Vaccines and Immune Response

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NIH

Outline: Vaccines and Immune Response

- Vaccines
- History of Vaccination
  - A Great Success for Public Health
  - Challenges for the Future
- Vaccine Mechanism
  - Humoral immunity via antibodies from B-cells
  - Cellular immunity via killing and help from T-cells
- Vaccine Construction
  - Types of vaccines
- Measurement of Immune Response
- Statistical Analysis of Immune Response
- Poliomyelitis vaccine development

Vaccines

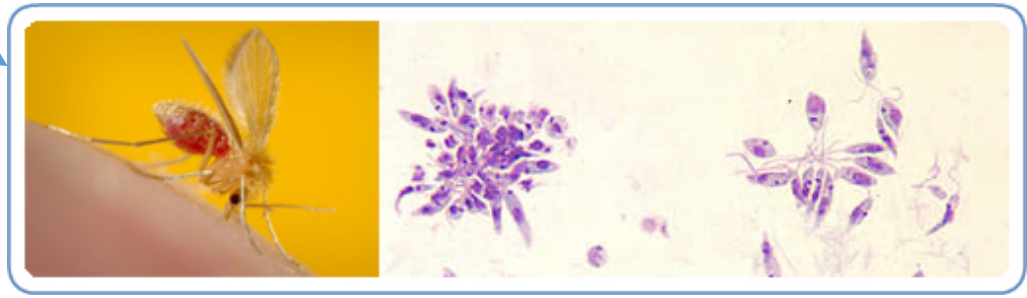
- A biological preparation that is used to induce immunity to a particular disease.

- One of the greatest public health inventions ever.
- A mystery revealed by the science method
- For me, a fascinating blend of biology, immunology, and statistical methodology.
- Lucky to be able to work in this area.

`Vaccination' in History

- People observed that, for select infections, people who recovered were subsequently immune
- Led to deliberate infections
  - China & Europe smallpox (variolation)
    - Dried pustules scratched on skin
  - Leishmanization
    - Lesion exudate scratched on buttocks
  - Chicken Pox parties
  - Zika infection for 10 year old girls?

FIGURES SHOWING VACCINATION PUSTULES
From a Chinese work on Vaccination



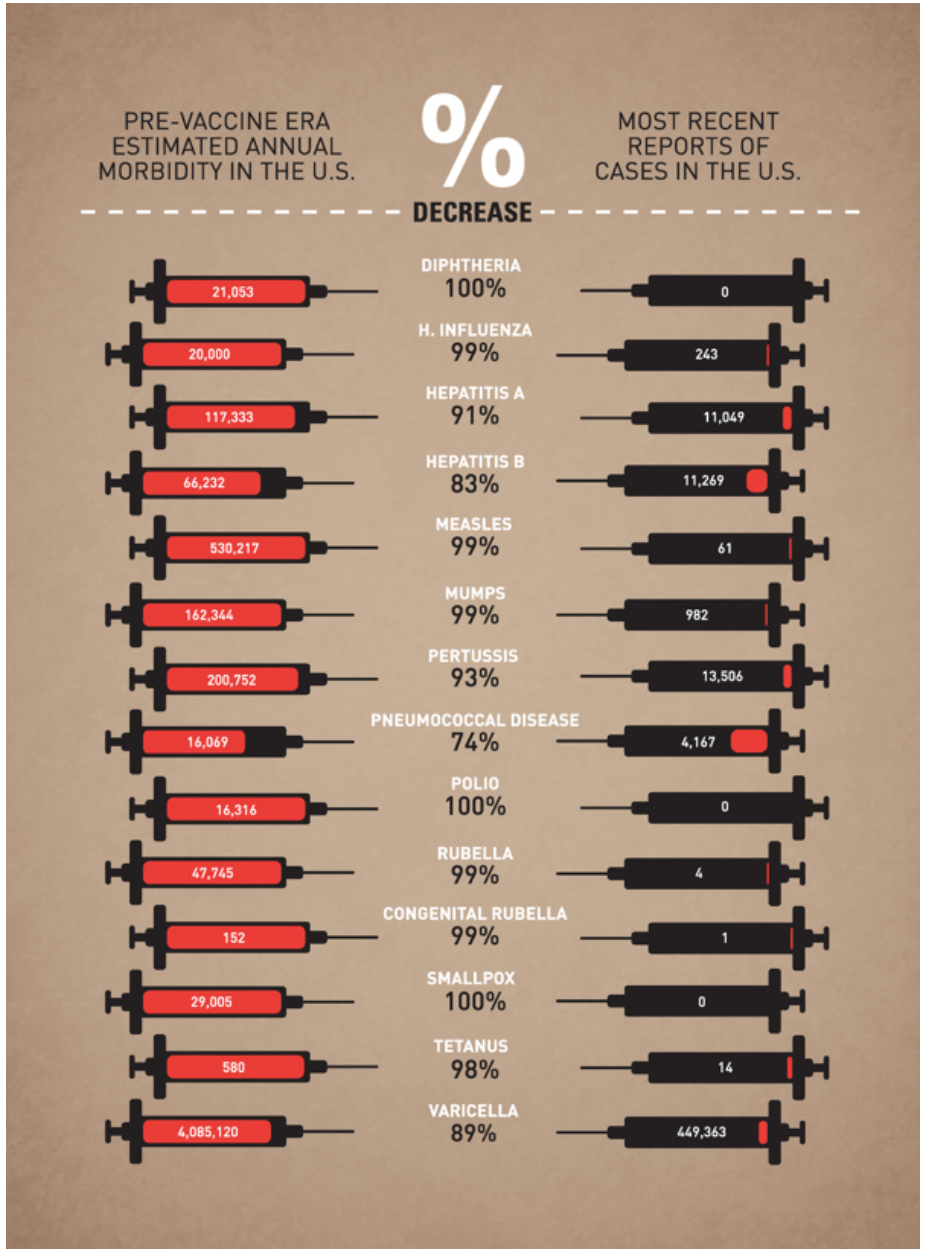
Jenner and Vaccination

- Jenner was a 18th century English physician
- Observed that milkmaids got cowpox but not smallpox
- Gave cowpox to a 9 year old boy waited a while then ``challenged" with variolation of smallpox virus (*variola*)
  - Success!
- Vaccination with cowpox compulsory in 1853
- Vaccination was controversial

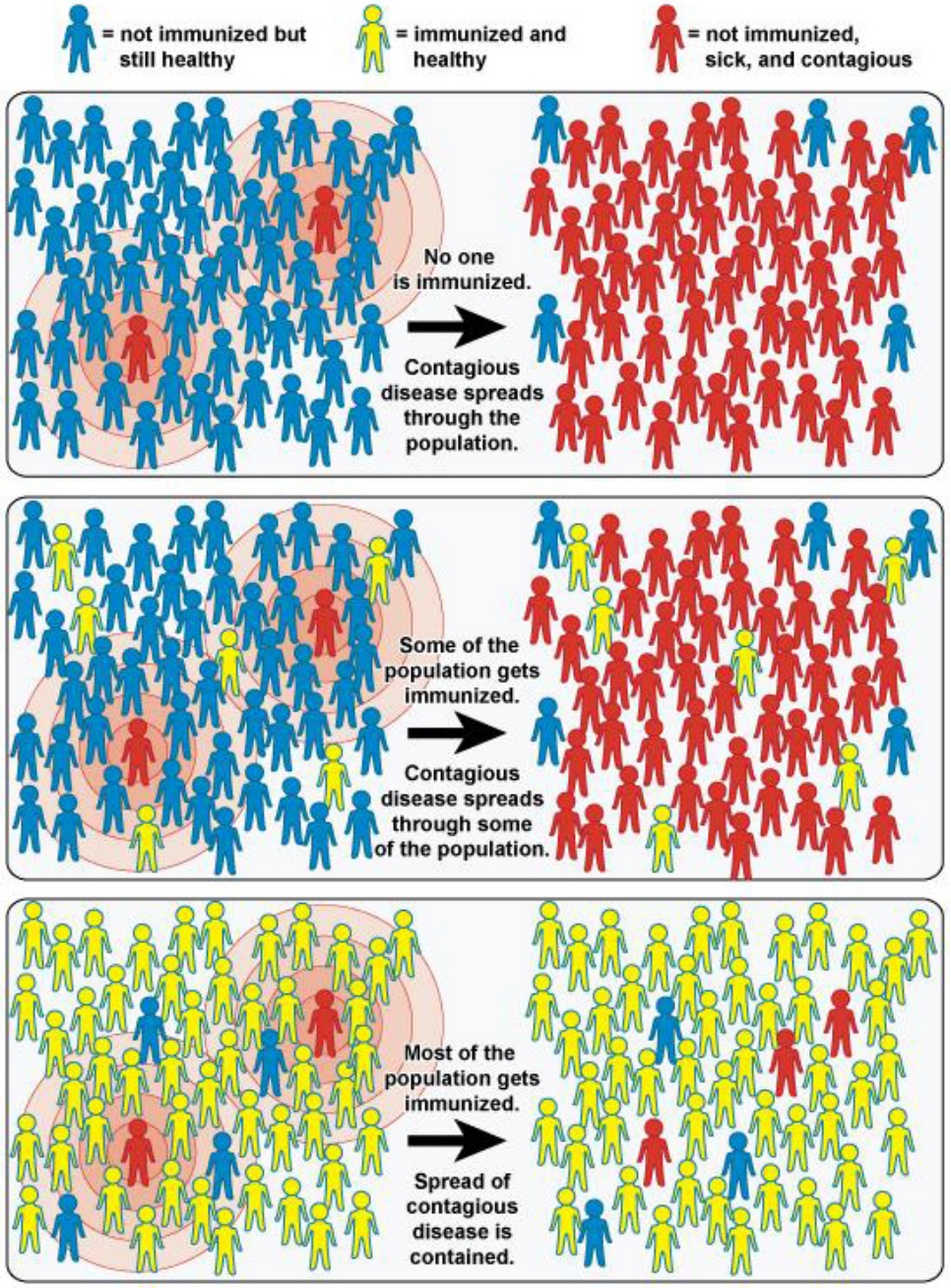
TABLE 1. Vaccine-preventable diseases

Disease	Year
Smallpox*	1798+
Rabies	1885+
Typhoid	1896+
Cholera	1896+
Plague	1897+
Diphtheria*	1923+
Pertussis*	1926+
Tetanus*	1927+
Tuberculosis	1927+
Influenza	1945&
Yellow fever	1953&
Poliomyelitis*	1955&
Measles*	1963&
Mumps*	1967&
Rubella*	1969&
Anthrax	1970&
Meningitis	1975&
Pneumonia	1977&
Adenovirus	1980&
Hepatitis B*	1981&
Haemophilus influenzae type b*	1985&
Japanese encephalitis	1992&
Hepatitis A	1995&
Varicella*	1995&
Lyme disease	1998&
Rotavirus*	1998&

* Vaccine recommended.
+ Vaccine developed
& Vaccine licensed



Vaccination has enormously improved Public Health



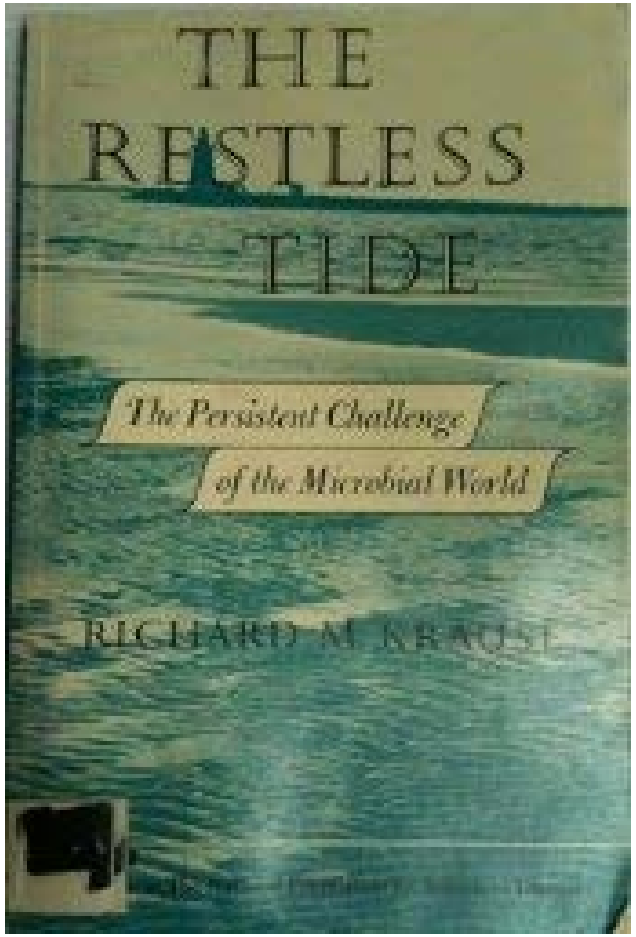
VACCINATIONS BENEFITS YOU . . . AND OTHERS

Vaccines work both directly and indirectly through *herd immunity.* If a sufficient number are immunized in a community the pathogen can't have much impact

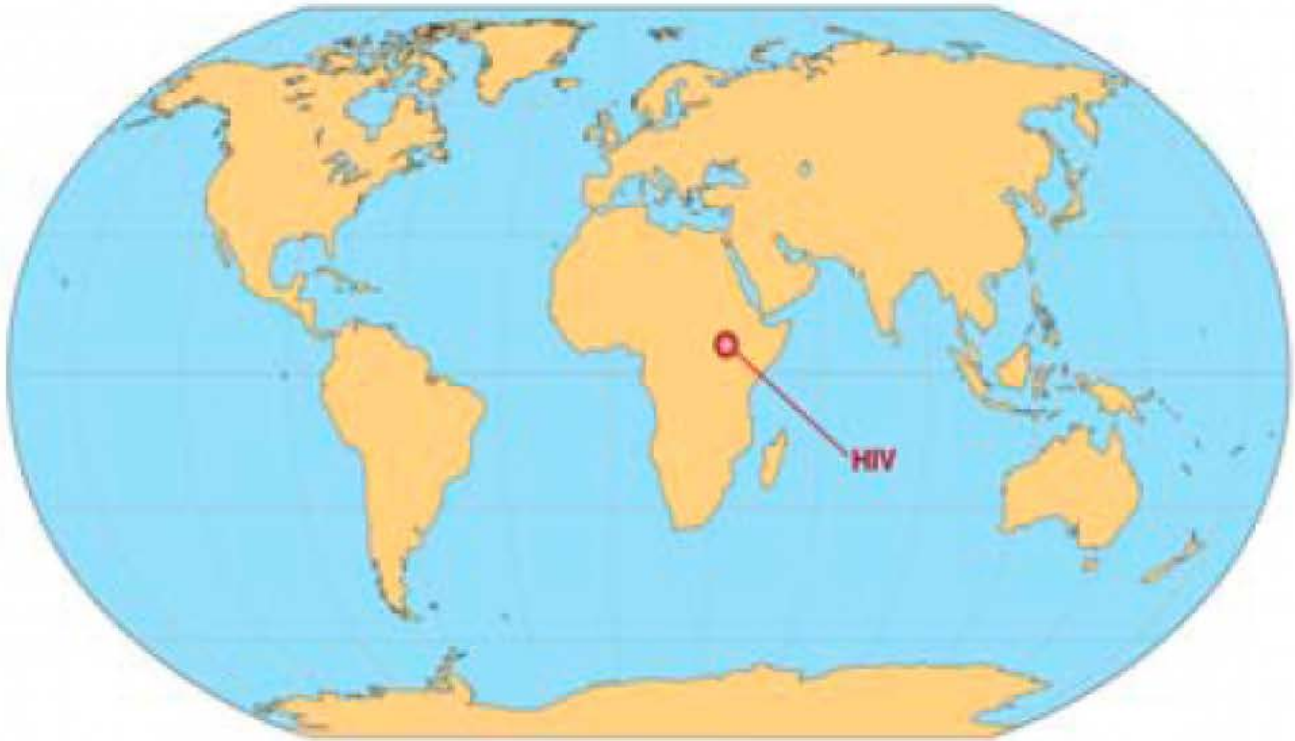
Those who can't vaccinated (immuno-compromised) rely on those who can get vaccinated for protection.

Misplaced complacency

- By the late 1970s infectious diseases were widely thought to be tamed.
  - Vaccines
  - Antibiotics
- Pathogens continually evolve in response to selection pressure---drugs, habitat, human immunity
- More interconnection, habitat exposure climate change---mixes things up
- Bio-warfare



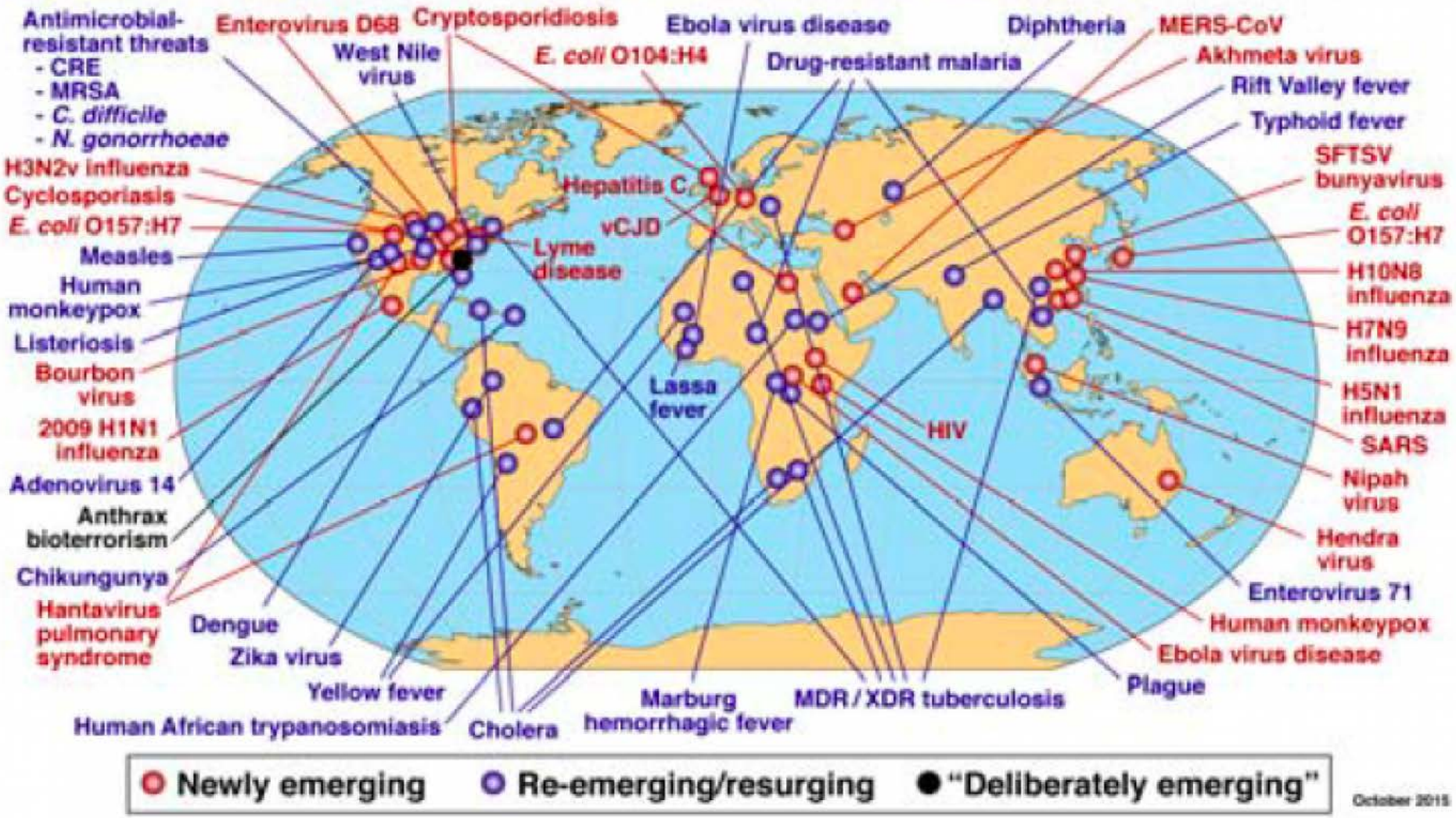
Global Examples of Emerging and Re-Emerging Infectious Diseases



Newly emerging Re-emerging/resurging "Deliberately emerging"

Dr. Fauci's slide circa 1985

Global Examples of Emerging and Re-Emerging Infectious Diseases



Dr. Fauci's slide
Today

Vaccine Challenges Today

- Tough infectious diseases
  - HIV
  - Malaria
  - Tuberculosis
- Rapid response to new emerging infectious diseases
  - West Nile
  - Ebola
  - Zika
- Outside the box
  - Cigarettes: (NicVAX) Opiods
  - Cancer: construct a unique vaccine for each patient based on their tumor
  - Mosquitoes as flying syringes

How do vaccines work?

