Serum Antibody Responses to Cholera: The *sero* in *sero*epidemiology

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Goals for session

- **Talk**
  1. Review the basis of testing serologic responses to *V. cholerae* infection
  2. Consider technical issues for seroepidemiologic studies, focusing on standardization
- **Discussion**
  1. What are some the potential advantages and challenges of seroepidemiologic studies of cholera disease burden? Similarly, what are the most and least useful likely applications?
  2. What are potential ways to improve approaches to serologic studies?
  3. Might these tools be considered part of a ‘laboratory package’ made available on request to improve research capacity in countries with a significant cholera burden?

V. cholerae infection confers durable protection

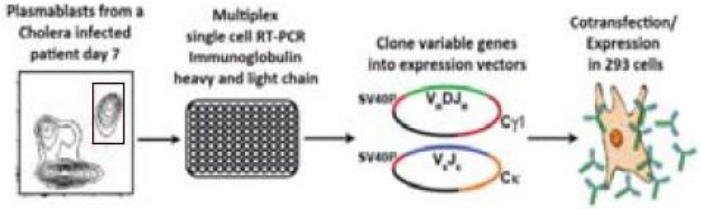
- Human challenge studies – U. of Maryland
- Longitudinal studies in endemic areas - Matlab
- Modeling of epidemiologic data in endemic areas
- Degree of protection appears in part to depend on serotype and other bacterial related factors
- Protection lasts longer than robust circulating (serum) antibody response (e.g. no perfect serum marker of past infection or immunity yet exists)

1. The antigenic repertoire of *V. cholerae?*

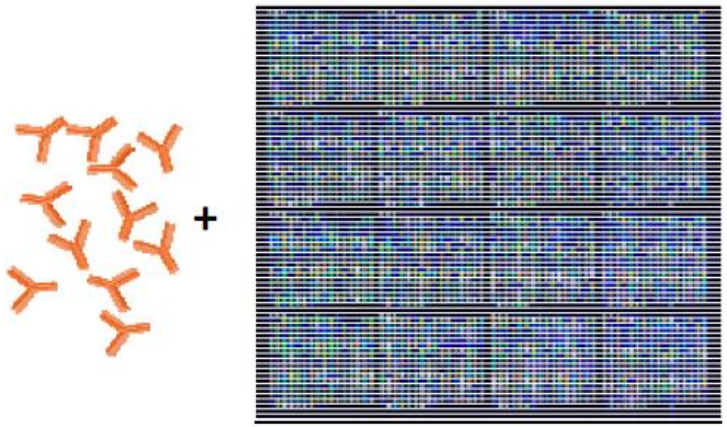
What is the antigenic repertoire of *V. cholerae*?

- Vibriocidal antibodies – largely target LPS
- Cholera toxin – primarily B subunit
- TcpA
- We evaluated plasmablast responses

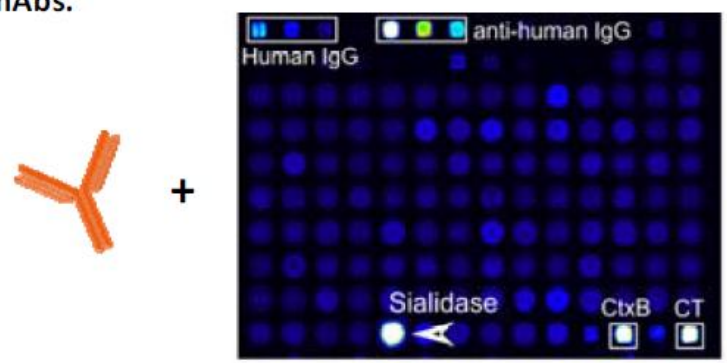
1. We generated 138 mAbs from patient plasmablasts.



