombinant VSVs expressing Ebola virus GP

rVSV-GP
Recombinant VSVs expressing Ebola virus GP

rVSV-GP
Recombinant VSVs expressing Ebola virus GP
rVSV expressing the SARS-CoV-2 spike

rVSV-SARS1 d19

rVSV-SARS2 wt
rVSV-SARS2 S passage selects for higher viral titers and a non-syncytiogenic replication phenotype
rVSV-SARS2 S passage selects for higher viral titers and a non-syncytiogenic replication phenotype
rVSV-SARS2 S is suitable for studies of viral entry and antibody neutralization.

\[ R^2 = 0.76 \]
High-throughput neut screening of COVID-19 donor mAbs

E. Dieterle, D. Haslwanter, R. Jangra
Identification of an ultrapotent NAb targeting the spike RBD

![Graph showing log mAb [nM] vs. % rVSV-SARS2 Infection (no mAb = 100%)](image)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI-56443</td>
<td>0.01973</td>
</tr>
<tr>
<td>ADI-56539</td>
<td>0.5118</td>
</tr>
<tr>
<td>ADI-56516</td>
<td>0.4992</td>
</tr>
<tr>
<td>ADI-56509</td>
<td>0.5961</td>
</tr>
<tr>
<td>ADI-56474</td>
<td>1.435</td>
</tr>
<tr>
<td>ADI-56453</td>
<td>3.941</td>
</tr>
<tr>
<td>ADI-56519</td>
<td>0.9999</td>
</tr>
<tr>
<td>ADI-56517</td>
<td>0.5261</td>
</tr>
<tr>
<td>ADI-56515</td>
<td>7.550</td>
</tr>
<tr>
<td>ADI-56519</td>
<td>4.664</td>
</tr>
<tr>
<td>ADI-56519</td>
<td>0.4355</td>
</tr>
<tr>
<td>ADI-56468</td>
<td>0.5272</td>
</tr>
<tr>
<td>ADI-56448</td>
<td>12.39</td>
</tr>
<tr>
<td>ADI-56532</td>
<td>5.055</td>
</tr>
<tr>
<td>ADI-56525</td>
<td>49.60</td>
</tr>
<tr>
<td>ADI-56467</td>
<td>2.903</td>
</tr>
<tr>
<td>Ctrl ADI-55688</td>
<td>0.1436</td>
</tr>
</tbody>
</table>

E. Dieterle, D. Haslwanter, R. Jangra
Identification of an ultrapotent NAb targeting the spike RBD

![Graph showing the % rVSV-SARS2 Infection (no mAb = 100%) against Log mAb [nM] for various mAbs and control.]

<table>
<thead>
<tr>
<th>mAb Code</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI-56443</td>
<td>0.01973</td>
</tr>
<tr>
<td>ADI-56448</td>
<td>12.39</td>
</tr>
<tr>
<td>ADI-56453</td>
<td>0.5272</td>
</tr>
<tr>
<td>ADI-56467</td>
<td>2.903</td>
</tr>
<tr>
<td>ADI-56468</td>
<td>0.5261</td>
</tr>
<tr>
<td>ADI-56474</td>
<td>1.435</td>
</tr>
<tr>
<td>ADI-56479</td>
<td>0.4355</td>
</tr>
<tr>
<td>ADI-56488</td>
<td>140 pM</td>
</tr>
<tr>
<td>Ctrl ADI-55688</td>
<td>0.1436</td>
</tr>
</tbody>
</table>

E. Dieterle, D. Haslwanter, R. Jangra
Where do we go from here?

- Screen and identify most potent NAbs from larger panel (>300)
- Further characterize NAbs (epitope, structural mode of binding, virologic mechanism of action)
- Optimize NAbs (binding affinity, stability, ‘developability’)
- Identify synergistic combinations and down-select to lead cocktails
- Discover novel, potent non RBD-binding NAbs (via use of alternative sorting antigens, other discovery approaches)
- Evaluate in animal challenge model(s) (WT Syrian hamster)
- Initiate preclinical development
Acknowledgments: Chandran Lab
Acknowledgments: Funding

[Logos for NIH, Albert Einstein College of Medicine, Montefiore, Fight NPC, and Defense Threat Reduction Agency]