- The immune system's memory of a prior infection allows a rapid response that can quickly destroy a pathogen's ability to replicate and disease is avoided.
- Vaccines exploit this by introducing a fake germ that induces the immune response, but doesn't cause disease
- When the real germ appears it is rapidly destroyed before causing disease

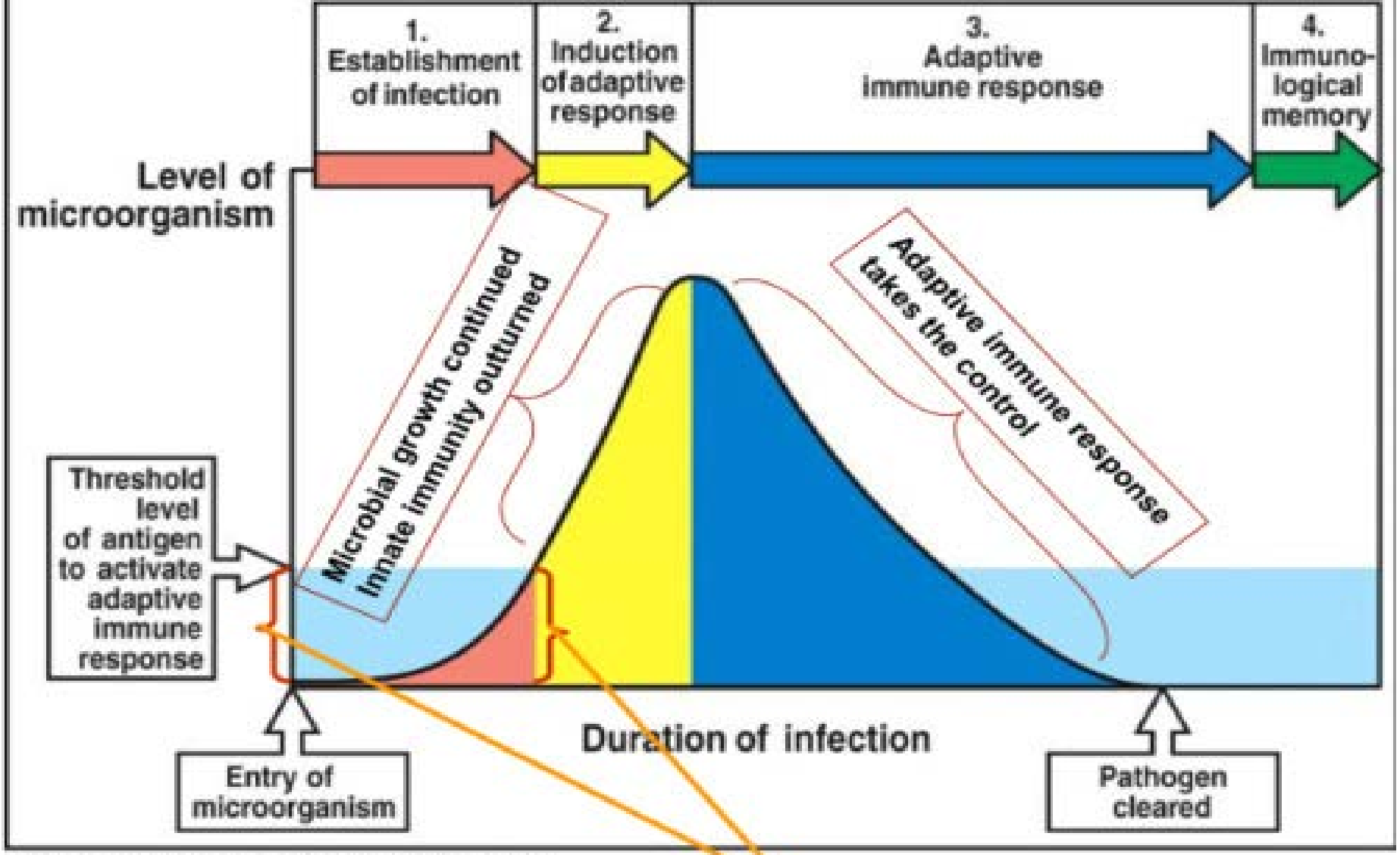
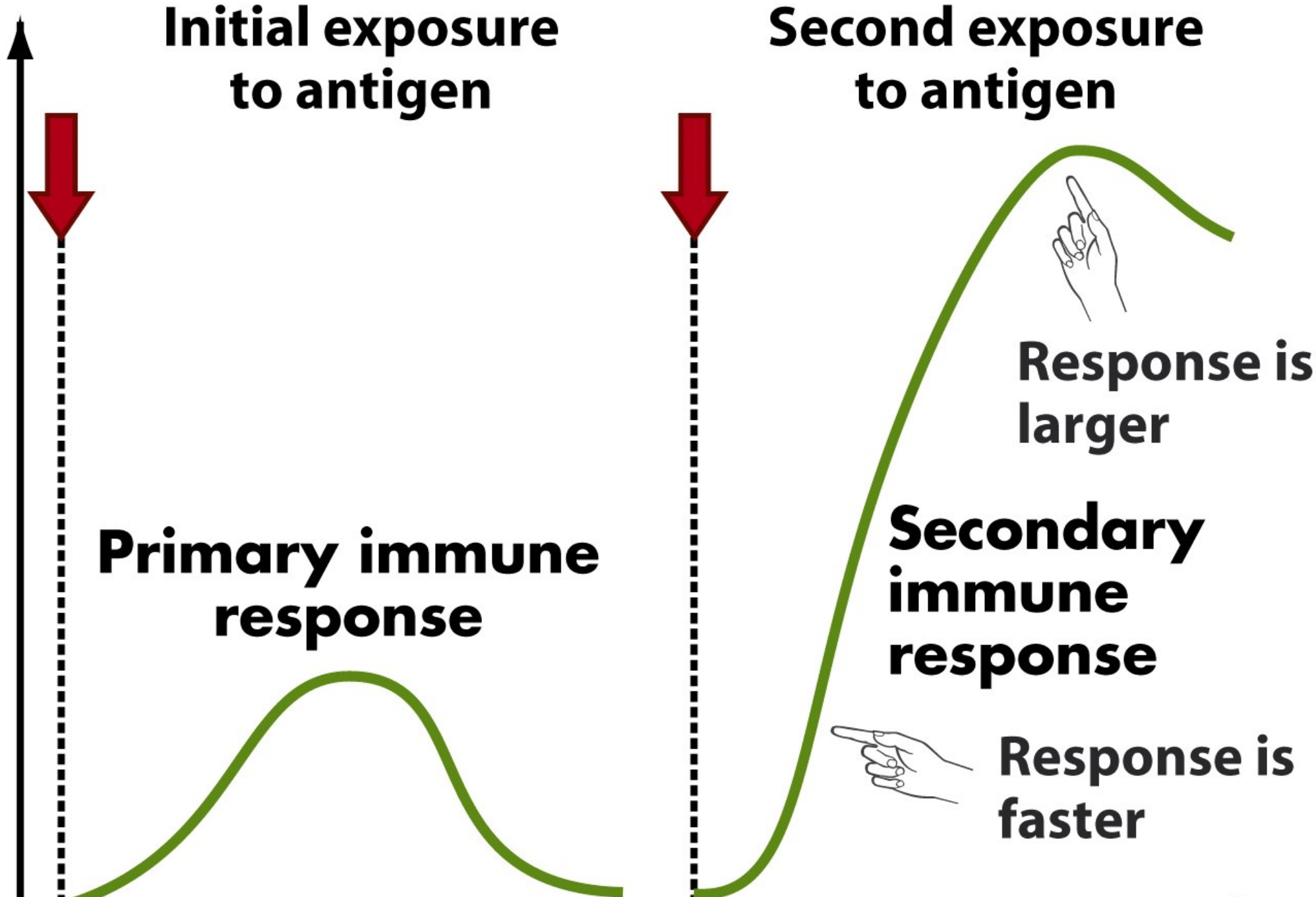
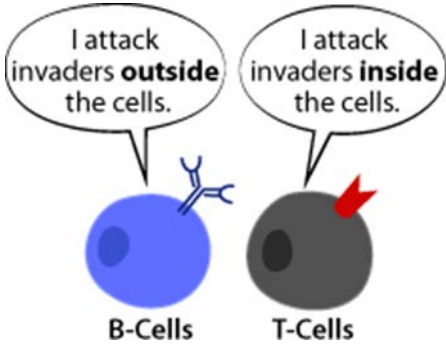


Figure 10-1 Immunobiology, 6/e. (© Garland Science 2005)

Innate immune response function at this stage to control most of the infection. Otherwise, we would have been suffering from different infections all the time.


Initial exposure to antigen
Second exposure to antigen
Primary immune response
Secondary immune response
Response is larger
Response is faster

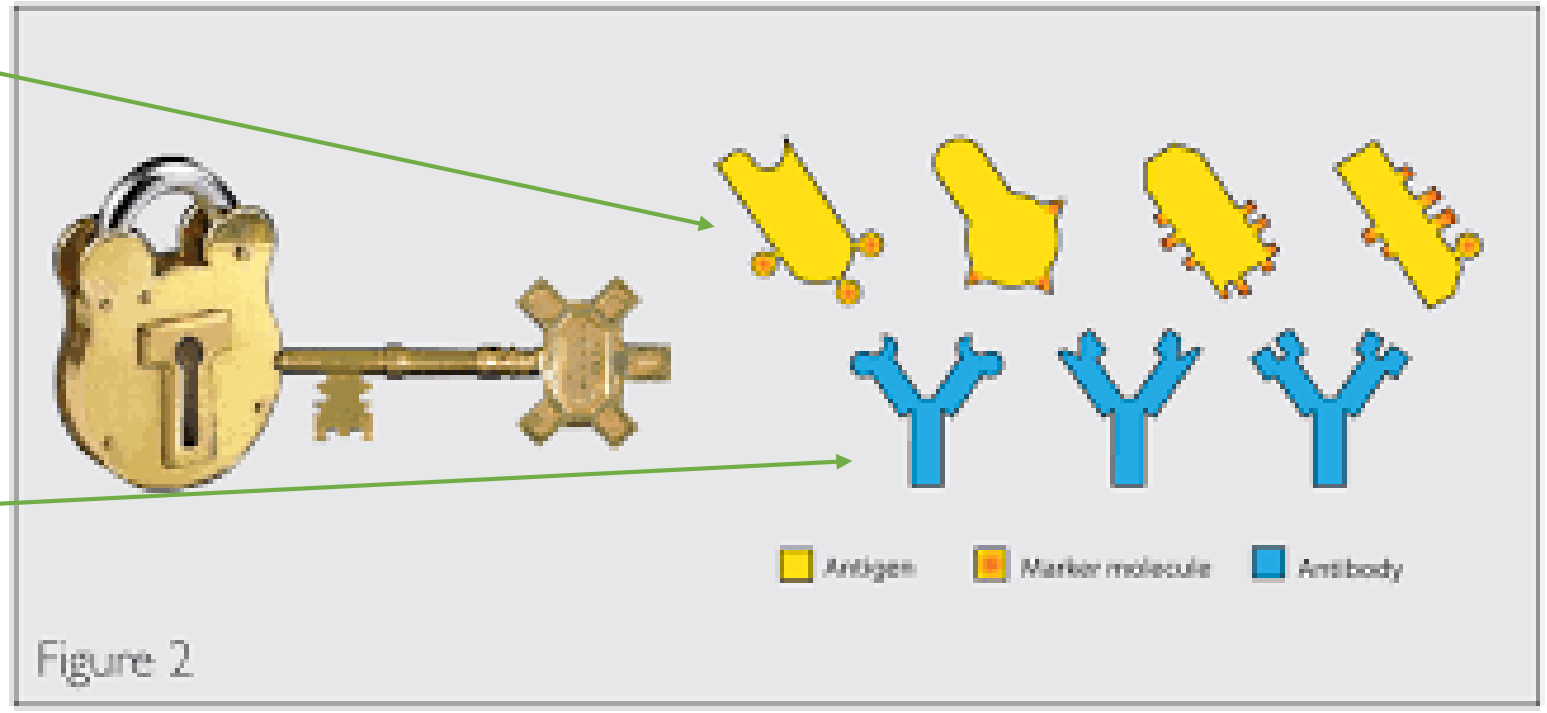
Adaptive Immune response to a pathogen



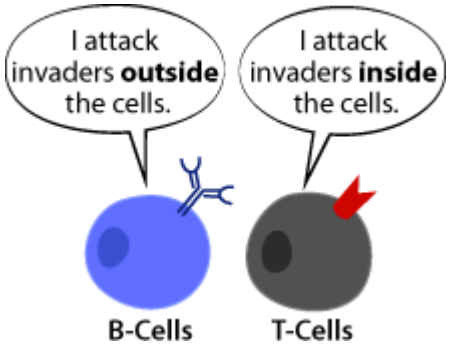
- B-cells and T-cells are immune cells.
  - 70,000 to 2,000,000 cells/ml in blood
- A cell recognizes a unique sequence of amino acids = part of a germ

Germ bits

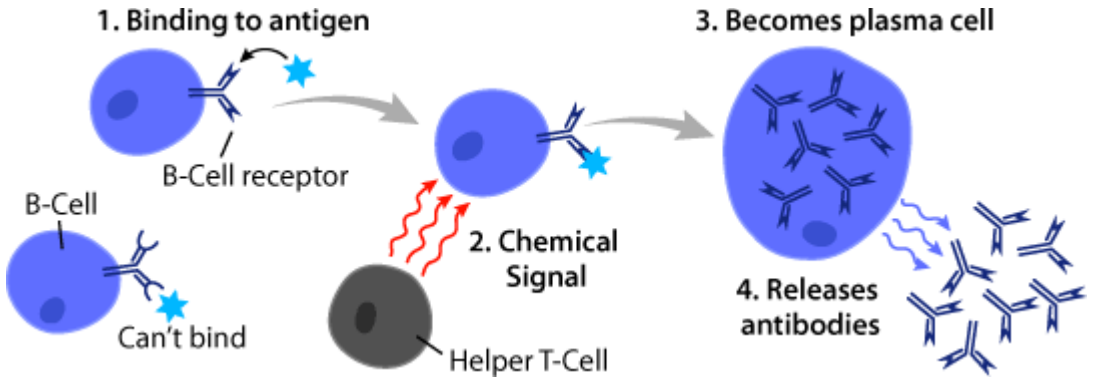
Molecules on outside of B-cells
That recognize germ bits



B-Cells

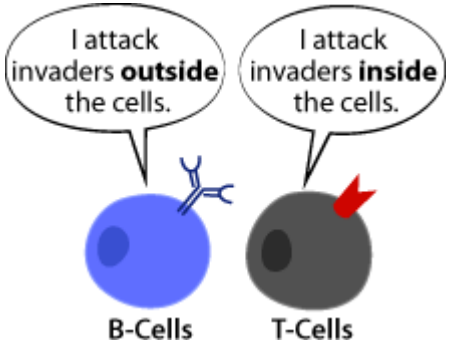


- B-cells work by identifying bits of extra-cellular germs
- Get signals from helper T-cells to expand and churn out antibodies

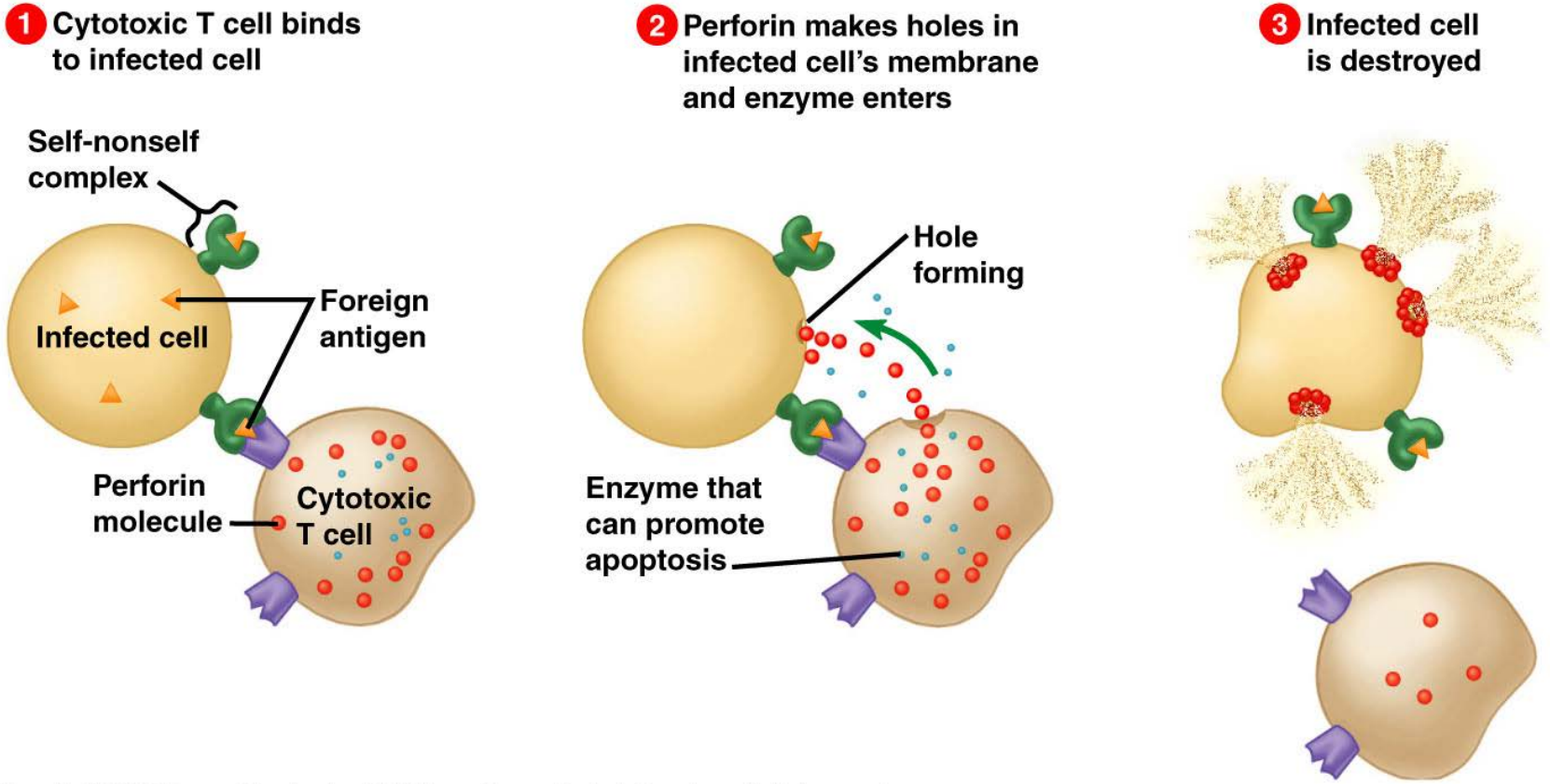


- Antibodies block germs or 'tag' them for killing
- After infection, some progeny become 'memory' B-cell for rapid response