2. We probed full 3,647 ORF arrays with pooled mAbs to generate a targeted antigen array.

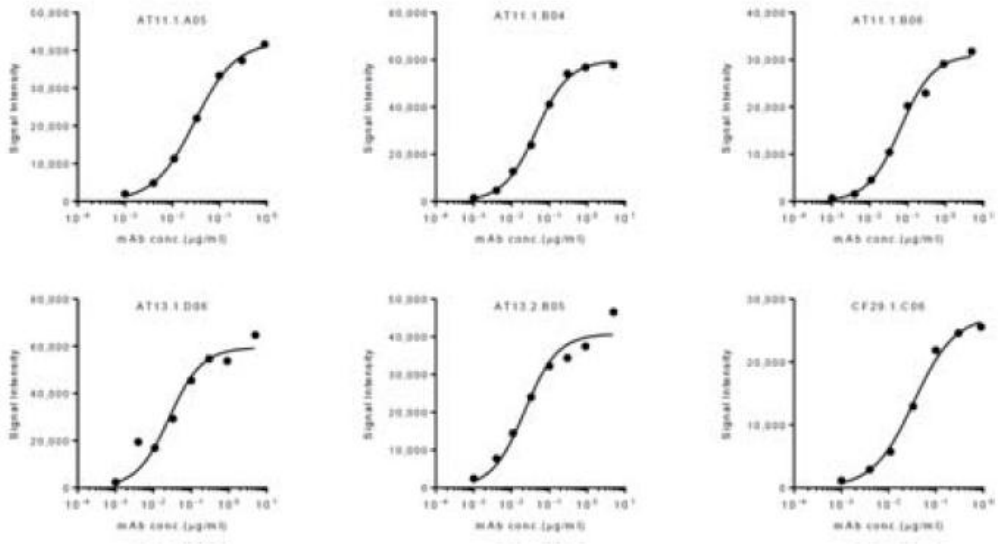


3. We probed a targeted 100 antigen array with individual mAbs.

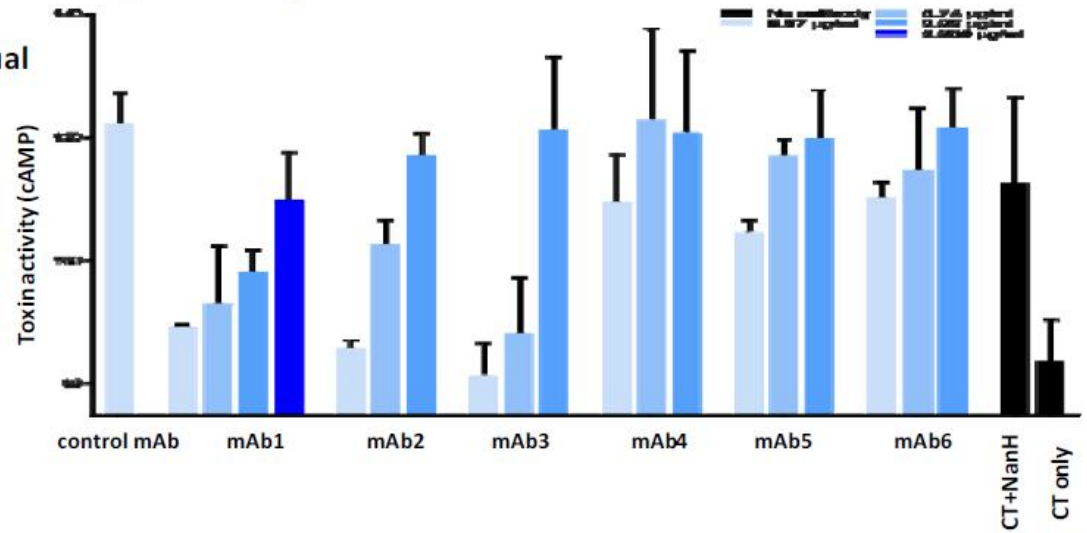


4. We identified six individual sialidase specific mAbs.

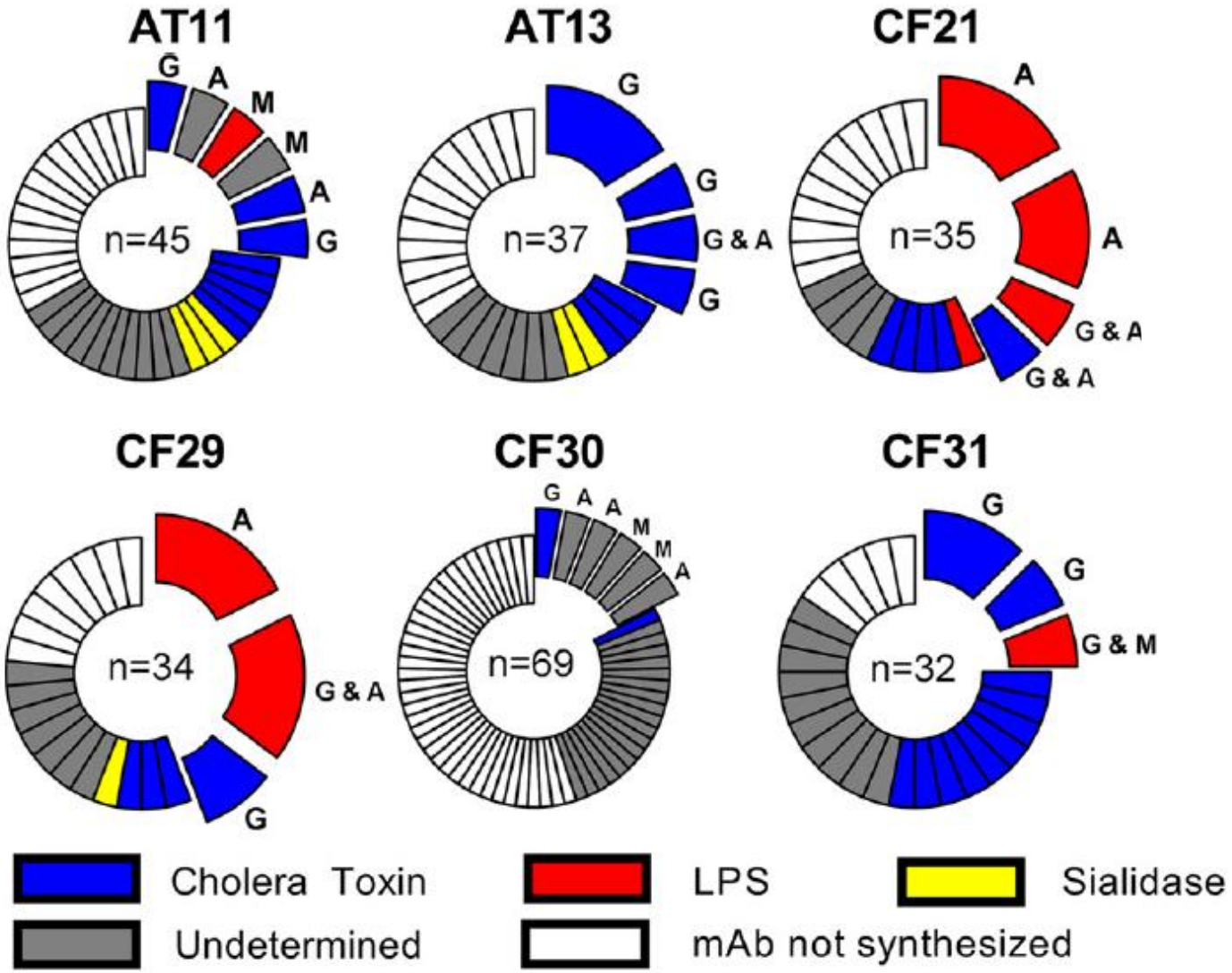
V. cholerae Sialidase specific mAb binding kinetics