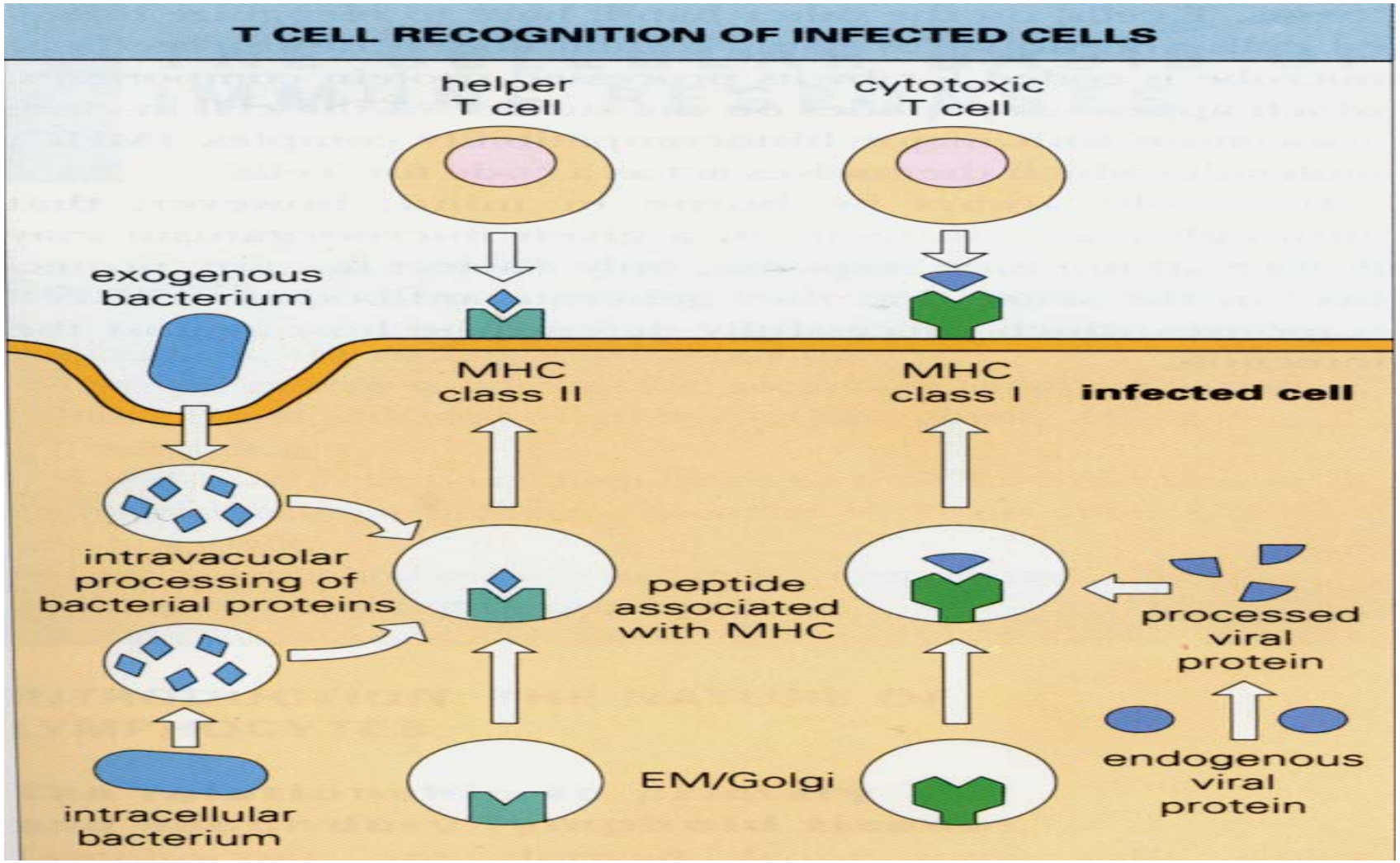
T-cells



- T-cells work by identifying germ infected cells and killing them



T-cell recognition, the details



MHC class I presentation
- *on all cells
- *sample inside cell
- * signal to kill (self) infected cell

MHC class II presentation
- * on some immune cells
- * sample outside cell
- * signal to help fight outside

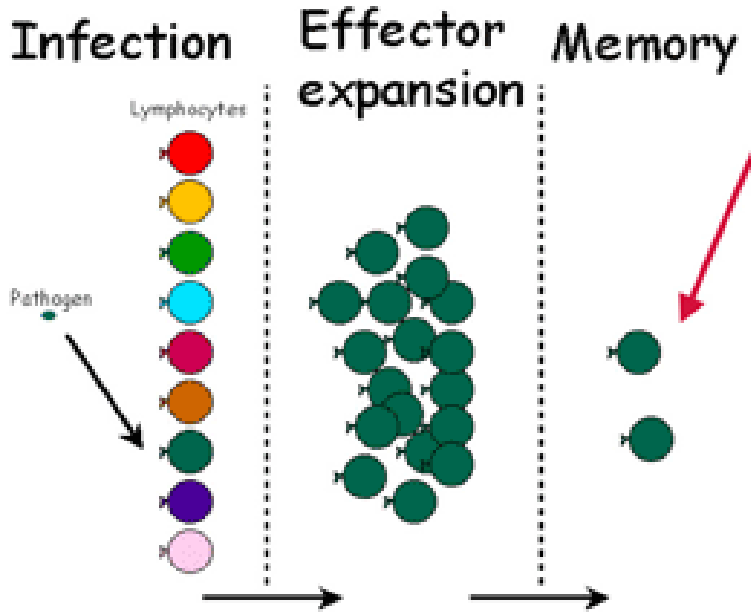
Immune Memory

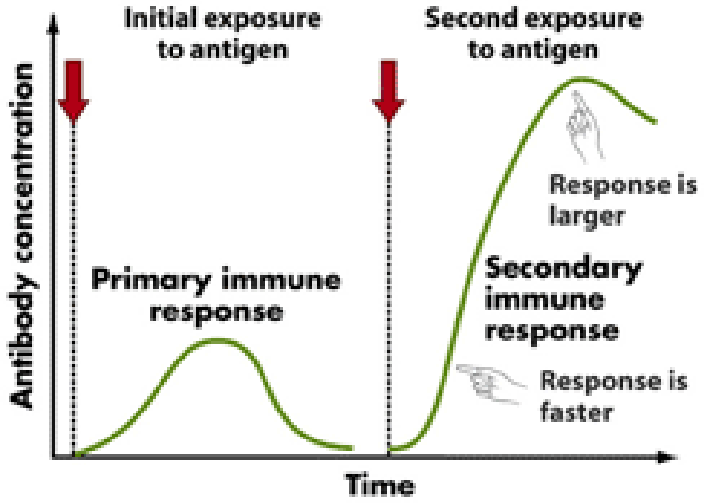
CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD
Andy Sewell, 2007

IMMUNE MEMORY

Once a lymphocyte has recognised antigen a foreign antigen it expands to eliminate the infection

Some cells then become long lasting >20 years 'memory' cells.





Memory cells respond very quickly to subsequent exposure to antigen

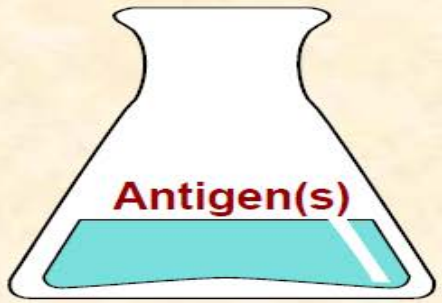
Types of vaccines (antigens)

- Goal is to trick the immune system to responding to a benign proxy for the germ. . . How?
- KILLED: Grow the germ and kill it
- ATTENUATED: Grow the germ in a hostile environment
  - Cold adapted strains can’t handle body heat (think polar bears in the Sahara)
  - Radiation messes up germs (think slow moving zombies)
- SUBUNIT: Grow the germ and snip out part of it
- VECTOR: Modify a benign virus to deliver germ bits

What's in a typical vaccine?

Active ingredients

Inactive ingredients



Weakened or killed germ or part of the DNA of the germ

Enhances the vaccine–induced immune response

Prevents bacterial or fungal growth

Maintains the vaccine's effectiveness during storage

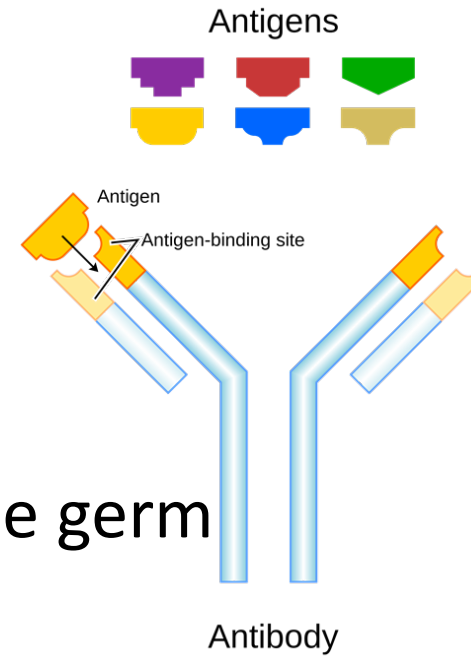
Immune Response Assays

- Measurement and analysis of the immune response to vaccination is a key feature of vaccine development
  - Identify which aspects of the immune system prevent disease
  - Via vaccination, enhance key aspects of the immune system
  - Identify a ‘correlate of protection’ e.g. antibody > threshold => protection)
- Many ways to measure the immune system
- Measurement involves biological systems, assays can be twitchy
  - Lots of statistical issues
  - Validation involves proving the assay works well using pre-specified criteria

Assays

- Antibodies: ELISA
  - Enzyme linked immuno-sorbent assay.
  - measures how the antibodies bind to their target antigen
- Antibodies: TZM-bl
  - Genetically engineered HIV infectable HeLa cells from Henrietta Lacks
  - light up with firefly biolumenescence when infected with HIV
- T-cells: ICS
  - intracellular cytokine staining measures secreted chemical signals from T-cells
  - e.g. ``come here''  ``do this'' ``have some poison''
- Antibodies: Standard Membrane Feeding Assay (SMFA)
  - Evaluate vaccine induced birth control for sexual-stage malaria parasites
- Antibodies: Binding Antibody Multiplex Assay (BAMA)
  - Efficiently evaluate binding of multiple antibody types at multiple sites

Measurement of Antibodies

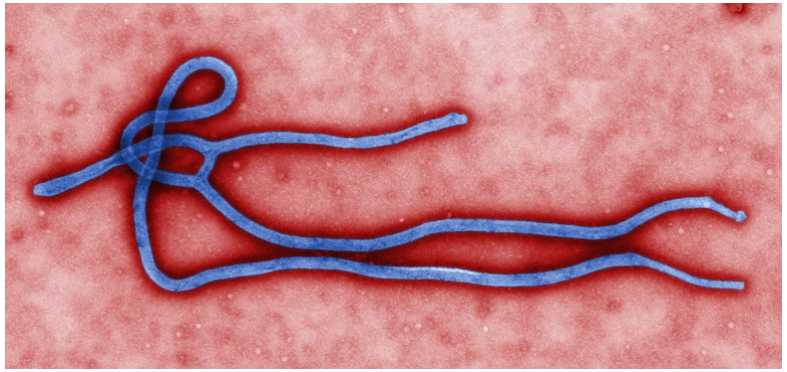


- Antibodies are produced by B-cells and circulate in blood
- An antibody binds to a unique string of 5-8 amino acids on the germ
  - Can neutralize the germ by preventing it from infecting a cell
  - Many antibodies can glom on germ and signal other cells to attack it
- Need to measure antibodies for the pathogen of interest
- Two ways to measure
  - Binding assay --- antibody sticks to its *cognate* antigen
  - Functional assay --- antibody prevents germ from infecting a cell

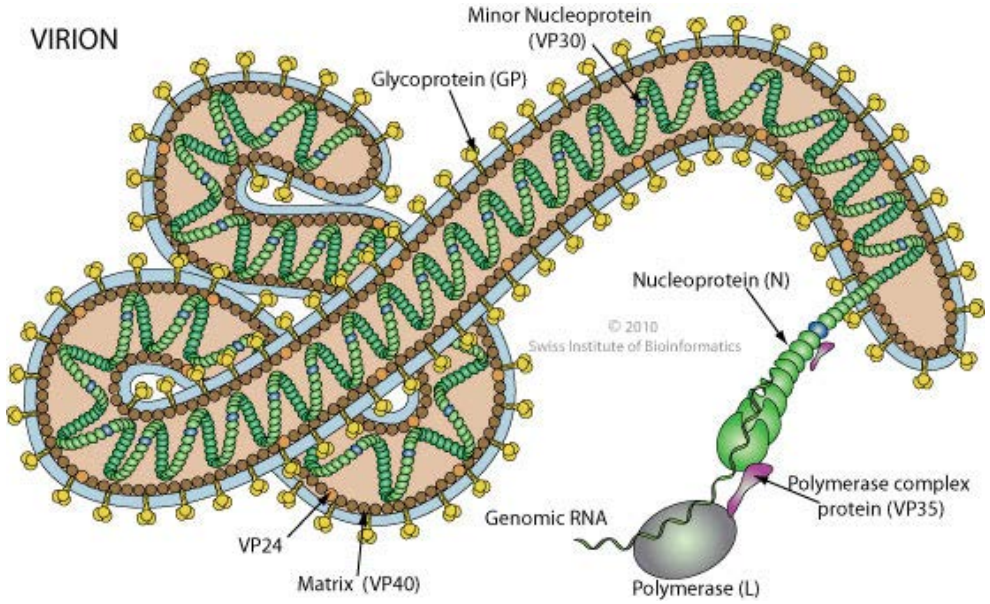
Ebola Vaccines

- Experiments suggested antibody response was correlated with survival in vaccinated monkeys exposed to disease
- Vaccine studies have looked at antibody response to see if vaccine is inducing an immune response
- Antibody responses measured in Ebola vaccine trials in West Africa

Antibody to Ebola virus

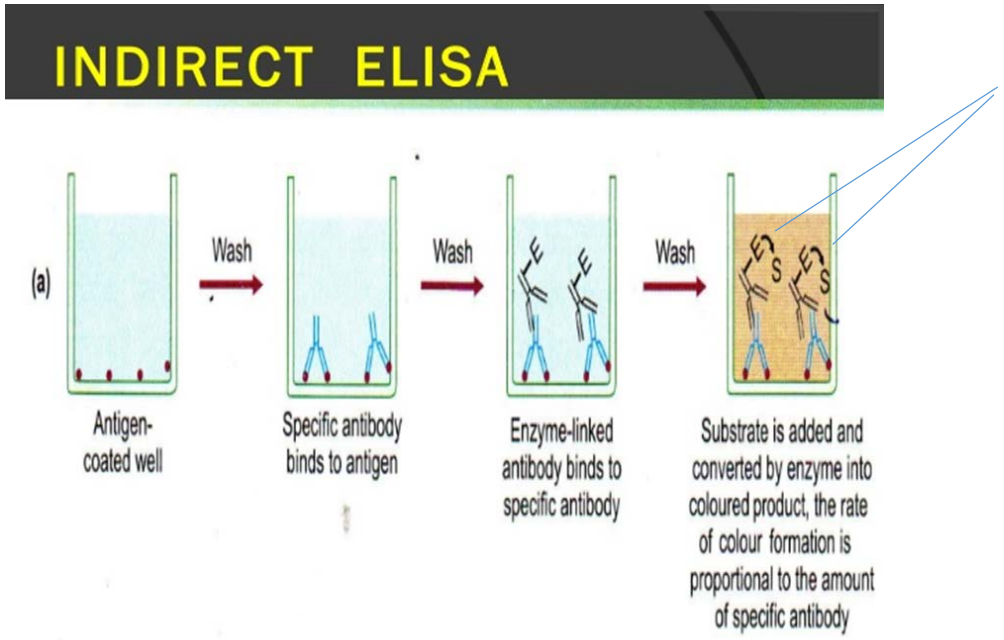


- Outer shell of Ebola virus is a glycoprotein GP (mixture of sugars and protein)
- Vaccines express bits of the GP to induce immune response
- Elisa assay used to measure the antibodies to GP



Binding antibody ELISA

- Enzyme-Linked ImmunoSorbent Assay performed in a 96-well plate
- Measures abundance of antibodies for a specific antigen (bit of Ebola)



Enzyme-Linked Immunosorbent Assay (ELISA)

96-well plate

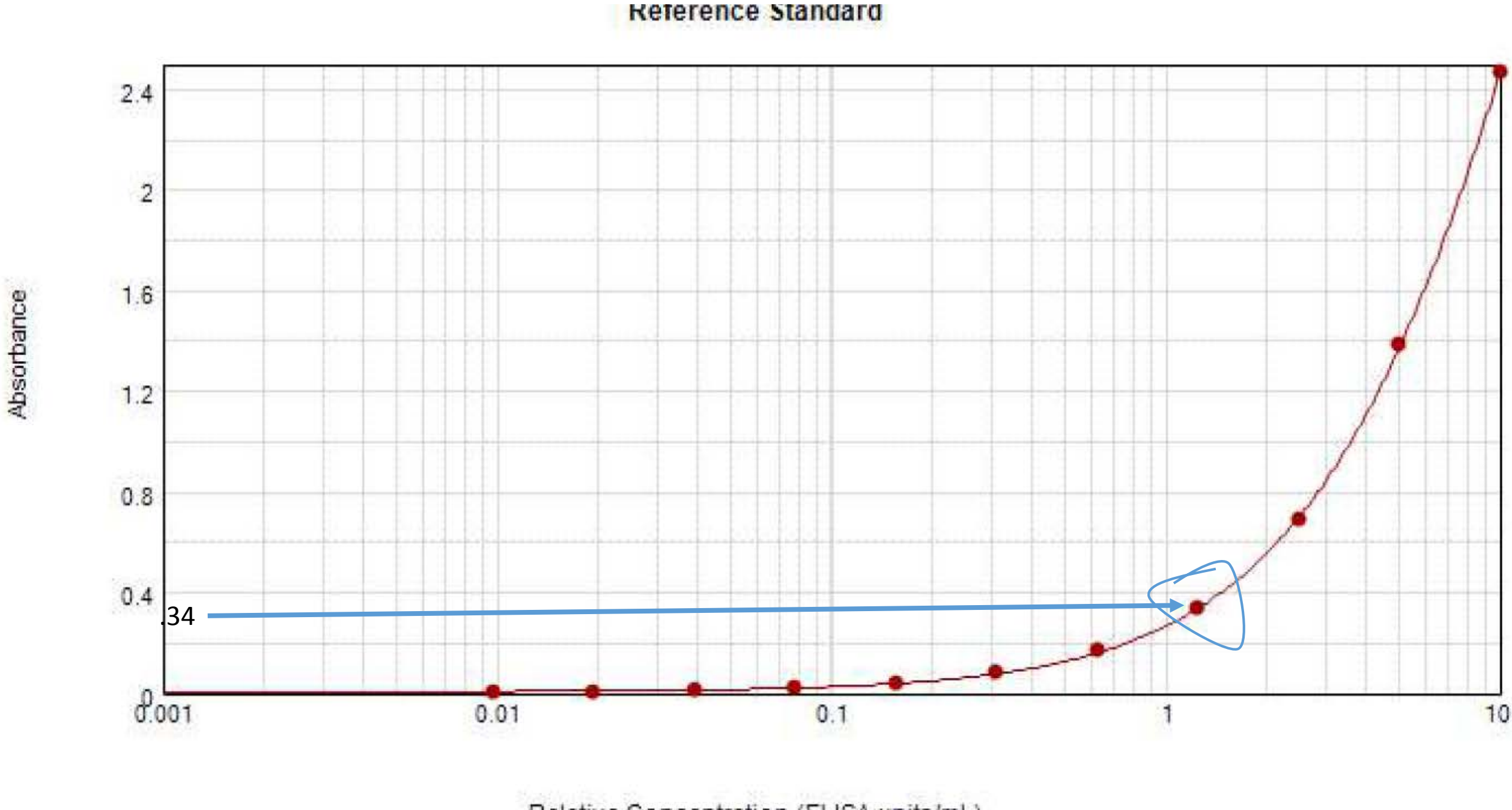
REFERENCE STANDARD

Each Well has a known concentration

OD values

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.671 0.048	1.509 0.042	0.767 0.040	0.416 0.039	0.217 0.038	0.121 0.038	0.082 0.037	0.061 0.037	0.054 0.040	0.048 0.039	0.049 0.042	0.130 0.037
B	2.353 0.046	1.347 0.048	0.697 0.040	0.341 0.038	0.201 0.038	0.113 0.038	0.075 0.038	0.059 0.038	0.049 0.038	0.045 0.037	0.043 0.039	0.111 0.039
C	1.075 0.041	1.066 0.042	0.690 0.043	0.616 0.040	1.446 0.043	1.222 0.042	0.426 0.040	0.548 0.040	0.323 0.039	0.350 0.039	2.507 0.048	1.136 0.042
D	0.666 0.040	0.574 0.040	0.428 0.039	0.290 0.040	0.929 0.043	0.788 0.041	0.245 0.040	0.267 0.039	0.176 0.039	0.189 0.039	1.577 0.044	0.579 0.040
E	0.316 0.039	0.306 0.039	0.191 0.039	0.190 0.039	0.515 0.040	0.342 0.040	0.156 0.039	0.199 0.052	0.117 0.038	0.117 0.039	0.873 0.041	0.294 0.039
F	0.161 0.039	0.150 0.039	0.119 0.041	0.125 0.038	0.246 0.039	0.172 0.039	0.087 0.038	0.097 0.039	0.082 0.039	0.238 0.193	0.436 0.040	0.166 0.039
G	0.101 0.038	0.109 0.039	0.075 0.041	0.082 0.039	0.143 0.039	0.111 0.042	0.073 0.039	0.106 0.038	0.060 0.038	0.073 0.039	0.288 0.039	0.099 0.039
H	0.065 0.038	0.067 0.038	0.066 0.038	0.064 0.038	0.098 0.038	0.103 0.039	0.056 0.038	0.054 0.038	0.054 0.038	0.053 0.038	0.154 0.039	0.070 0.038

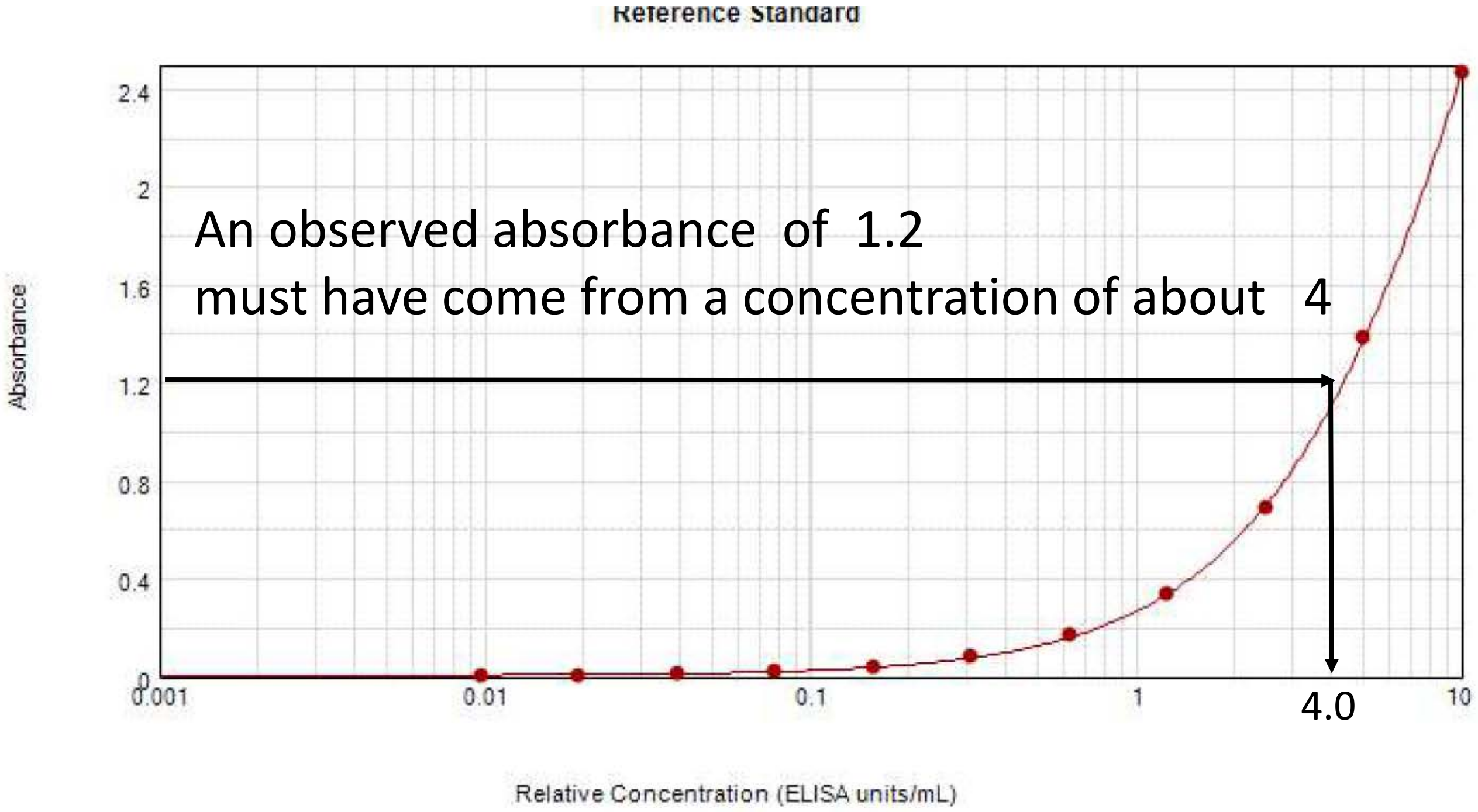
(.416+.341)/2 - (.039+.038)/2 = .34

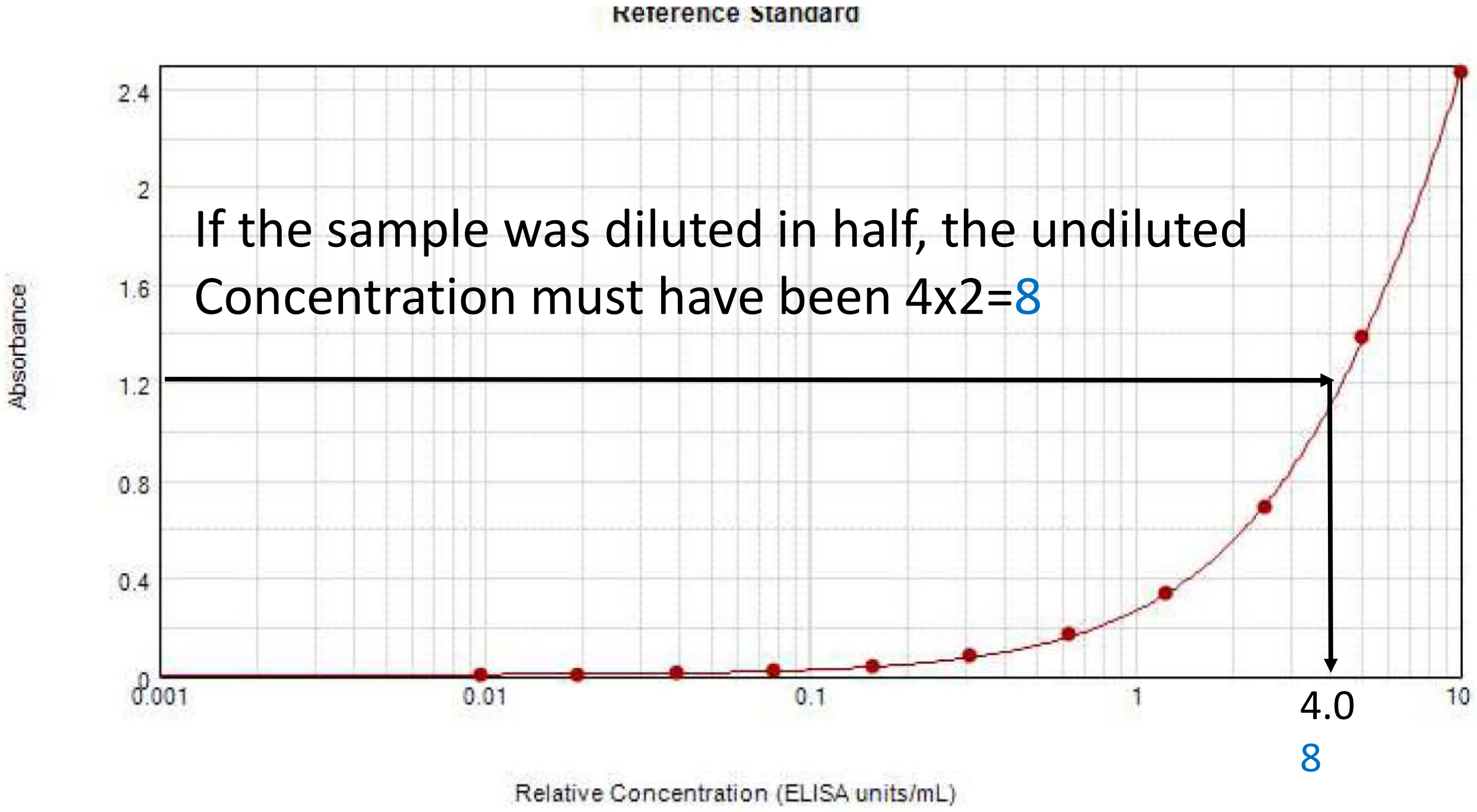

Reference Standard
Absorbance
2.4
2
1.6
1.2
0.8
0.4
0
.34
0.001
0.01
0.1
1
10

ODvalues

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.671 0.048	1.509 0.042	0.767 0.040	0.416 0.039	0.217 0.038	0.121 0.038	0.082 0.037	0.061 0.037	0.054 0.040	0.048 0.039	0.049 0.042	0.130 0.037
B	2.353 0.046	1.347 0.048	0.697 0.040	0.341 0.038	0.201 0.038	0.113 0.038	0.075 0.038	0.059 0.038	0.049 0.038	0.045 0.037	0.043 0.039	0.111 0.039
C	1.075 0.041	1.066 0.042	0.690 0.043	0.616 0.040	1.446 0.043	1.222 0.042	0.426 0.040	0.548 0.040	0.323 0.039	0.350 0.039	2.507 0.048	1.136 0.042
D	0.666 0.040	0.574 0.040	0.428 0.039	0.290 0.040	0.929 0.043	0.788 0.041	0.245 0.040	0.267 0.039	0.176 0.039	0.189 0.039	1.577 0.044	0.579 0.040
E	0.316 0.039	0.306 0.039	0.191 0.039	0.190 0.039	0.515 0.040	0.342 0.040	0.156 0.039	0.199 0.052	0.117 0.038	0.117 0.039	0.873 0.041	0.294 0.039
F	0.161 0.039	0.150 0.039	0.119 0.041	0.125 0.038	0.246 0.039	0.172 0.039	0.087 0.038	0.097 0.039	0.082 0.039	0.238 0.193	0.436 0.040	0.166 0.039
G	0.101 0.038	0.109 0.039	0.075 0.041	0.082 0.039	0.143 0.039	0.111 0.042	0.073 0.039	0.106 0.038	0.060 0.038	0.073 0.039	0.288 0.039	0.099 0.039
H	0.065 0.038	0.067 0.038	0.066 0.038	0.064 0.038	0.098 0.038	0.103 0.039	0.056 0.038	0.054 0.038	0.054 0.038	0.053 0.038	0.154 0.039	0.070 0.038

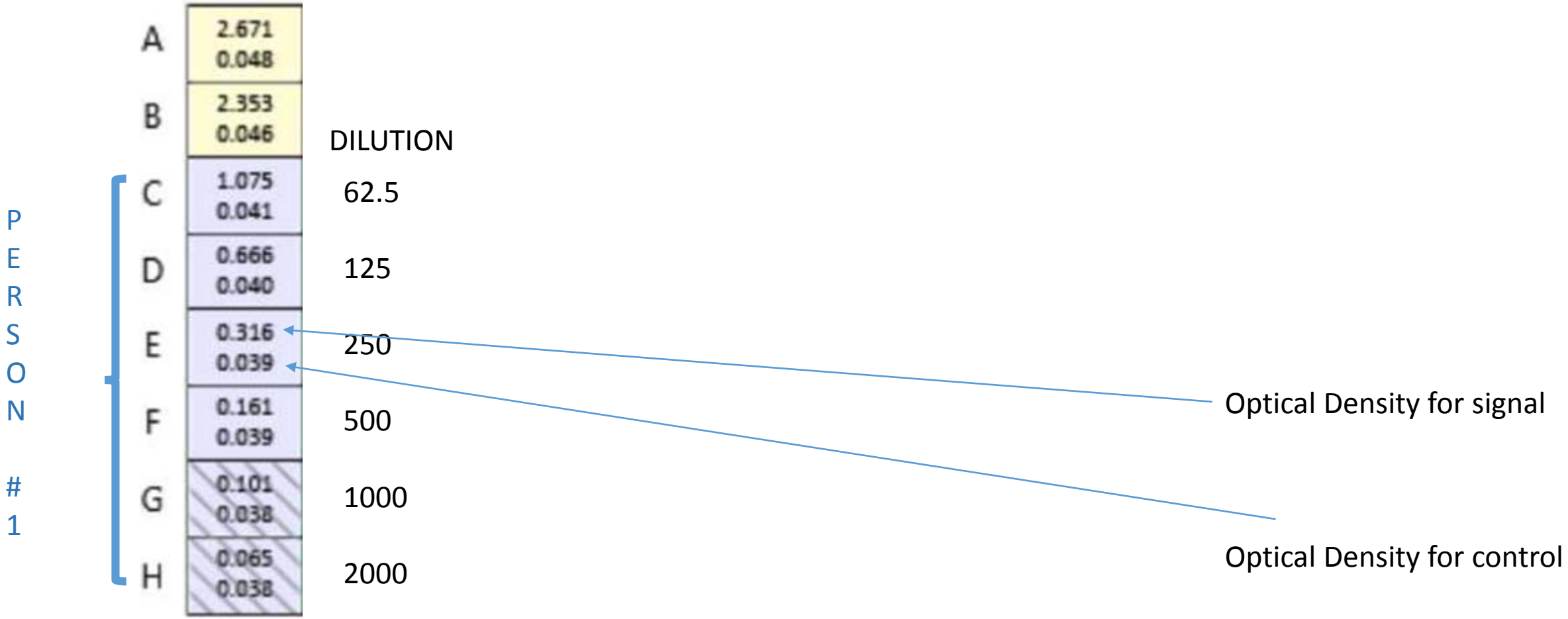
1.22 - .042 about 1.2


Reference Standard
Absorbance
2.4
2
1.6
1.2
0.8
0.4
0
0.001
0.01
0.1
1
10
An observed absorbance of 1.2
must have come from a concentration of about 4
4.0
Relative Concentration (ELISA units/mL)

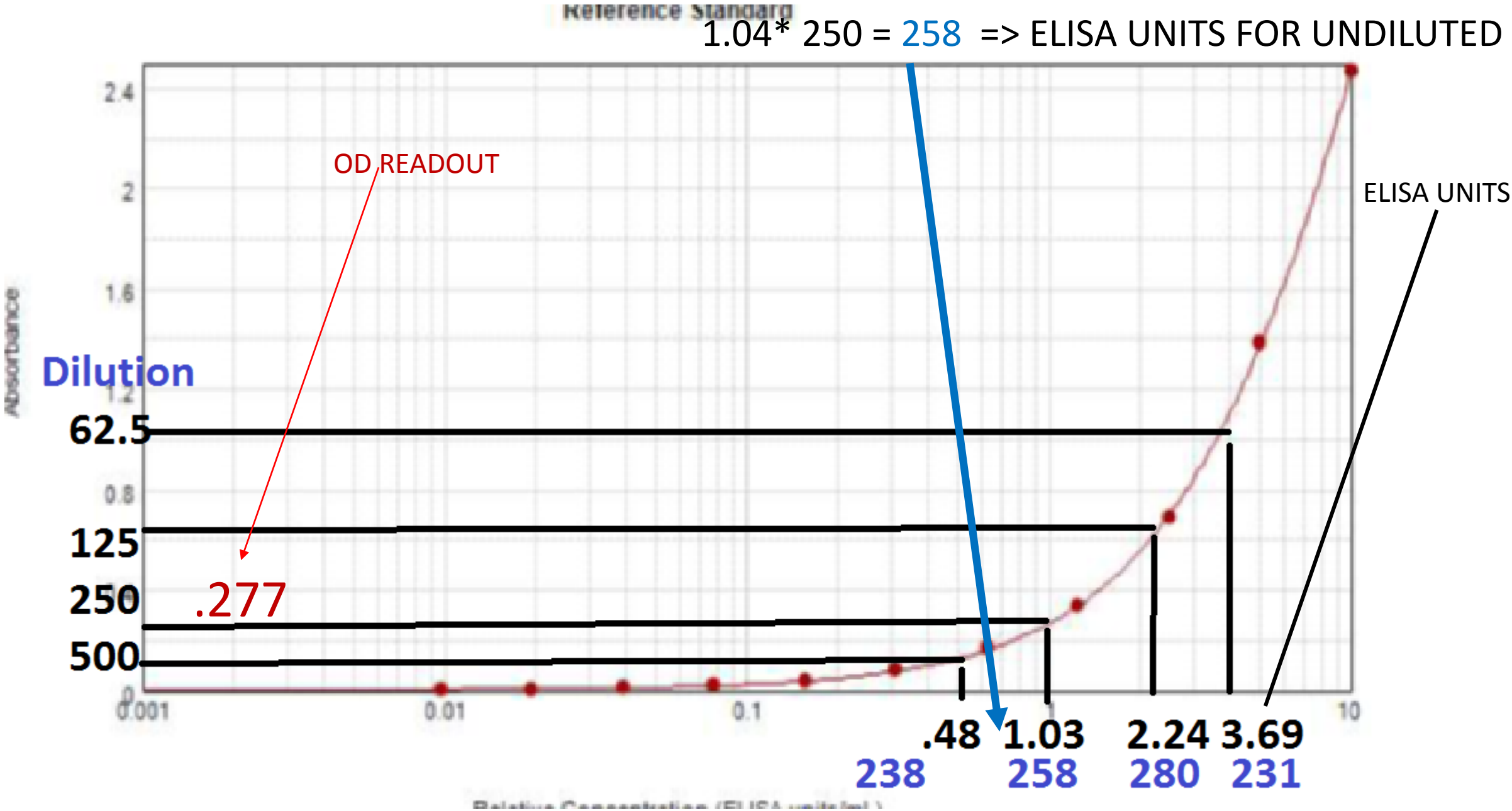


If the sample was diluted in half, the undiluted Concentration must have been 4x2=8

Optical Density for Subject 1's sera at different dilutions



.316 - .039 = .277


Reference Standard
1.04* 250 = 258 => ELISA UNITS FOR UNDILUTED
OD READOUT
ELISA UNITS
Absorbance
2.4
2
1.6
1.2
0.8
0
Dilution
62.5
125
250
500
.277
0.001
0.01
0.1
1
10
.48
1.03
2.24
3.69
238
258
280
231

Readout for person 1

Sample	Wells	Values	Result	Dilution	Adj.Result
1	C1	1.035	3.69	62.5	230.84
2	D1	0.626	2.24	125.0	279.77
3	E1	0.277	1.03	250.0	257.76
4	F1	0.122	0.48	500.0	238.16
5	G1	Masked	Masked	1000.0	Masked
6	H1	Masked	Masked	2000.0	Masked

AVG = 251.63

Masked values are too low or aberrant to be credible and excluded.