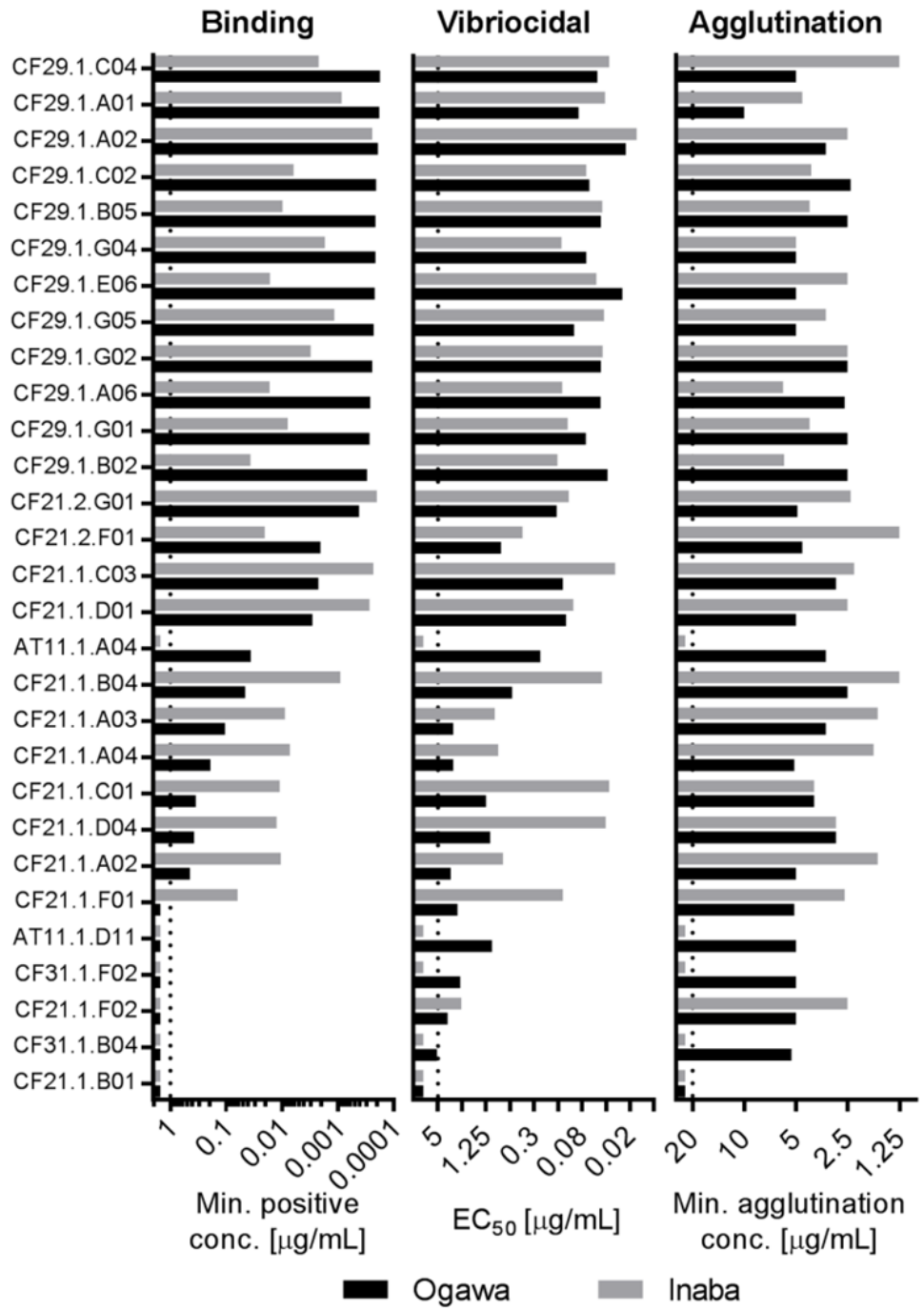


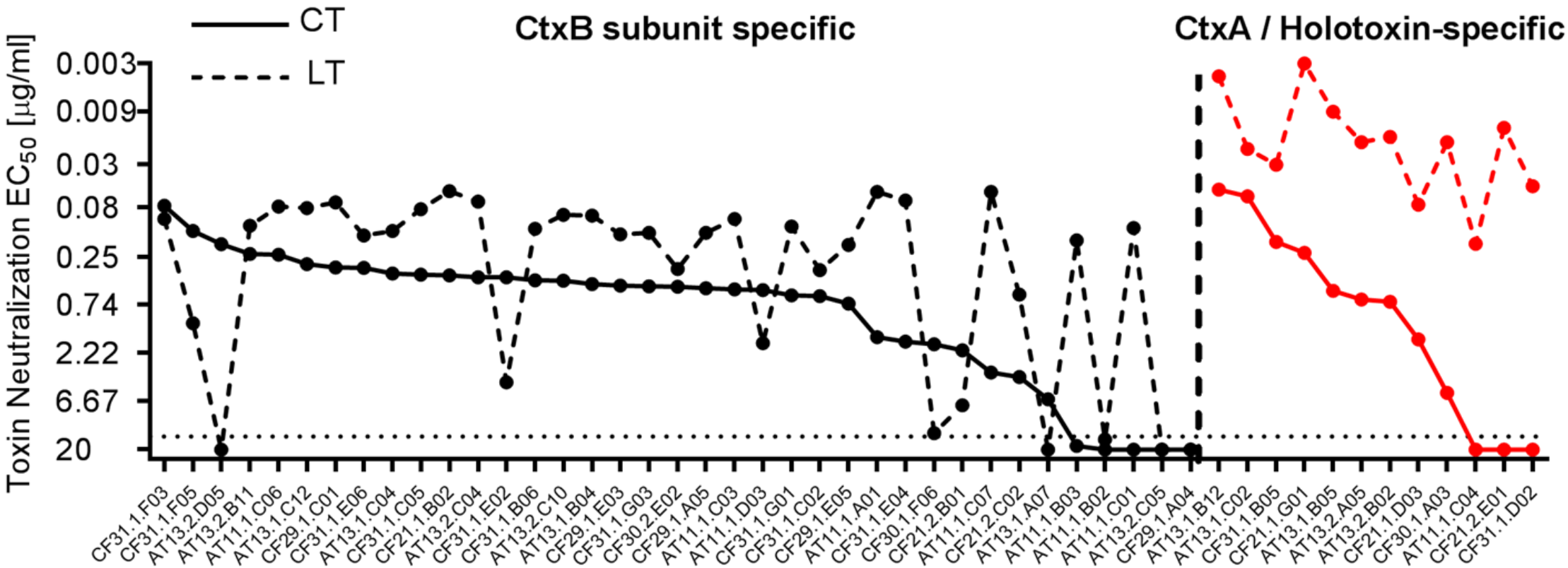
5. We demonstrated that anti-sialidase mAbs block the cholera toxin potentiating effects of sialidase in vitro.