- lower readout than negative control
- sample coefficient of variation ($S/\bar{X}$) improves a lot with their elimination

Positivity Criteria

- Vaccine studies like to report the response or ‘take’ rate
  - A relic from variolation when a ‘take’ could be observed if pustule formed where scratched?
  - Take => you’ll be protected?
- Responder definition 1
  - Take Y from unvaccinated controls & determine mean + 2 std = c
  - If readout $Y > c$ => responder
- Responder definition 2
  - Take placebo group 1 month change $Y_1 - Y_0$ & determine mean + 2 std = c
  - If vaccine $Y_1 - Y_0 > C$ => Responder

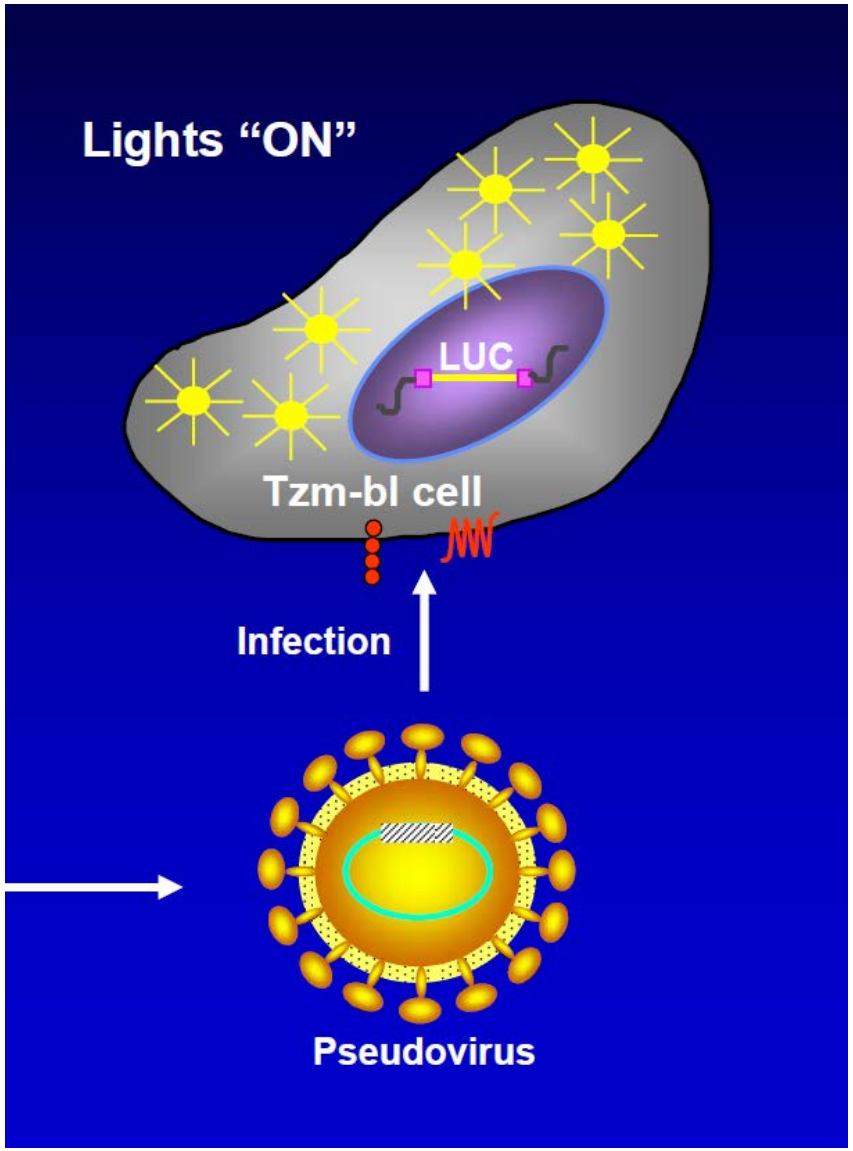
Analysis

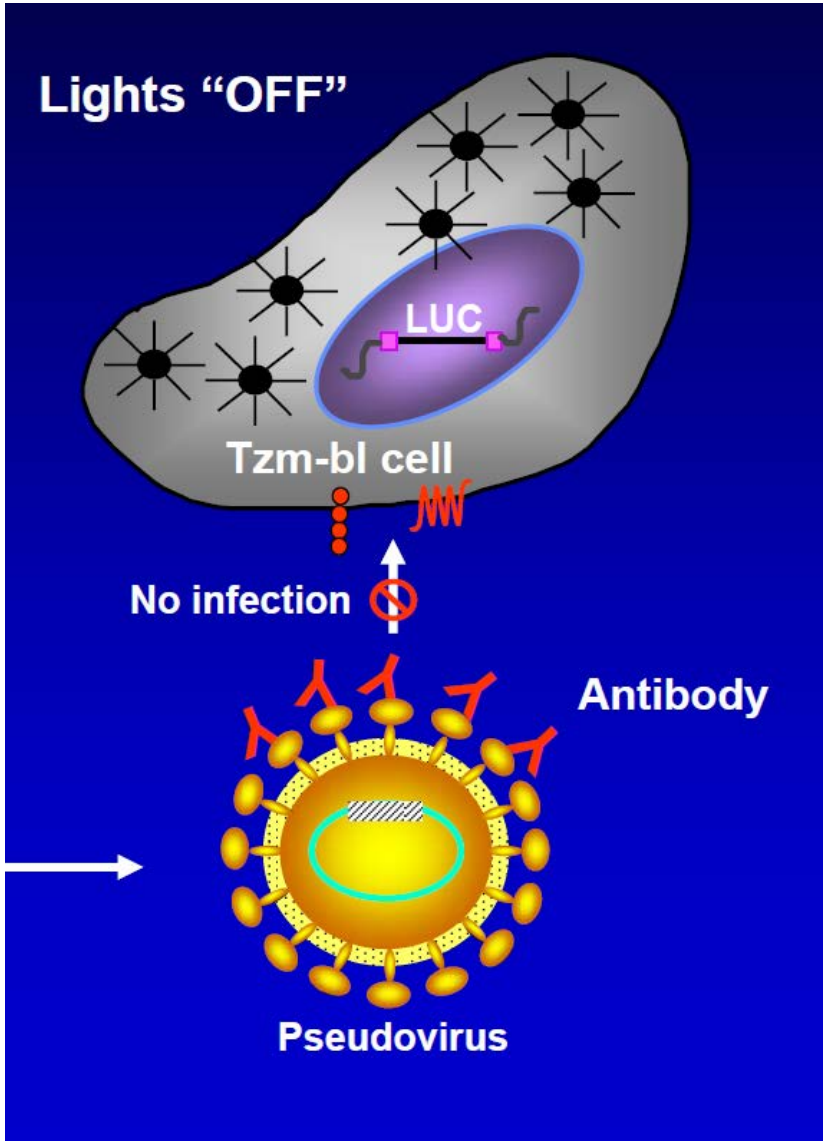
- Use t-test to determine if the mean Y differs between groups
- Use Fisher's exact test to determine if the response rate differs between groups

Functional antibody assay: TZM-bl

- TZM-bl cells are genetically engineered HIV-infectable cells that contain a gene for luciferase which makes fireflies glow
  - Lucifer – light-bearer (lucem ferre)
- If HIV infects a TZM-bl cell HIV replication within the cell turns on the luciferase gene.
- Mix serum from vaccinees, TZM-bl cells, and HIV-like virions in wells at various concentrations

How the TZM-bl Assay works





Envelope Pseudotyped Virus TZM-bl Neutralization Assay

Different amounts of pseudo-virus

virus

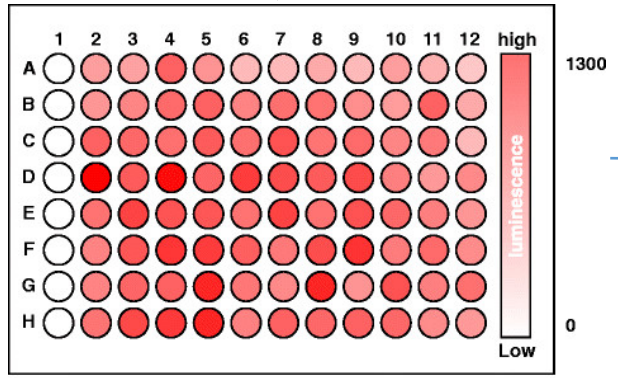
\+

antibodies γ

HIV LTR — Luciferase

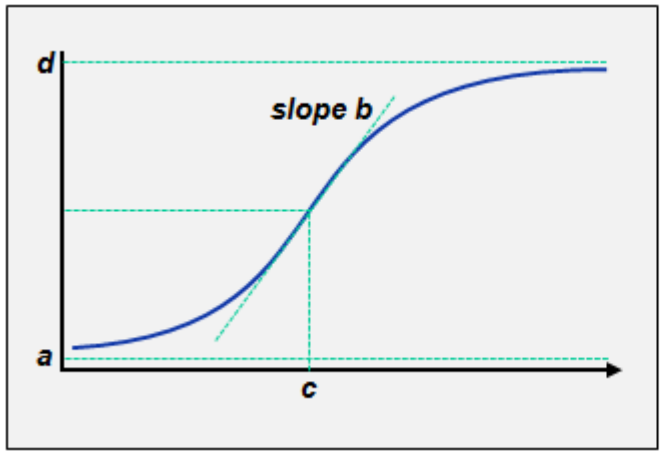
TZM-bl cells

incubate (48 hrs)

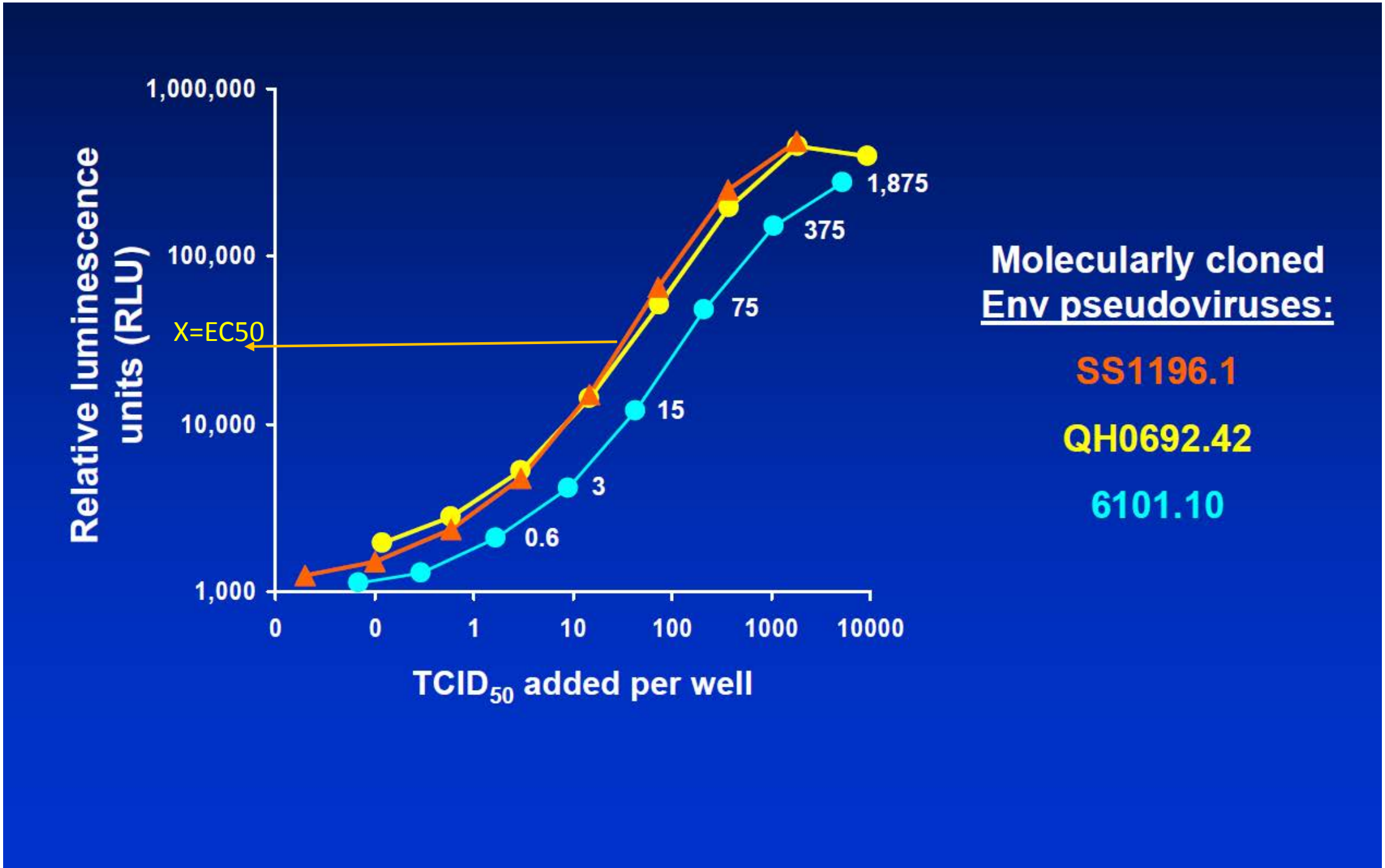


n = 88, vector amount 1 µg, average 727, standard error 21

Fit a 4 parameter logistic curve via leas squares



a = minimum asymptote
b = steepness of the cu
c = inflection point
d = maximum asymptot


1,000,000
100,000
10,000
1,000
Relative luminescence units (RLU)
X=EC50
1,875
375
75
15
3
0.6
0
0
1
10
100
1000
10000
TCID50 added per well
Molecularly cloned
Env pseudoviruses:
SS1196.1
QH0692.42
6101.10

Analysis of TZM-bl readout

- Interest in how much antibody is needed for protection
- Gave 21 animals different amounts of antibody
  - Measured amount in blood using TZM-bl assay - X
  - Animals were 'challenged' with SHIV and infection status recorded
    Y = 1 if infected Y = 0 uninfected

- Used maximum likelihood to estimate a, b of probit regression

$$P(Y = 1) = \Phi(a + b X)$$

Standard Normal Cumulative Distribution Function

TZM-bl	Infection	Old assay
3.2582	0	2.0899
3.6101	0	2.0899
	0	1.6021
3.1715	0	1.6021
2.9314	0	1.1761
2.9256	1	1.2553
3.0066	0	1.2553
2.5736	0	1.1139
2.6198	0	1.0792
	1	0.8451
1.9655	0	0.7782
2.4441	1	0.8451
1.9834	1	0.4771
	1	0.6021
	0	0.6021
	0	0.9031
	0	0.699
	1	0.6021
	1	0.4771
	1	0.4771
	1	0.4771

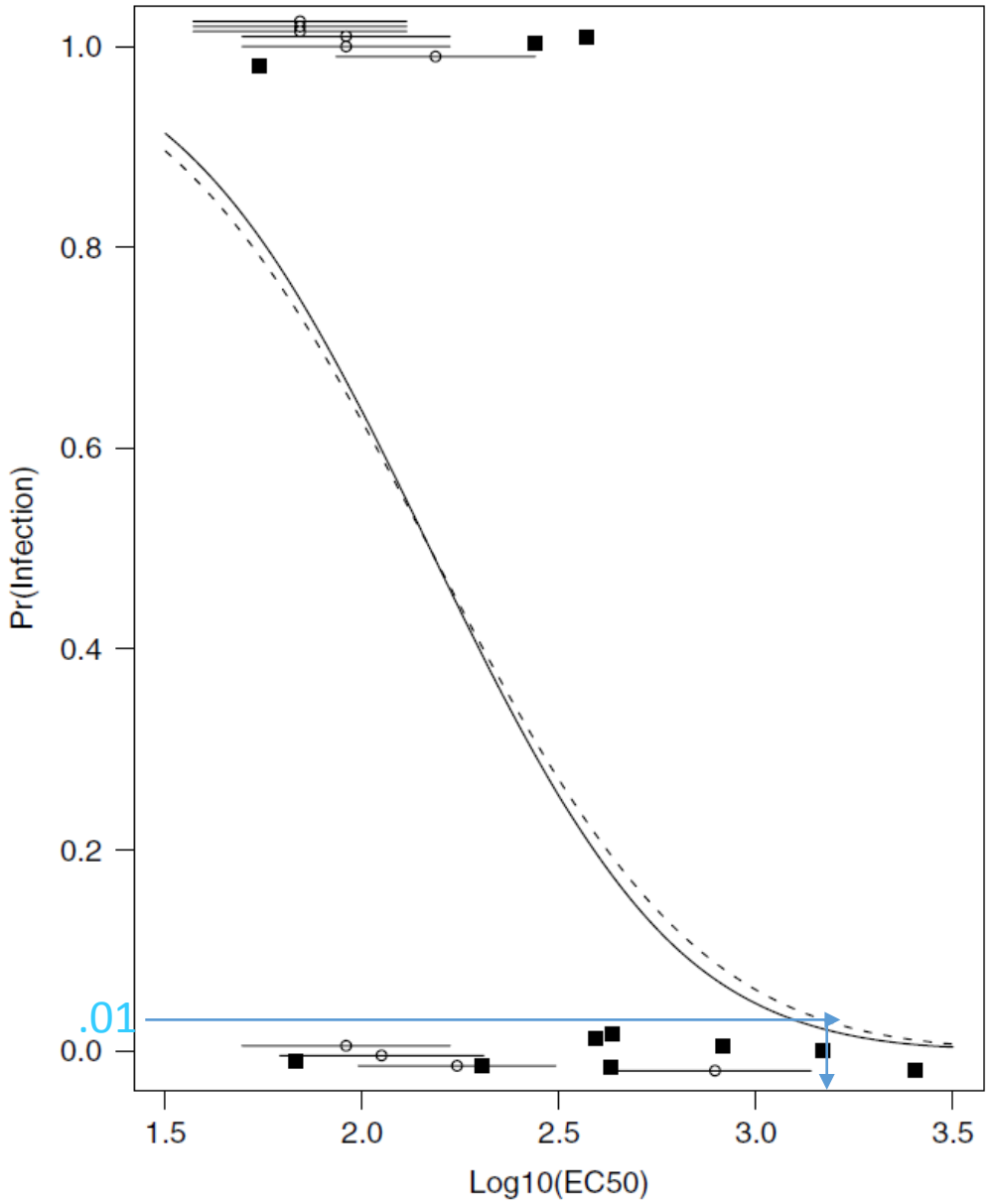


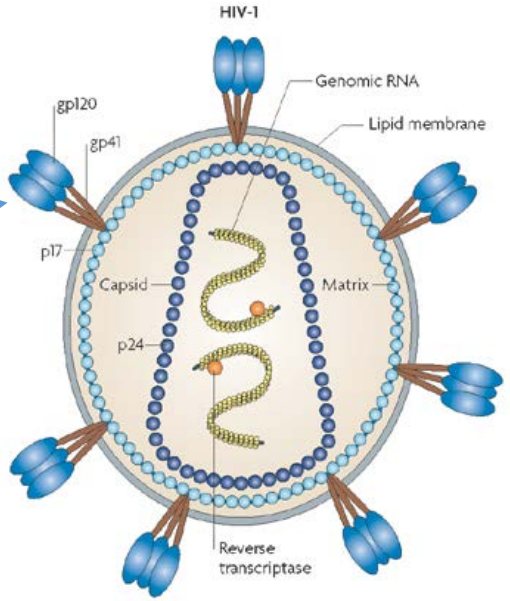
Table 1

Pseudo-likelihood estimates for the ID_{50} and ID_1. Confidence intervals are obtained by profiling the pseudo-likelihood ratio test statistic.