V. cholerae antigens: O antigen and toxin.




Binding
Vibriocidal
Agglutination
CF29.1.C04
CF29.1.A01
CF29.1.A02
CF29.1.C02
CF29.1.B05
CF29.1.G04
CF29.1.E06
CF29.1.G05
CF29.1.G02
CF29.1.A06
CF29.1.G01
CF29.1.B02
CF21.2.G01
CF21.2.F01
CF21.1.C03
CF21.1.D01
AT11.1.A04
CF21.1.B04
CF21.1.A03
CF21.1.A04
CF21.1.C01
CF21.1.D04
CF21.1.A02
CF21.1.F01
AT11.1.D11
CF31.1.F02
CF21.1.F02
CF31.1.B04
CF21.1.B01
1
0.1
0.01
0.001
0.0001
Min. positive conc. [μg/mL]
5
1.25
0.3
0.08
0.02
EC50 [μg/mL]
20
10
5
2.5
1.25
Min. agglutination conc. [μg/mL]
Ogawa
Inaba


CT
LT
CtxB subunit specific
CtxA / Holotoxin-specific
Toxin Neutralization EC50 [µg/ml]
0.003
0.009
0.03
0.08
0.25
0.74
2.22
6.67
20
CF31.1.F03
CF31.1.F05
AT13.2.D05
AT13.2.B11
AT11.1.C06
AT13.1.C12
CF29.1.C01
CF31.1.E06
AT13.1.C04
CF31.1.C05
CF21.1.B02
AT13.2.C04
CF31.1.E02
CF31.1.B06
AT13.2.C10
AT13.1.B04
CF29.1.E03
CF31.1.G03
CF30.2.E02
CF29.1.A05
AT11.1.C03
AT11.1.D03
CF31.1.G01
CF31.1.C02
CF29.1.E05
AT11.1.A01
CF31.1.E04
CF30.1.F06
CF21.2.B01
AT11.1.C07
CF21.2.C02
AT13.1.A07
AT11.1.B03
AT11.1.B02
AT11.1.C01
AT13.2.C05
CF29.1.A04
AT13.1.B12
AT13.1.C02
CF31.1.B05
CF21.1.G01
AT13.1.B05
AT13.2.A05
AT13.2.B02
CF21.1.D03
CF30.1.A03
AT11.1.C04
CF21.2.E01
CF31.1.D02

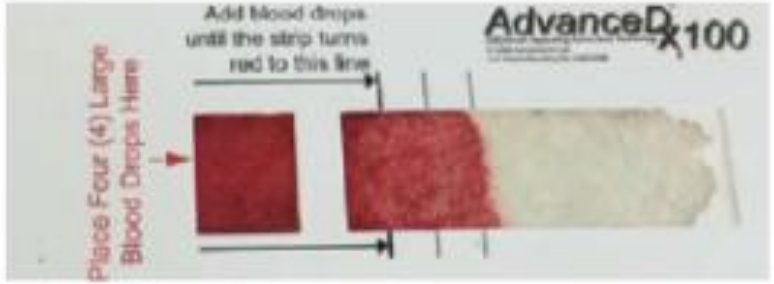
2. Technical considerations for seroepidemiologic studies

Development of improved methods for seroepidemiology

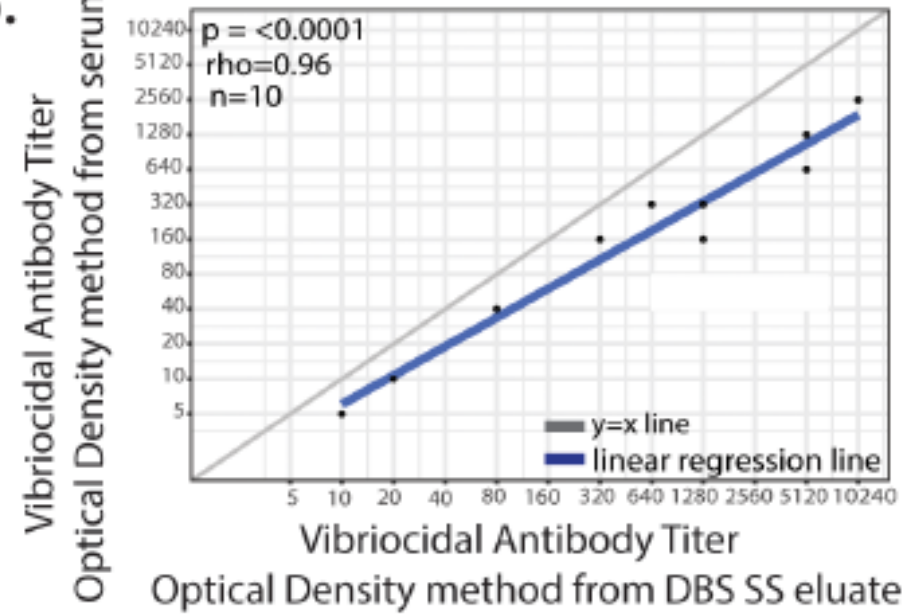
- Seroepidemiology starts with understanding the ability of serologic tests to accurately predict past infection
  - Work by Andrew Azman, Daniel Leung and colleagues (discussed yesterday)
  - Symptomatic vs. asymptomatic
  - Vaccination vs. infection
  - Cross-sectional vs. longitudinal
- Ease of application
  - Dried blood spot
- **Assay standardization**
  - **Standard protocol**
  - **High titer serum vs. mAb**

Ease of application: Dried Blood Spots

A.



B.



PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Dried Blood Spots for Measuring *Vibrio cholerae*-specific Immune Responses

Anita S. Iyer[1], Andrew S. Azman[2,3], Malika Bouhenia[4], Lul O. Deng[5], Cole P. Anderson[1], Michael Graves[1], Pavol Kováč[6], Peng Xu[6], Edward T. Ryan[7,8,9], Jason B. Harris[7,10], David A. Sack[11], Francisco J. Luquero[11,12], Daniel T. Leung[1,13]*

Assay standardization

- Especially important for seroepidemiology, especially cross-sectional surveys
- Also relevant to vaccine immunogenicity and bridging studies
- **Protocols vary significantly between laboratories!**
- In addition unintended variation may significantly impact responses
- ‘Internal standard’ approach with high titer serum is flawed
  - Blood borne pathogens
  - Limited quantities, not completely reproducible

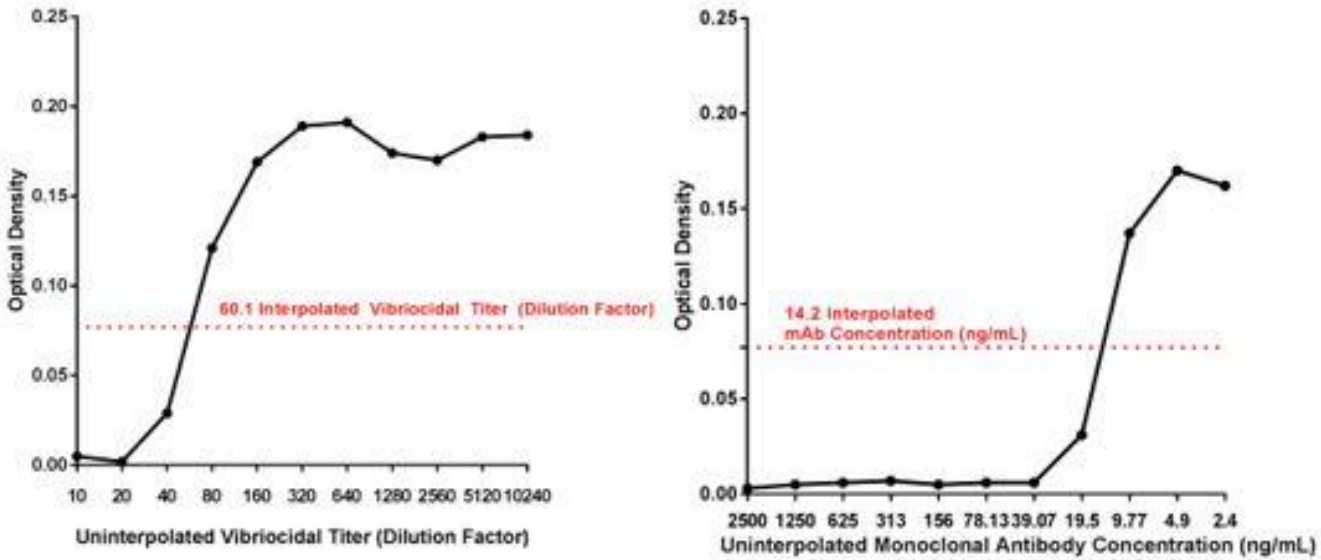
Typical set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	Growth Control	Sample 1 (1:10)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
B	Growth Control	Sample 1 (1:10)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
C	Growth Control	Sample 2 (1:10)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
D	Growth Control	Sample 2 (1:10)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
E	Saline Control	Sample 3 (1:10)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
F	Saline Control	Sample 3 (1:10)	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
G	Saline Control	mAb (2500ng/mL)	1250	625	312.5	156.25	78.125	39.0625	19.53125	9.765625	4.8828125	2.44140625
H	Saline Control	mAb (2500ng/mL)	1250	625	312.5	156.25	78.125	39.0625	19.53125	9.765625	4.8828125	2.44140625

Fig 1

● ● ● ● Optical Density at 50% killing of O1 *Vibrio cholerae*

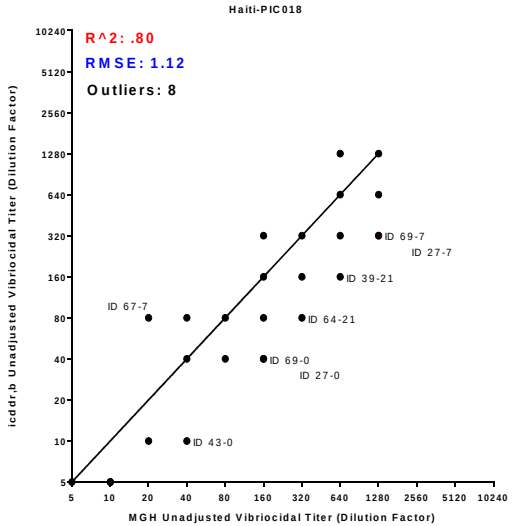
A.

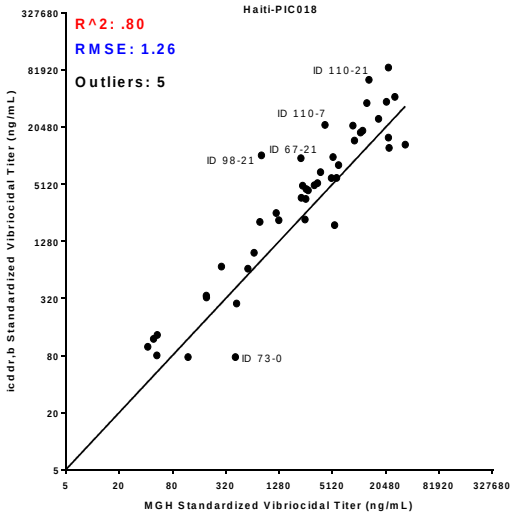


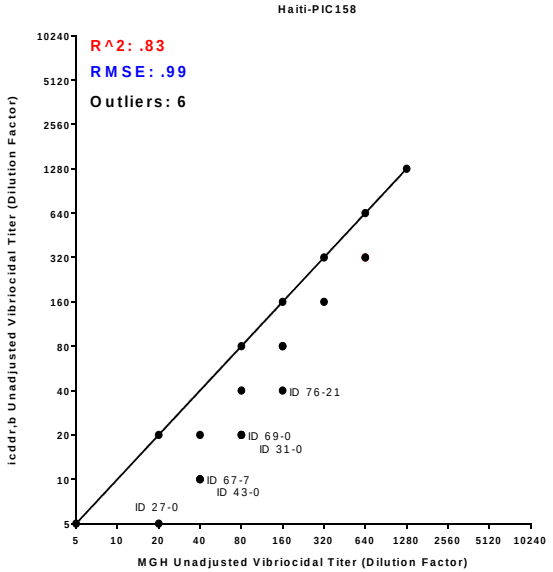
B.