		95% profile CI	
Parameter	Estimate	Lower	Upper
ID_{50}	2.17	1.46	2.62
ID_1	3.32	2.67	8.04

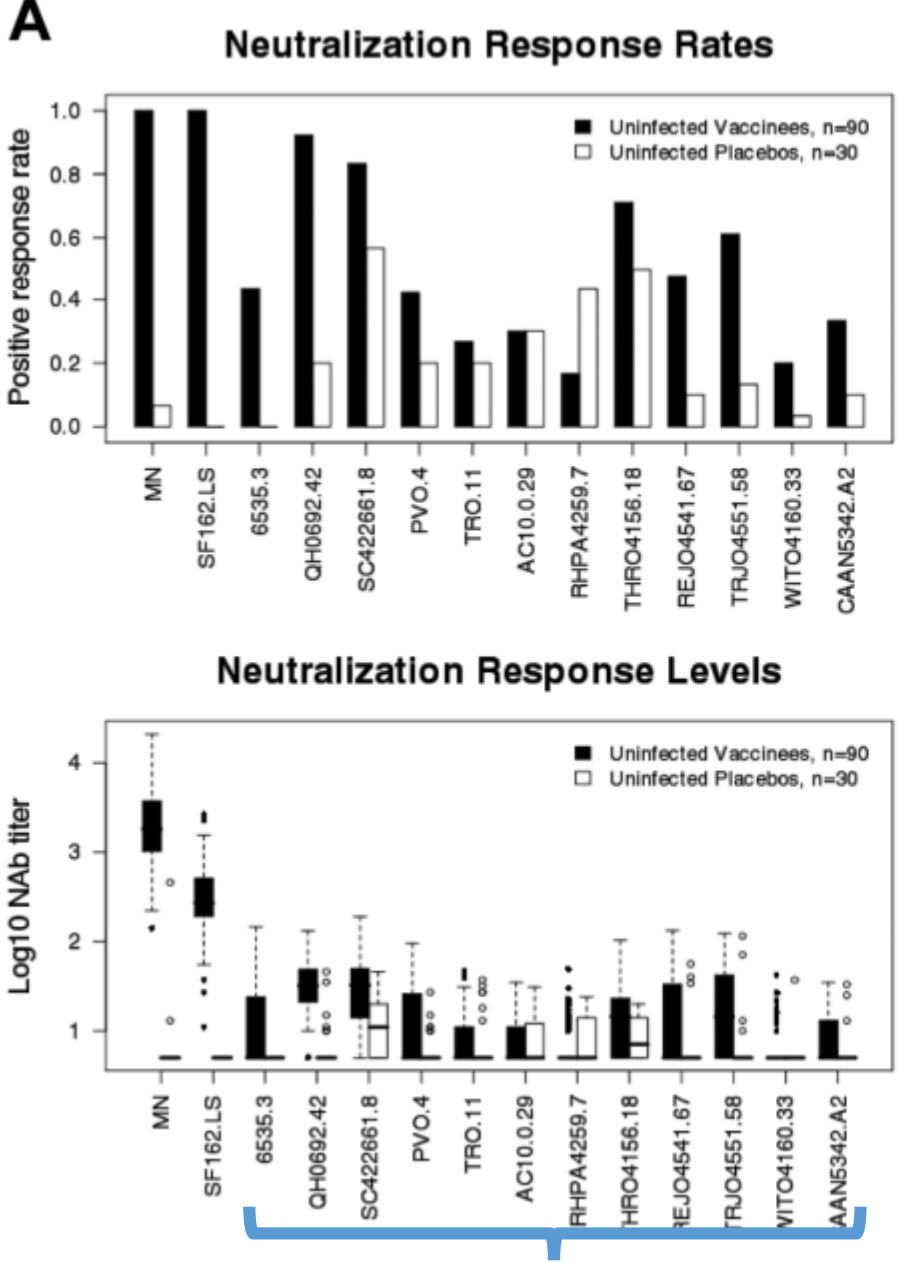
Target for a vaccine may be to achieve 3.32 units of Antibody

TZM-bl assay in a human vaccine trial



- VAX004 was the first phase III HIV vaccine trial
  - Used 2 gp120 proteins from 2 HIV-1 strains
  - Overall there was no efficacy

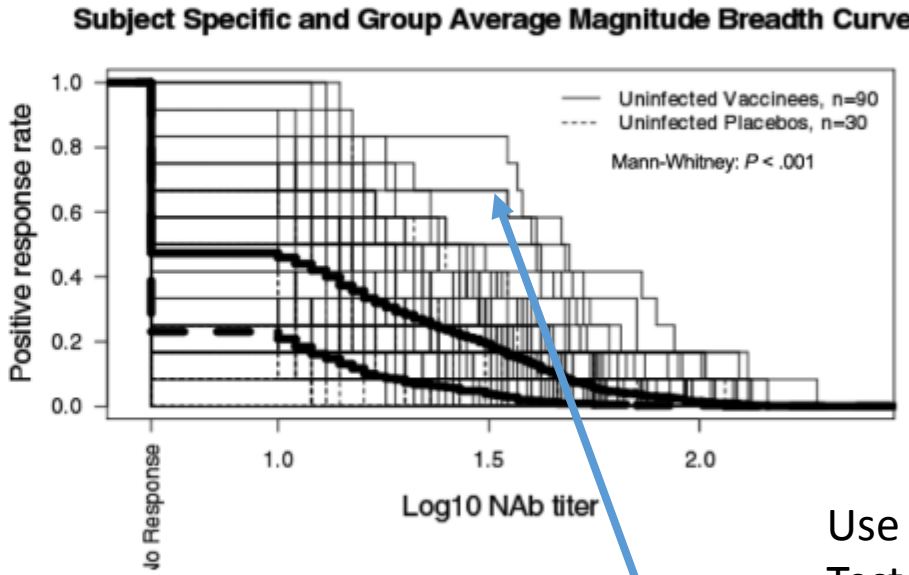
- Use the TZM-bl assay to characterize the immune response of an inefficacious vaccine.
  - Evaluate 14 types of HIV virus: HIV-1_{MN}, SF162.LS, and 12 'tier 2' viruses



Tier 2----Harder to neutralize

Gilbert et al 2010

Tier 2 viruses

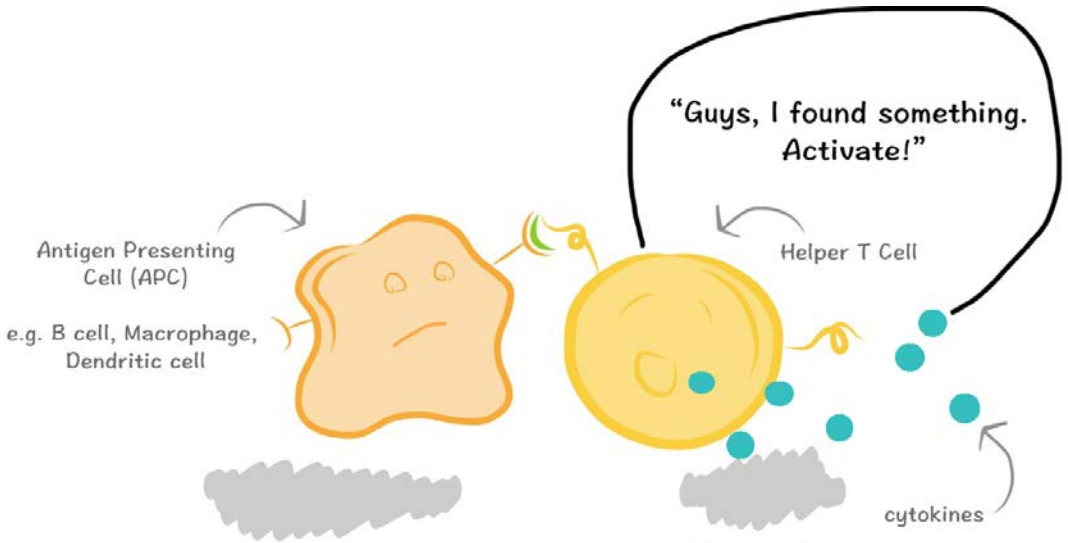


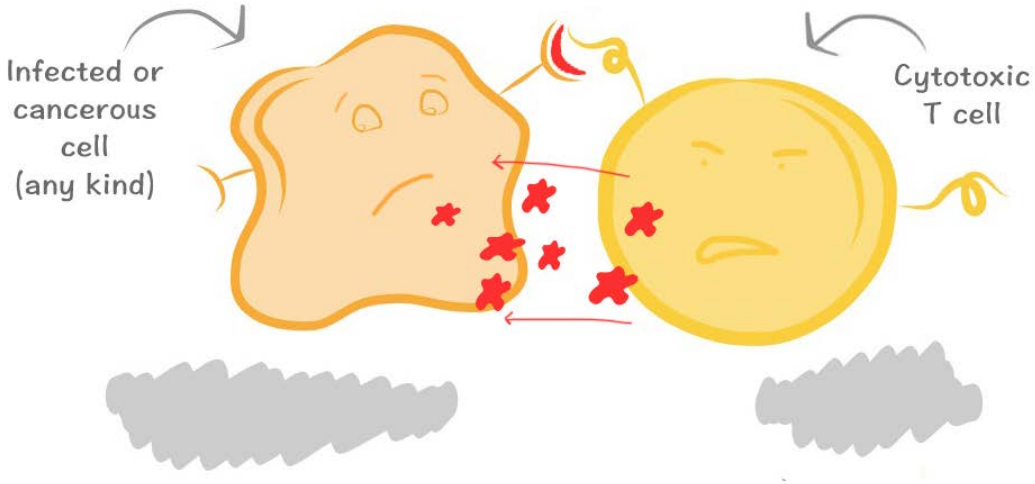
Use Mann-Whitney Test to compare AUCs between groups

This vaccinee had a TZM-bl value X of 1.6 or better for 8 of 12 tier 2 viruses

Measurement of T-cells

- T-cells secrete chemicals
  when confronted with a cell
  that displays part of *their* germ
- The chemicals either
  - Signal other cells to come and destroy
  - Kill an infected cell
- Assays ‘annoy’ T cells with e.g. HIV
  antigen and look for chemicals, if
  present, T-cell recognized HIV….

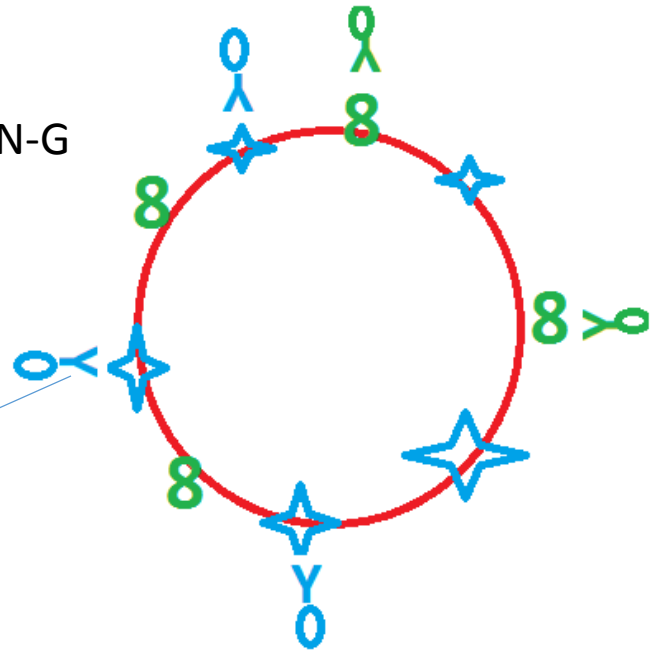




Flow cytometry measurement of T cells

- Sample circulating T-cells from blood of a vaccine trial subject
- Annoy the T-cells with HIV-peptides and see if they secrete chemicals
- Make fluorescently labeled antibodies to attach to chemicals and T-cell molecules

Stimulated CD8+ T cell that secretes IFN-G

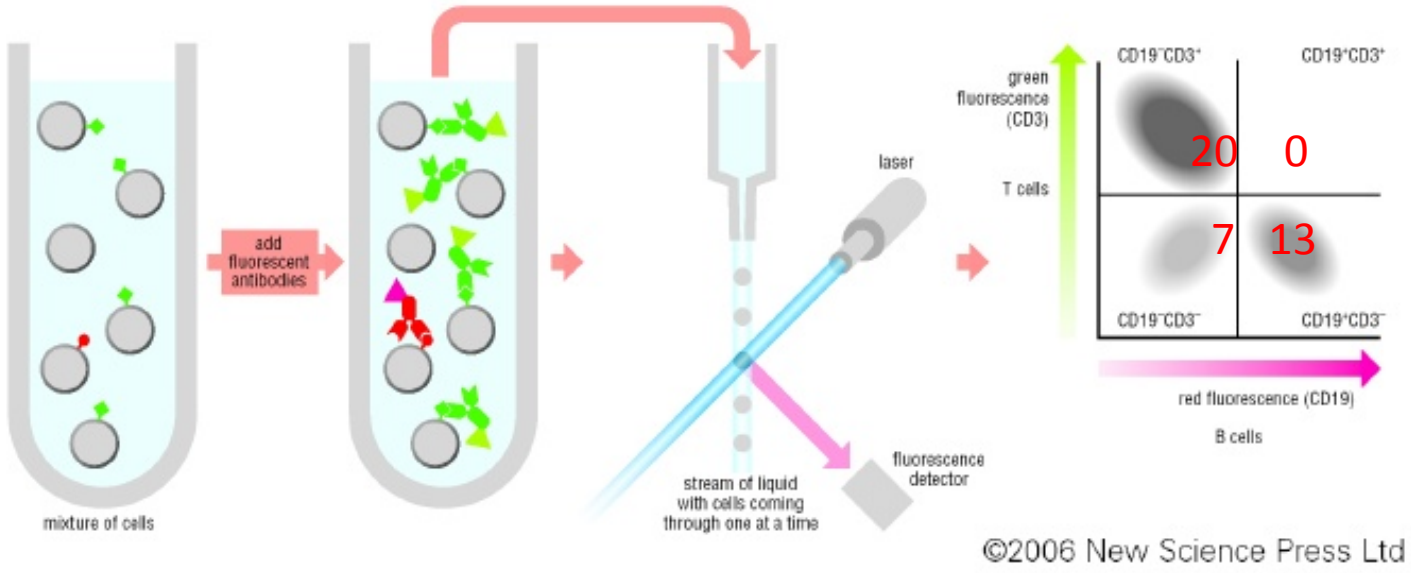


Fluorescent antibody to CD8

Fluorescent antibody to cytokine intefereon-gamma

Flow cytometry measurement of T cells

Use of monoclonal antibodies recognizing CD antigens by flow cytometry



Flow cytometry: measure the amount of a protein on the surface (or inside) individual cells; measure the numbers of particular types of cells in blood (etc.)

Count # of cells in each quadrant

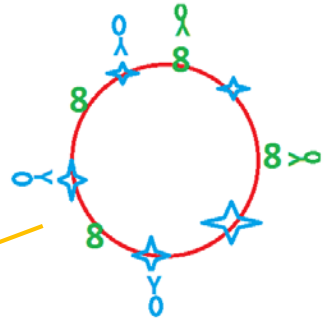
CD19 -	CD3 –	7
CD19+	CD3 -	13
CD19-	CD3 +	20
CD19+	CD3 +	0

Table 1 Sample Data for one blood sample from one patient, CD8 cells only, in response to background and peptide stimulation (columns). The rows represent the set of 2^5 mutually exclusive outcomes of positive and negative results for the five cytokines

PatientID	Combo	Background	ENV	GAG	NEF	POL	TRV
1481	$7+g+b+2+a+$	0	1	53	11	10	53
1481	$7+g+b+2+a-$	0	0	21	11	13	34
1481	$7+g+b+2-a+$	5	20	298	77	97	198
1481	$7+g+b+2-a-$	18	67	658	311	228	370
1481	$7+g+b-2+a+$	0	0	0	0	0	0
1481	$7+g+b-2+a-$	1	0	1	0	0	0
1481	$7+g+b-2-a+$	1	0	0	1	2	3
1481	$7+g+b-2-a-$	1	7	23	15	4	29
1481	$7+g-b+2+a+$	1	0	0	0	0	0
1481	$7+g-b+2+a-$	5	2	3	4	8	8
1481	$7+g-b+2-a+$	4	9	7	0	4	5
1481	$7+g-b+2-a-$	222	380	458	393	380	297
1481	$7+g-b-2+a+$	0	0	0	0	0	0
1481	$7+g-b-2+a-$	28	36	12	19	34	33
1481	$7+g-b-2-a+$	0	1	1	0	0	2
1481	$7+g-b-2-a-$	242	357	253	269	209	248
1481	$7-g+b+2+a+$	0	0	1	1	0	2
1481	$7-g+b+2+a-$	3	3	3	0	0	0
1481	$7-g+b+2-a+$	6	5	5	2	2	21
1481	$7-g+b+2-a-$	13	29	46	33	31	58
1481	$7-g+b-2+a+$	0	0	0	0	0	0
1481	$7-g+b-2+a-$	0	3	0	0	0	3
1481	$7-g+b-2-a+$	0	0	0	1	0	1
1481	$7-g+b-2-a-$	8	45	23	29	89	100
1481	$7-g-b+2+a+$	0	0	0	0	0	0
1481	$7-g-b+2+a-$	1	12	0	3	5	5
1481	$7-g-b+2-a+$	4	5	2	1	2	2
1481	$7-g-b+2-a-$	1269	1898	1476	1242	1443	984
1481	$7-g-b-2+a+$	1	1	0	1	0	0
1481	$7-g-b-2+a-$	113	424	44	89	87	88
1481	$7-g-b-2-a+$	64	85	24	25	32	39
1481	$7-g-b-2-a-$	199351	219871	207308	204533	157181	192849

Different parts of HIV

Counts of different CD8+ Killer T-cells



CD8+ T-cells that secrete IFN-g but nothing else

Simple Analysis of ICS T-cells

PatientID	Combo	Background	ENV	GAG	NEF	POL	TRV
1481	$7-g+b-2-a-$	8	45	23	29	89	100

Patient 1481's Readout for CD8 positive T-cells that recognize TRV:
100/195379 - 8/201401 = .00047 = Y

Can do a univariate t-test of outcome Y in vaccinees versus placebos

Can do a multivariate t-test to see if the vector of outcomes differ between vacinees and placebos

Mixture model Analysis of ICS T-cells
To classify 'responders' borrows information

396,788 T cells from `Fred`

	Positive (for IF-gamma alone)	Negative (for IF-gamma alone)
Stimulated	100	195279
Unstimulated	8	201401

- Let p^s be the true probability a stimulated cell is positive
- Let p^u be the true probability an unstimulated cell is positive
- $Y^s = 100 \sim$ Binomial (p^s ,195379)
- $Y^u = 8 \sim$ Binomial (p^u ,201409)

- Assume

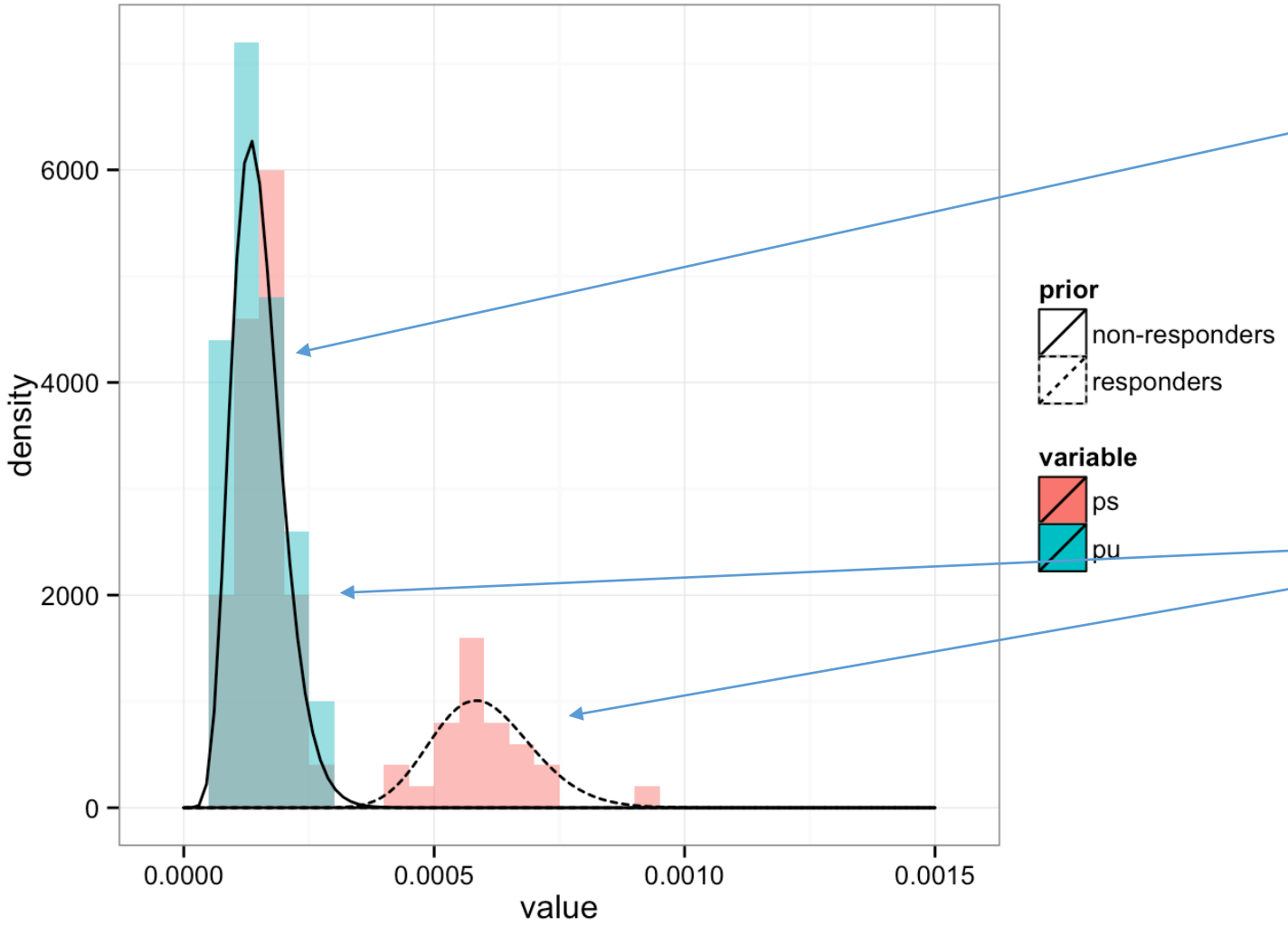
  p^s follows Beta(a^u, b^u) if non-responder i.e. R=0

  p^s follows Beta(a^s, b^s) if responder i.e. R=1

  } Mixture

  p^u follows Beta(a^u, b^u) distribution

Beta distributions for p for all subjects



A non responder `draws'
p^u for Y^u # unstimulated cells
and for
Y^s # stimulated cells

A responder `draws'
p^u for Y^u # unstimulated cells
p^s for Y^s # stimulated cells