Unadjusted Vibriocidal Titer (Dilution Factor): 1:40 **Adjusted Vibriocidal Titer (Dilution Factor): 1:60.1**	**Unadjusted mAb Concentration (ng/mL): 19.5** **Adjusted mAb Concentration (ng/mL): 14.2**
Standardized Vibriocidal Titer (Standard Units) = Adjusted Vibriocidal Titer (Dilution Factor) * Adjusted mAb Concentration (ng/mL) **853.4 (Standard Units) = 60.1 (Dilution Factor) * 14.2 (ng/mL)**	

Between-lab variation







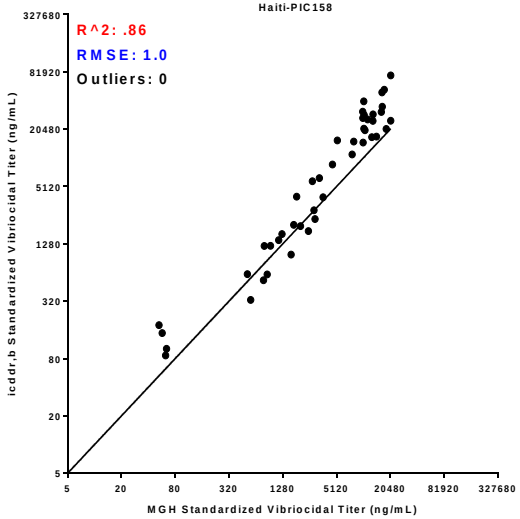


Fig. 2

Guinea Pig Complement Variation

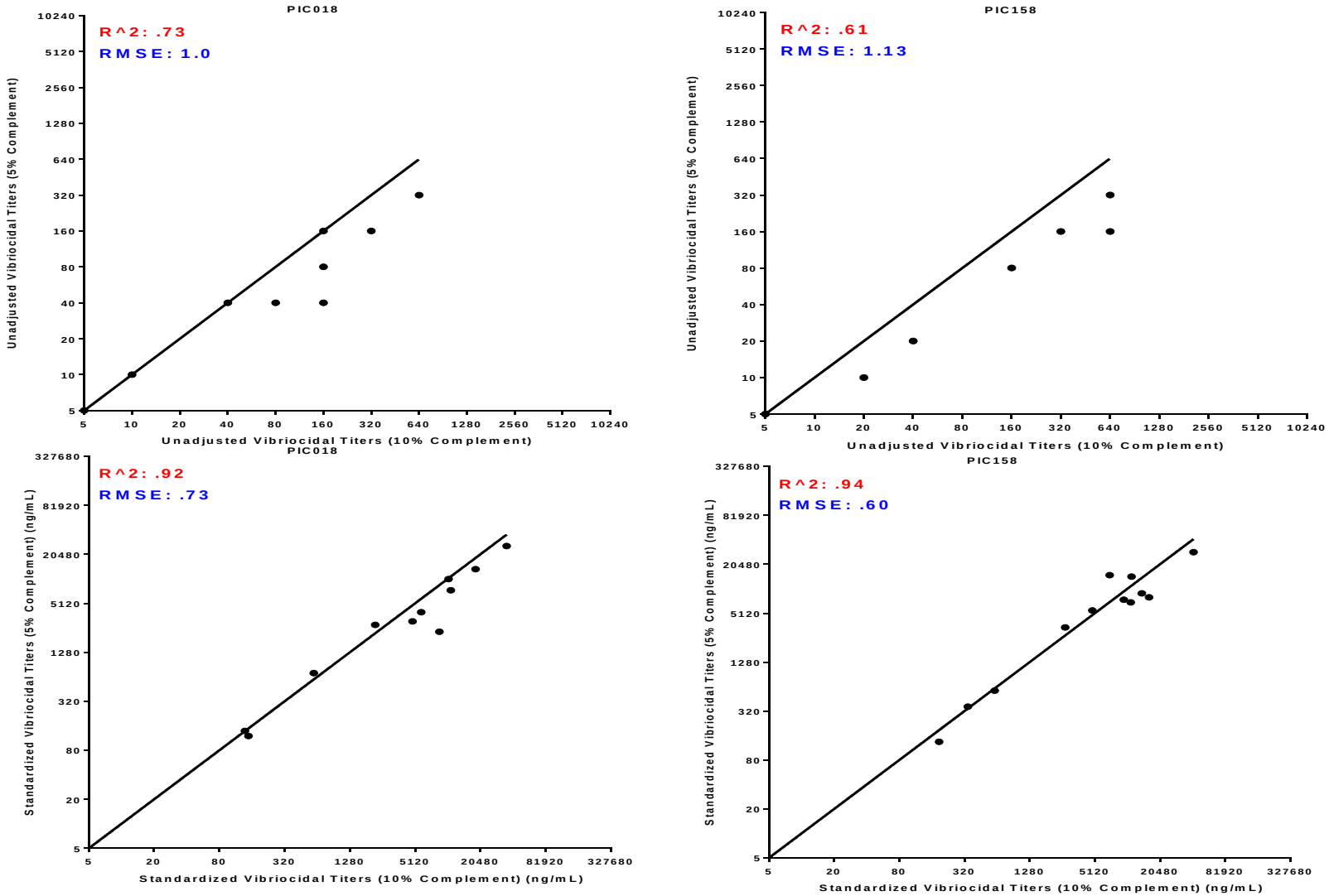


Fig. 3

•We varied experimental complement percentages to induce further variance.

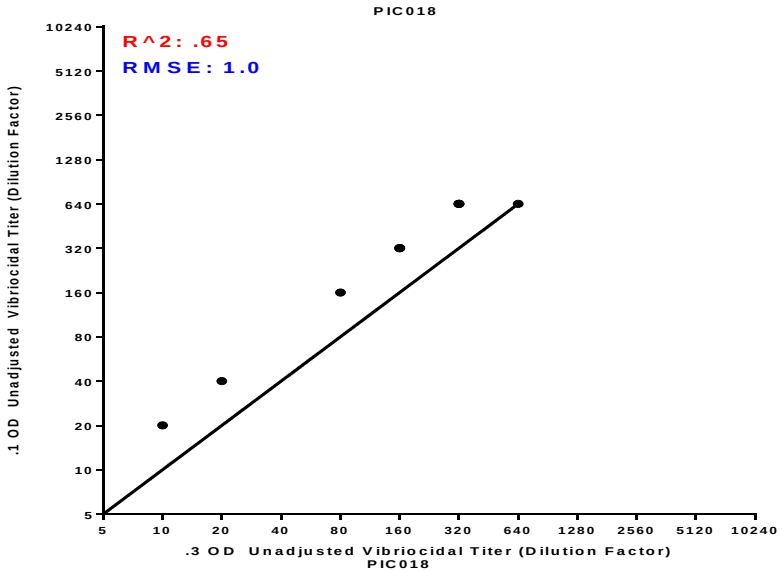
•A standard vibriocidal plate has a complement-bacteria-saline solution of 3mL with 300µl of complement, or 10 percent of the total volume.
•By varying the amount of complement to 5%, or 150µl, we would theoretically reduce the efficacy of the serum/mAb samples in killing the *V. cholerae*, decreasing the titers, which would induce variation that can be standardized by the monoclonal.

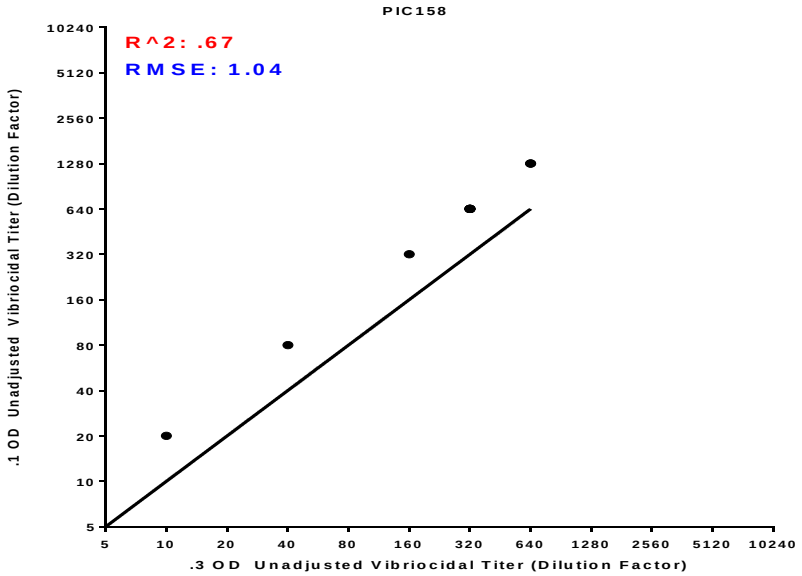
•With this first example completed, we moved onto varying the bacterial concentration.