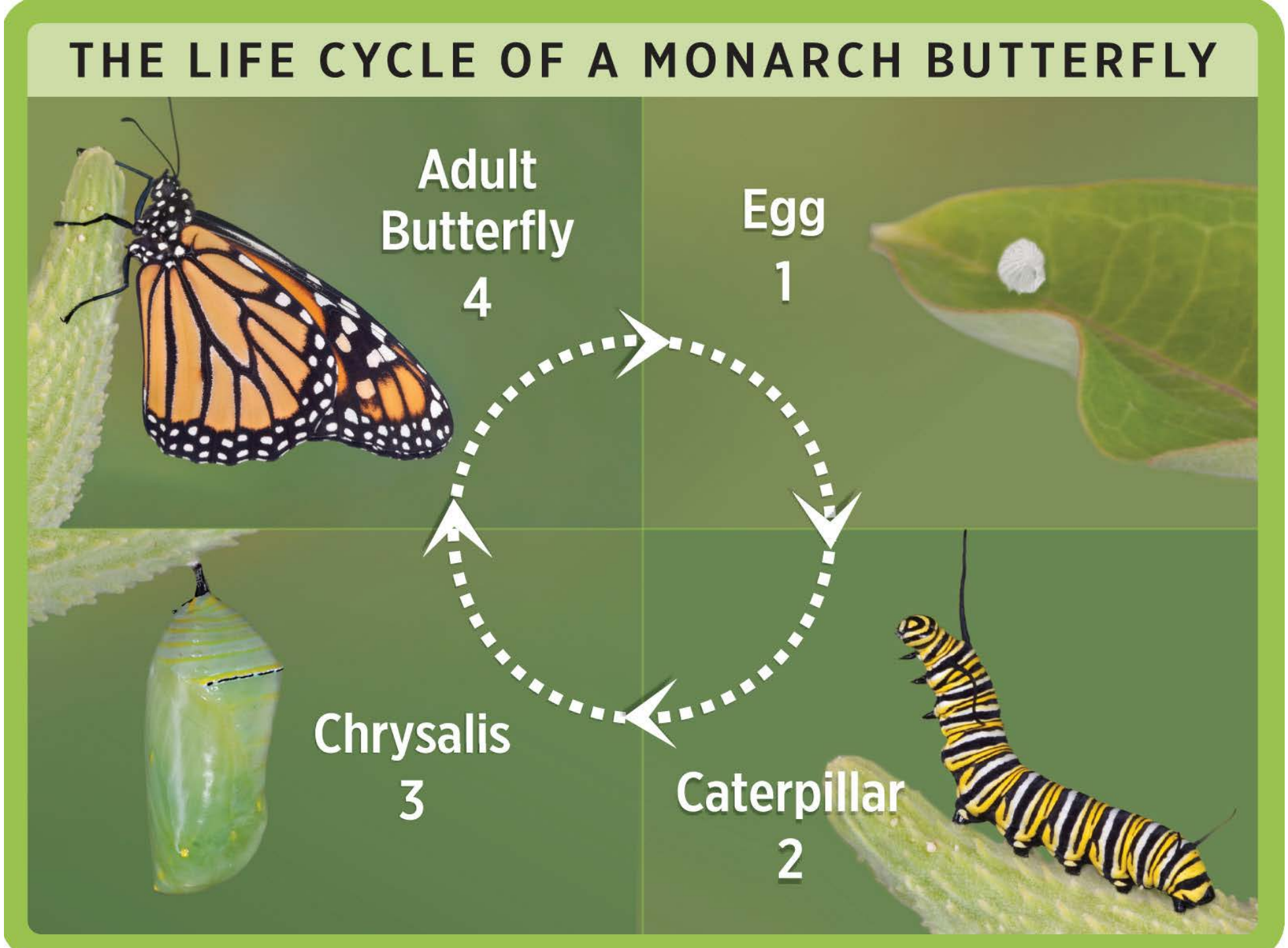
MIMOSA Analysis

- Each person is a non-responder (R=0) or responder (R=1)
- Can estimate $\Pr(R=1) = \Pr(p^u < p^s)$

  if large enough, classify as a responder
- Can be more sensitive and specific than using Fisher's exact test

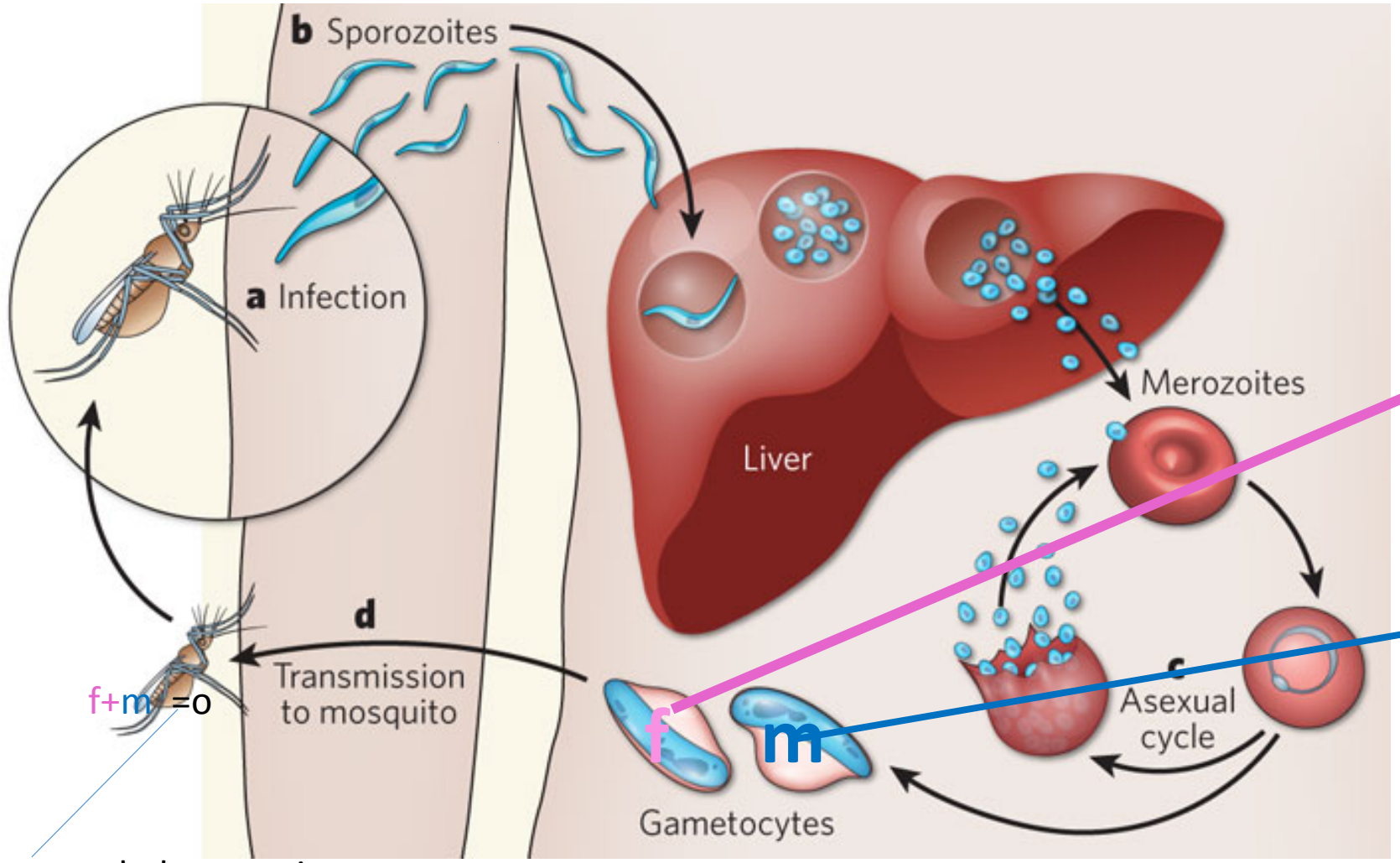
  For a subject ``Fred"
  - Borrows strength from other subjects via use of commonly estimated

    $\text{Beta}(a^u, b^u)$ and $\text{Beta}(a^s, b^s)$
  - Blends it with 2x2 table for Fred's cell counts
- Fisher's exact test only uses Fred's table to classify Fred.


THE LIFE CYCLE OF A MONARCH BUTTERFLY
Adult Butterfly 4
Egg 1
Chrysalis 3
Caterpillar 2

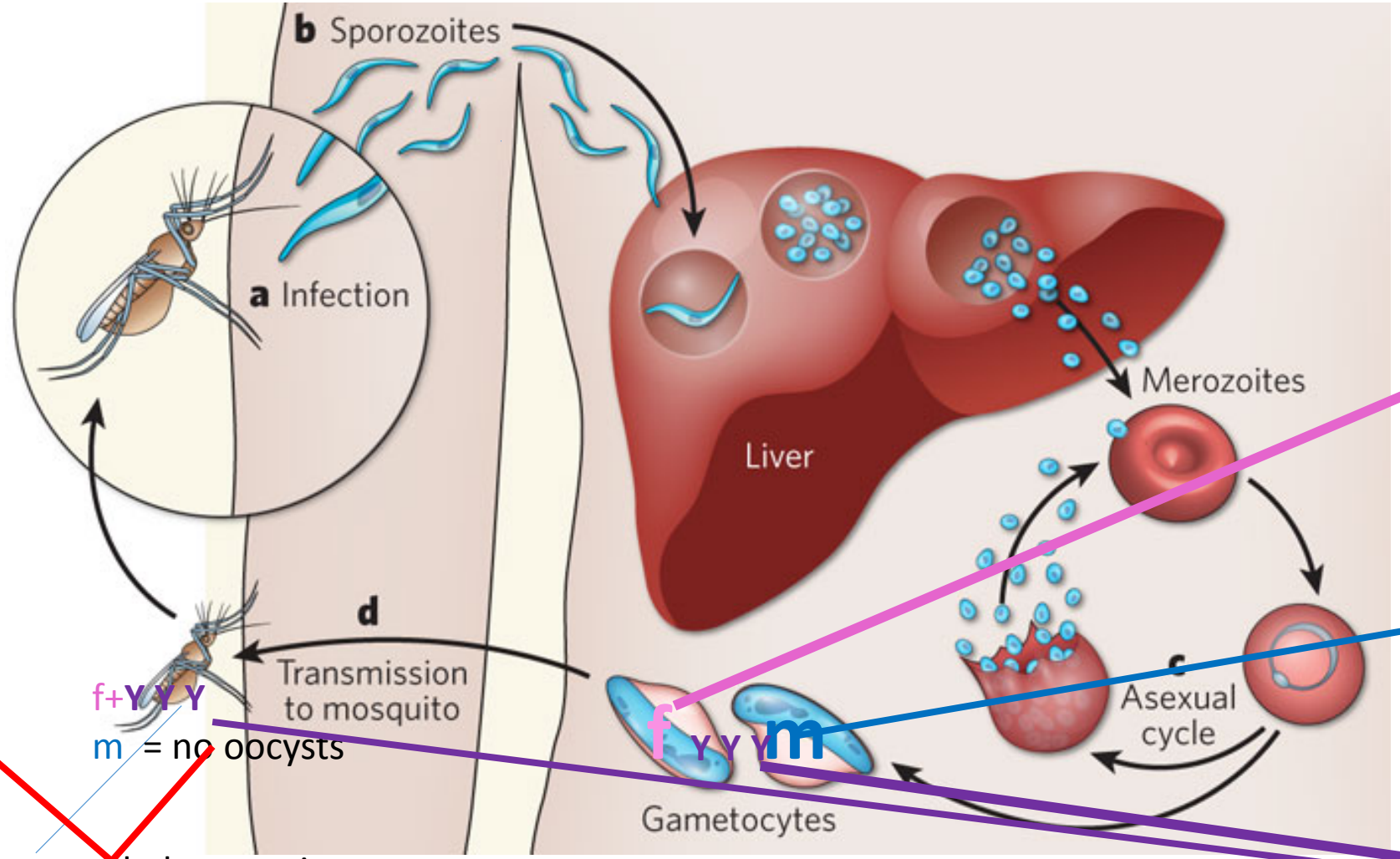
Kafka

Malaria Life Cycle



Oocysts = baby parasites

Antibodies break the life cycle



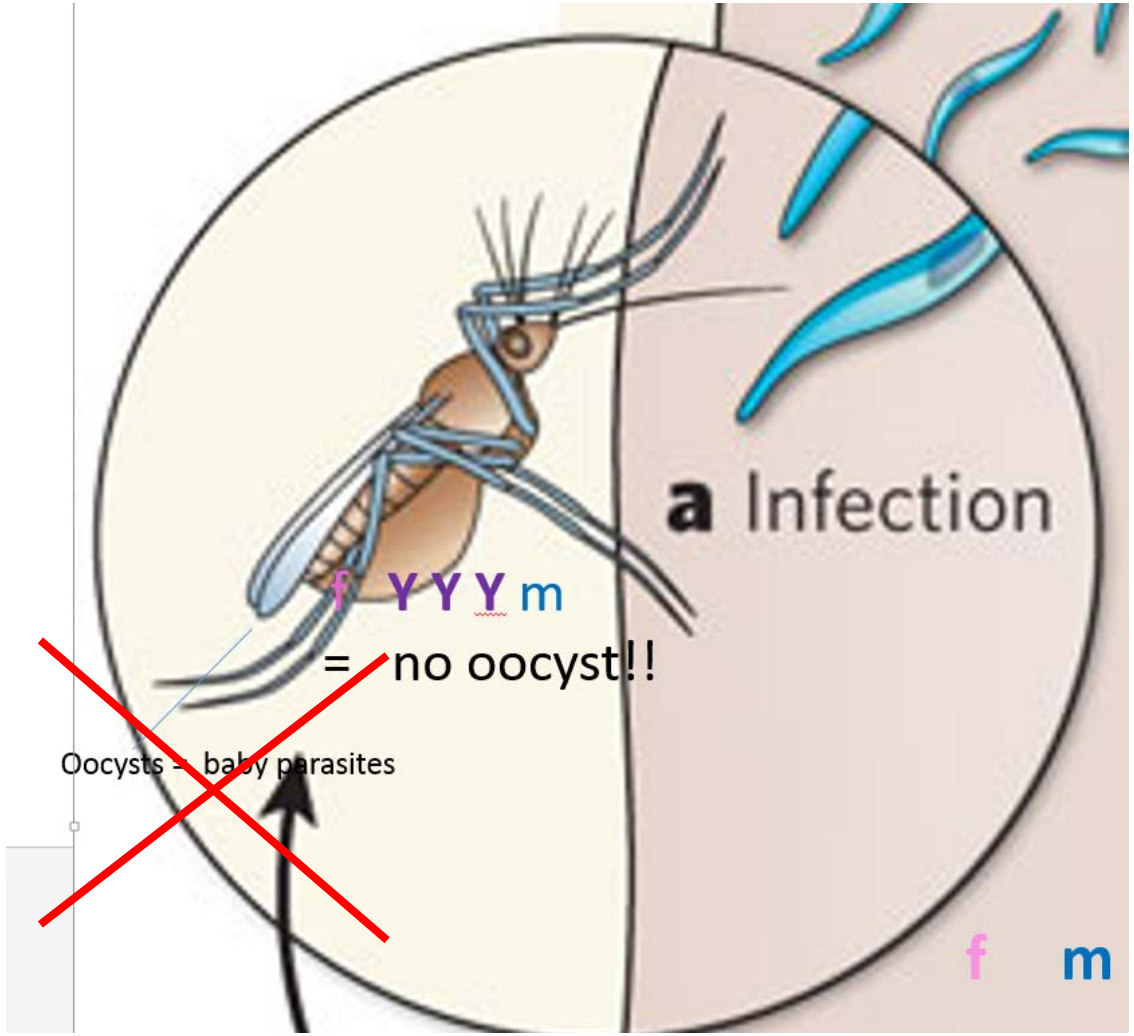
female

male

Oocysts = baby parasites

Vaccine induced antibodies

Antibodies: Barrier method of parasite birth control



Standard Membrane Feeding Assay

- Gametocyte (**f**/**m**) to oocyst to sporozoite in mosquito
- Vaccinated humans produce antibodies **Y Y**
- Mosquito sucks up blood and
  - **m f Y Y f m Y Y f f Y Y m**
- Antibodies prevent oocyst progeny from gametocyte parents **f m**
- Use a Membrane Feeding Assay to see how well antibodies block oocyst development

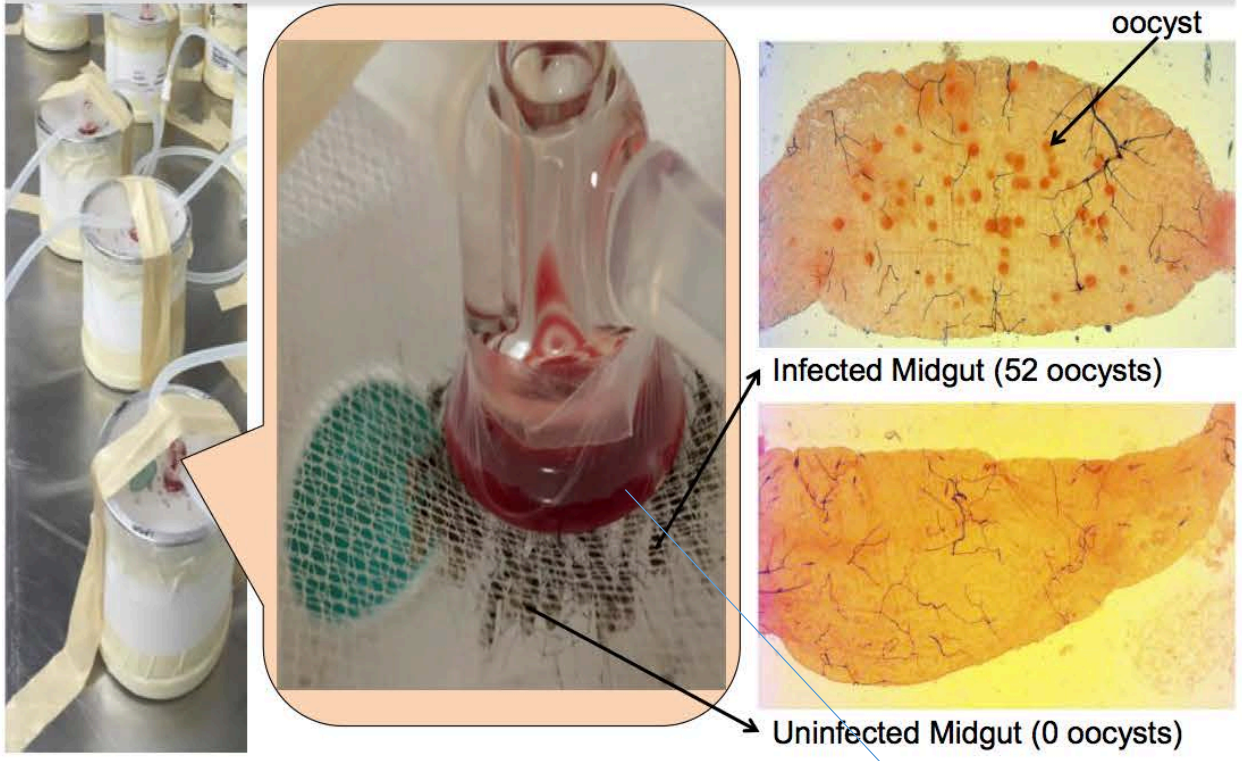
Assay Readouts for SMFA

EMPIRICAL

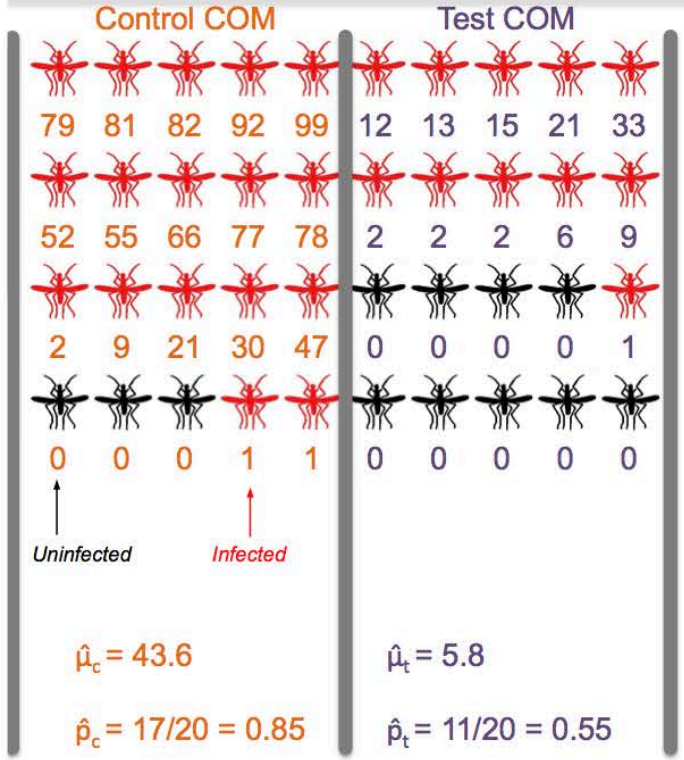
COMs on a Feed-day

Mosquitoes feeding in a COM (zoomed)