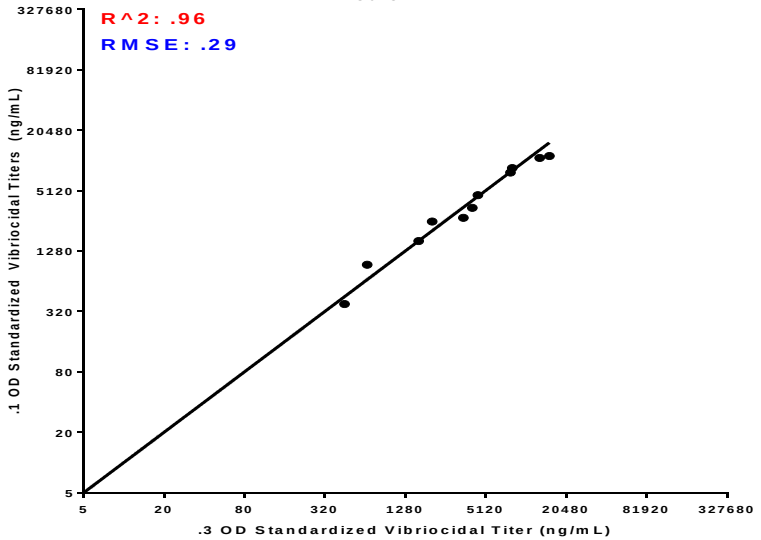
Bacterial Concentration Variation

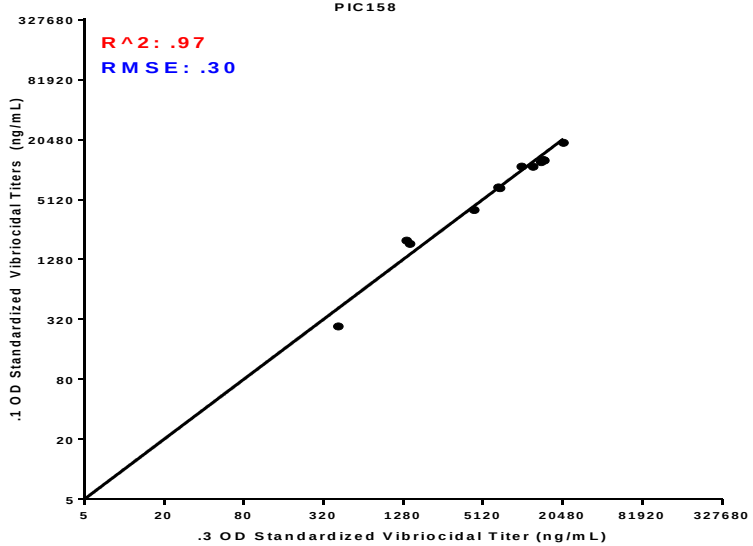
— Y=X Slope

Fig. 4





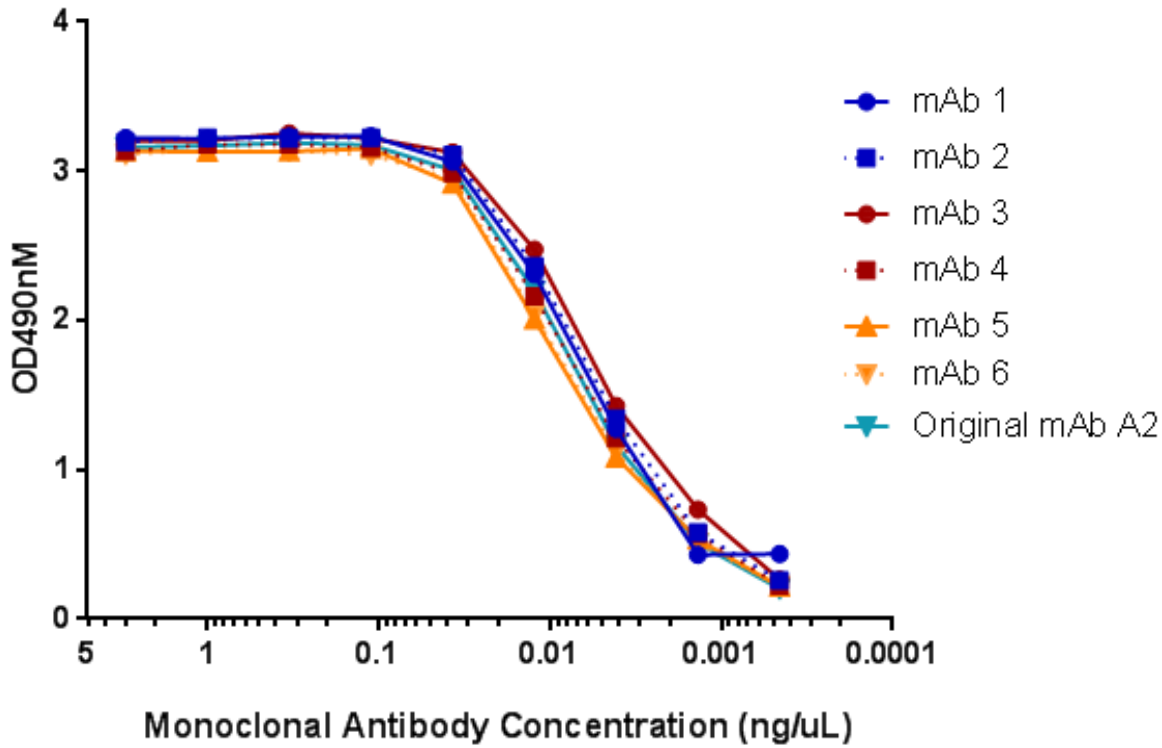


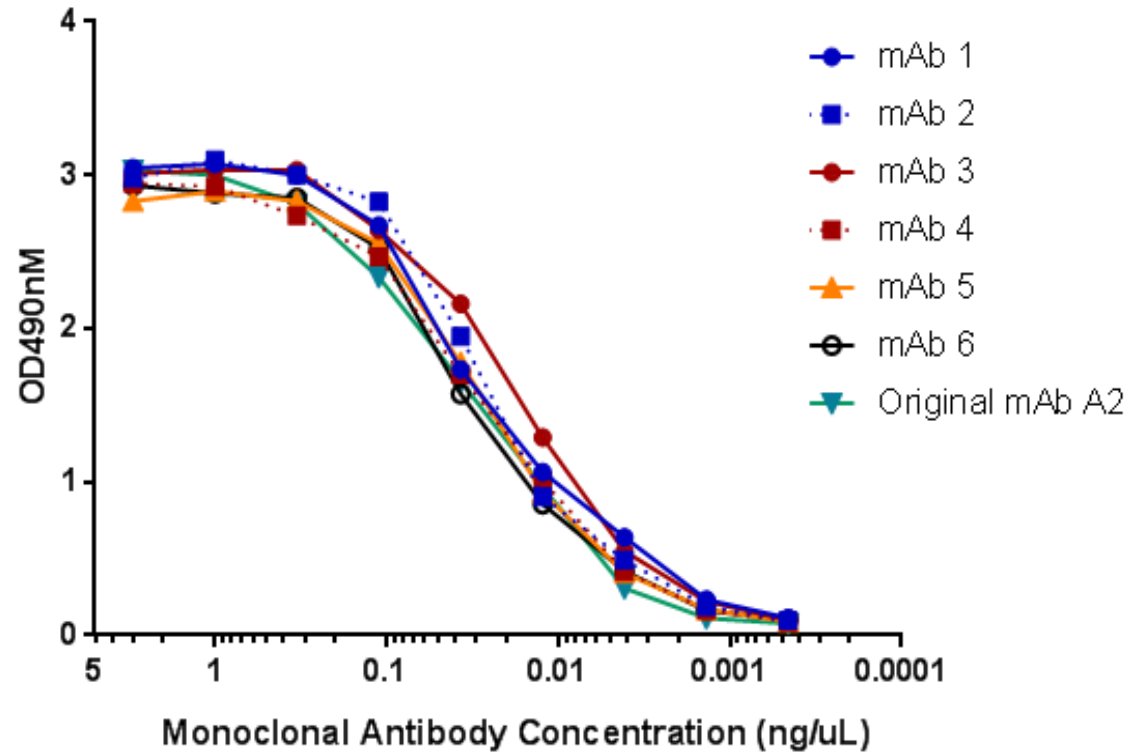


• Next, we varied experimental bacterial concentration percentages to induce further variance.

•A standard vibriocidal plate has a normalized bacterial concentration of .3OD. By reducing the amount of bacteria to an OD of .1, we would theoretically increase the titers and induce variance, which could be standardized by the monoclonal.

Reproducible by transient transfection





Discussion

1. What are some of the potential advantages and draw backs of seroepidemiologic studies of cholera disease burden?

2. Similarly, what would be the most and least useful applications for seroepidemiologic studies?

3. What are some potential ways to improve approaches to serologic studies?

4. How might this figure into our discussion of a ‘laboratory package’ to improve capacity in countries with a significant cholera burden?

Team:

- iccdr,b/MGH team
  - **Firdausi Qadri, Taufiq Bhuiyan**, Ashraful Khan, Fahima Chowdhury
  - Edward Ryan, Richelle Charles, Stephen Calderwood, Regina LaRocque, Ana Weil
- Utah –
- Emory – Jens Wramm**Daniel Leung* (modeling and DBS with Francisco and Andrew)**ert, Robert Kauffman
- UCI – Phillip Felgner, Rie Nakajima
- PaxVax/CVD103-HgR Challenge – Marc Gurwith, Douglas Haney, Mike Levine, Marcelo Sztein
- Partners In Health, Zanmi Lasante/MGH team – **Louise Ivers**, Patrick Almazor, Gregory Jerome, Ralph Ternier, Brie Falkard, Wilfredo Matias