Mosquitoes dissected, oocysts counted (2 midguts shown)



20 Mosquitoes per COM with oocyst counts



Corresponding readouts for this SMFA

TRA:

$$100 \times \left(1 - \frac{\hat{\mu}_t}{\hat{\mu}_c}\right) = 86.7$$

TBA:

$$100 \times \left(1 - \frac{\hat{p}_t}{\hat{p}_c}\right) = 35.3$$

Blood + Gametocyte (f/m) + vaccine trial subject's antibodies

Assay Analysis

i j k
day container mosquito

- Use a zero-inflated negative binomial mixture model for the counts

$$P(Y_{ijk} = y_{ijk}) = \begin{cases} \pi + (1-\pi)\left(1+\frac{\lambda_{ij}}{\theta}\right)^{-\theta}, & y_{ijk} = 0 \\ (1-\pi)\,\frac{\Gamma(y_{ijk}+\theta)}{\Gamma(y_{ijk}+1)\Gamma(\theta)}\,\frac{\left(\frac{\lambda_{ij}}{\theta}\right)^{y_{ijk}}}{\left(1+\frac{\lambda_{ij}}{\theta}\right)^{y_{ijk}+\theta}}, & y_{ijk} = 1, 2, \ldots \end{cases}$$

- Use model to estimate E(Y) = μ and P(Y>0) = p for Control COM and Serum COM. Form TRA, TBA

TRA:

$$100 \times \left(1 - \frac{\hat{\mu}_t}{\hat{\mu}_c}\right) =$$

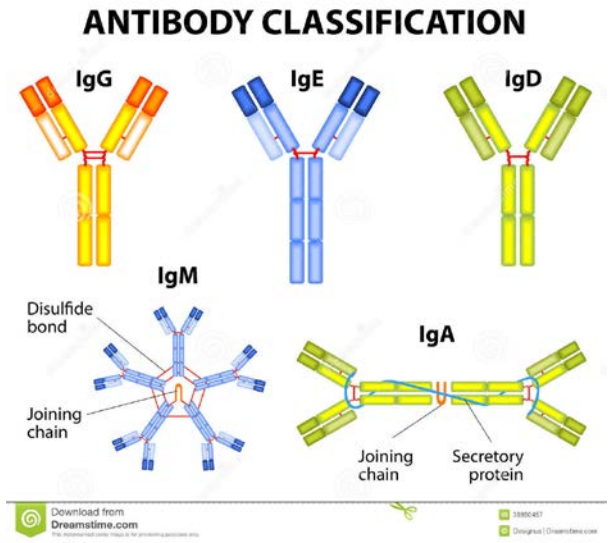
TBA:

$$100 \times \left(1 - \frac{\hat{p}_t}{\hat{p}_c}\right) =$$

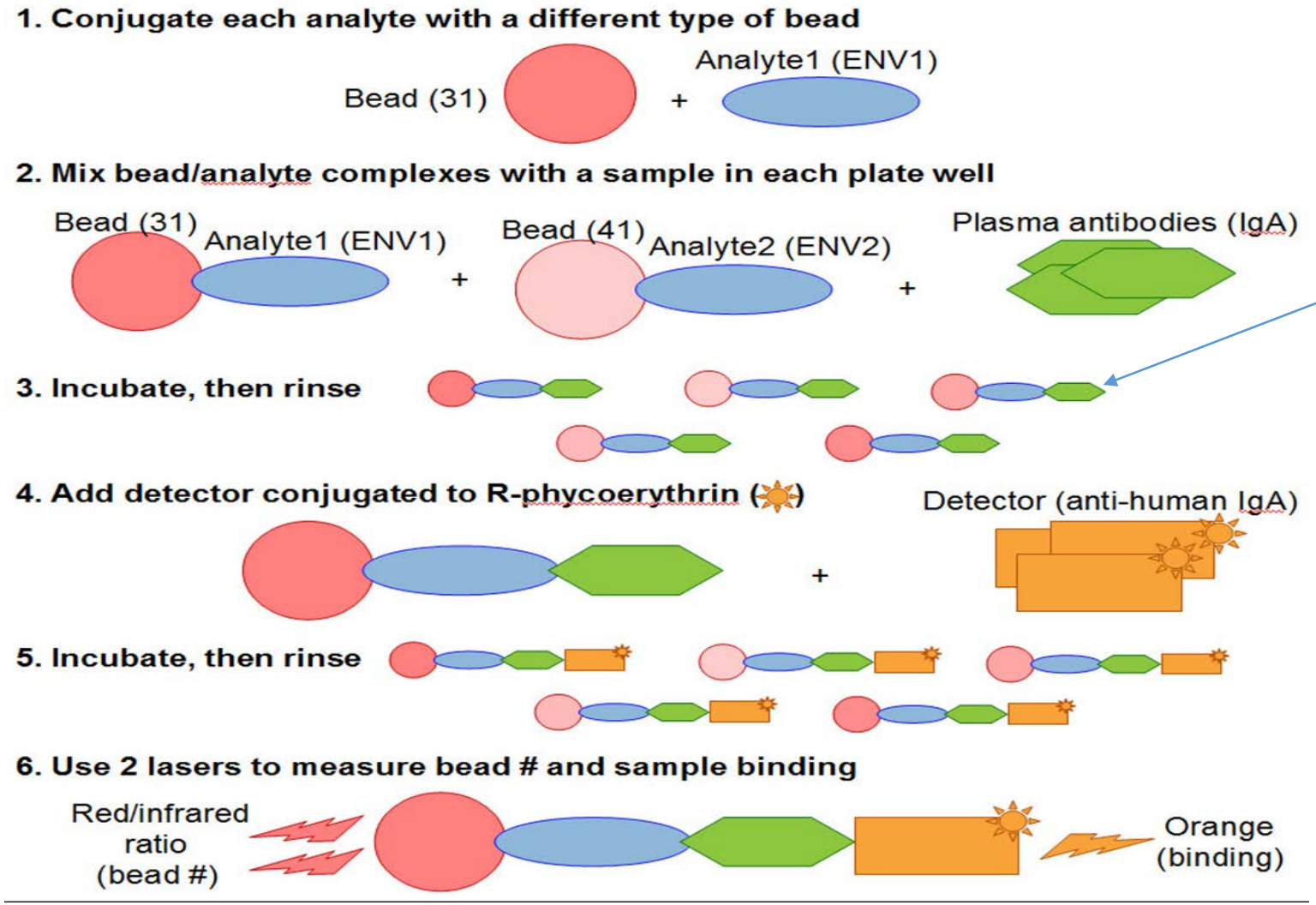
Binding Antibody *Multiplex* Assay

- Simultaneous evaluation of multiple parameters *within each well*
  - Multiple antigens --- different bits of Ebola
  - Multiple types of antibodies ---- different classes different function
- e.g. IgA type antibody recognizes gp120 region of HIV
- Used in immune correlates analysis of an HIV vaccine to efficiently evaluate many antigen/antibody types

Haynes et al 2012



BAMA details



IgA type antibody recognizes Env2

Eckels et al 2013

Design your multiplex experiment

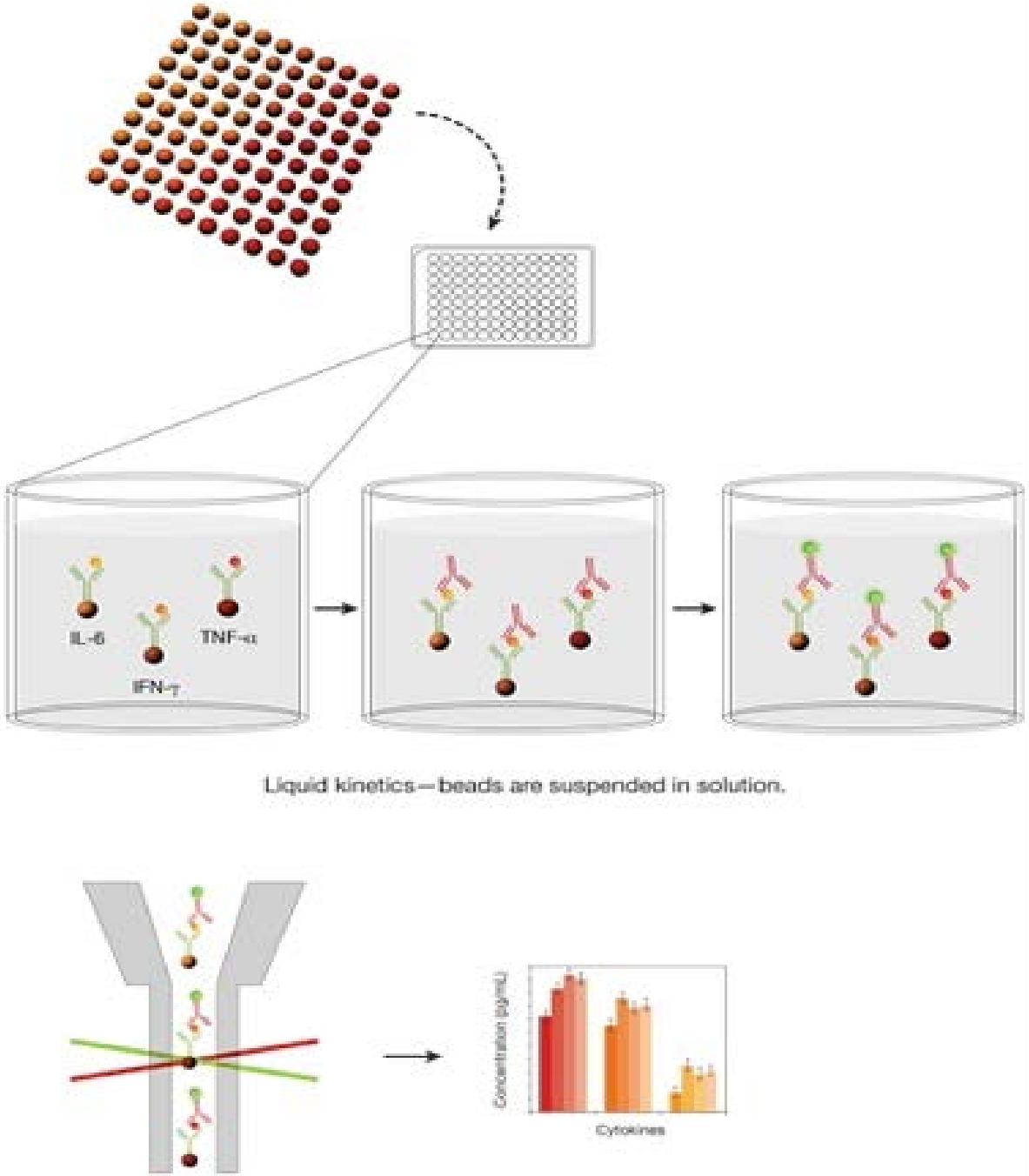
Choose from up to 100 unique, highly specific beads, ideal for microplate-based analysis.

Perform your assay

With 3 simple incubations and 3 wash steps, your assay is complete.

Get rapid results

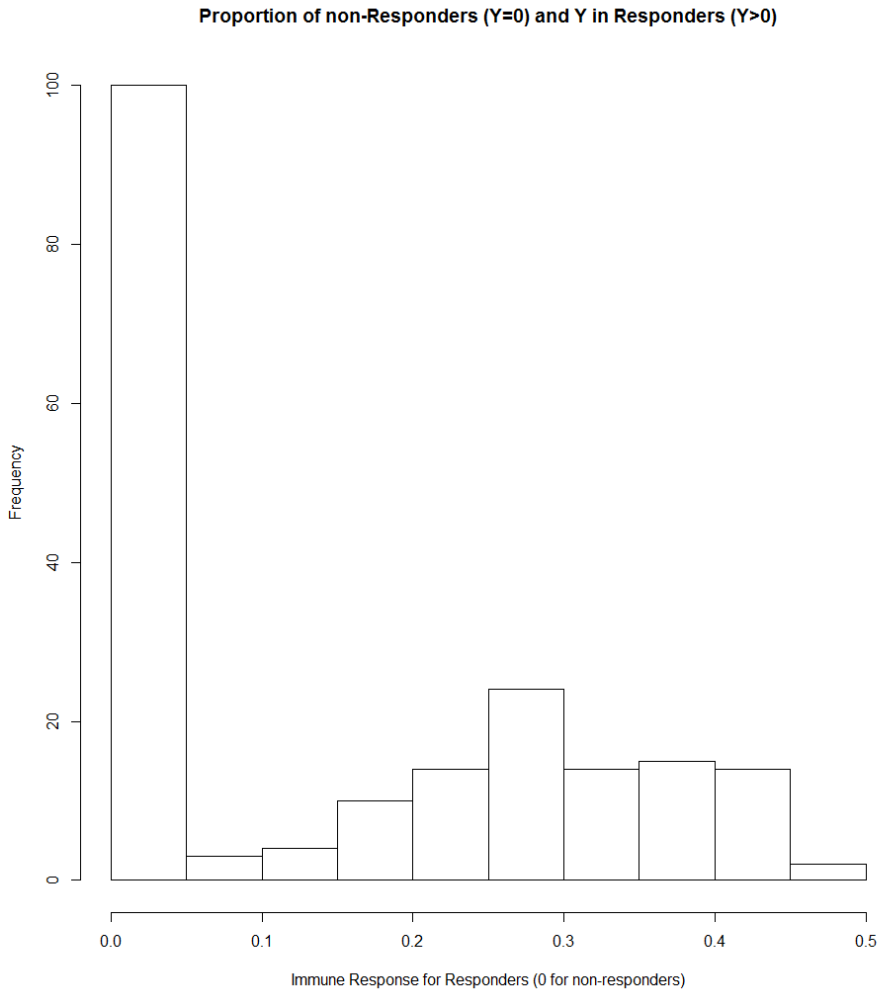
Powerful Luminex® data acquisition and analysis provide fast answers.



Liquid kinetics—beads are suspended in solution.

Analysis of Immune Response

- The immune response can be decomposed as
  - R = 1 if respond 0 otherwise
  - Y = magnitude of response in responders
- How to compare a mixture distribution between vaccinees and placebos?



Hu-Proschan two-part test

- Let Z_P be the test that the proportions of responders are the same in vaccine and placebo groups.
- Let Z_Y be the test that the average immune response Y is the same in the vaccine and placebo groups.
- Form an overall test

$$Z = \frac{Z_P + Z_Y}{\sqrt{2}}$$

- Weighted versions of the test can be incorporated.

Which assay is better?

- Prevail 1 used two assays to measure antibody response to Ebola vaccine
  - ADI was wide-spread and available
  - FANG had advanced validation
- How to tell which is better?
  - Evaluate, dynamic range, sensitivity, specificity etc.?
  - See which has a smaller p-value for the vaccine vs placebo comparison
    - Would be more powerful assay for a future study.

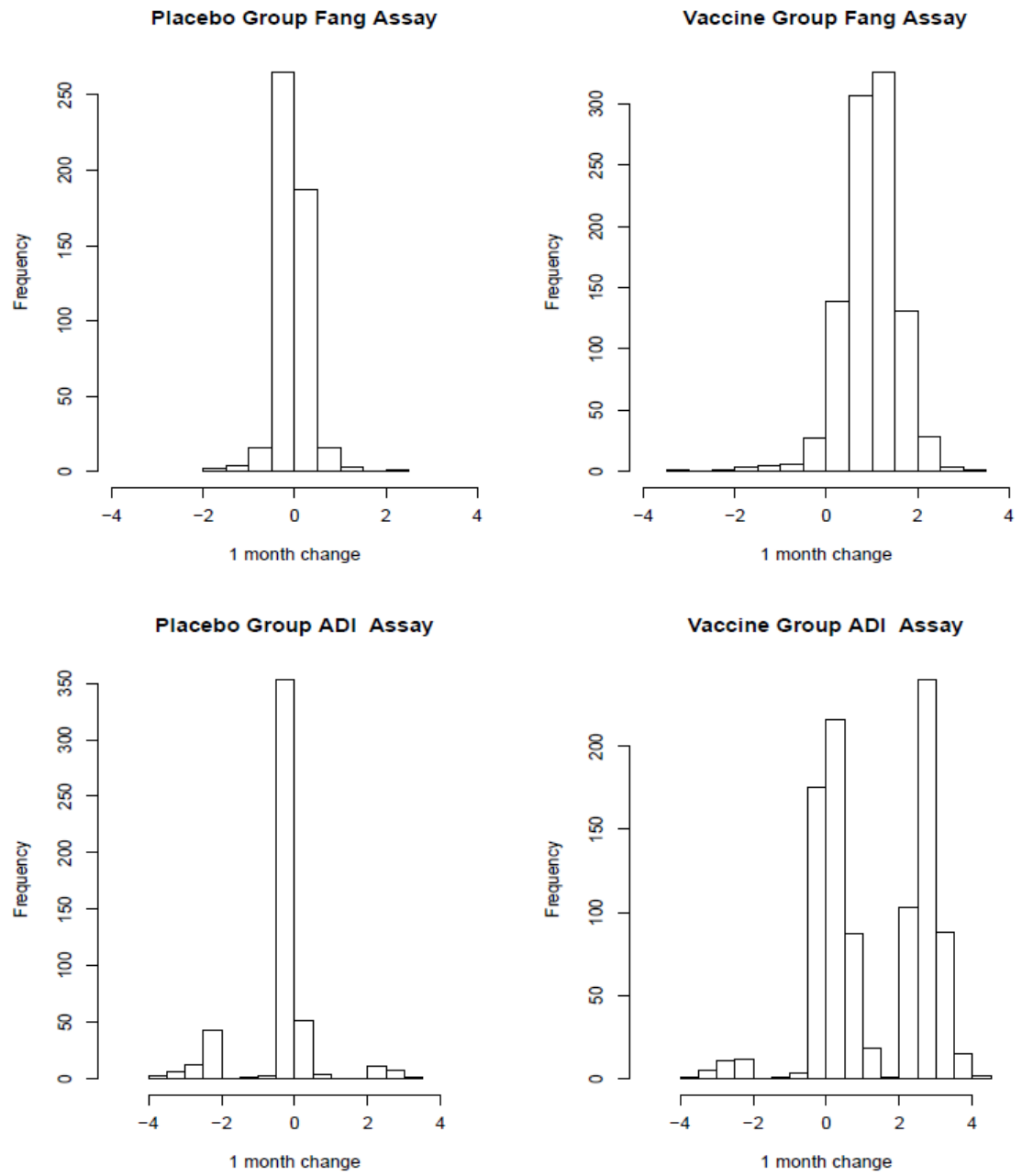


Figure 2: Histogram of 1 month log change in ELISA EU/ml readout from FANG and ADI

Results

Table 3: Evaluation of the 1 month post vaccination change in log10 ADI and ELISA assay readouts used in the Prevail 1 Ebola Vaccine Trial. Sample averages, standard deviations (in parenetheses) and Welch's t-statstics are reported.

Assay	Vaccine	Placebo	Welch's t
FANG	0.964	-0.043	41.15
	(0.613)	(0.326)	
ADI	1.314	-0.221	23.33
	(1.469)	(1.024)	
	N=978	N=494	

FANG has a much bigger test statistic (smaller p-value). How to interpret? Is it reliably bigger?

Power for a future study

- Can show that the sample size required to power a future study is proportional to the square of Welch's t-statistic
  - $(\text{T-FANG} / \text{T-ADI- T})^2 = (41.15/23.33)^2 = 3.11$
- A trial with ADI would require about 3 times the sample size of a trial using FANG.
- Can use bootstrap or profile methods to construct a 95% confidence interval of (2.51, 3.88)
- FANG is reliably much better than ADI.

Summary

- Vaccines are a great success for public health
- Vaccines induce adaptive or memory immunity from specific bits of a pathogen
  - B-cells make antibodies that fight pathogens outside cells
  - T-cells kill infected cells
- Clever assays can measure immunity and help with vaccine development
  - Help understand what exactly causes protection
  - Identify goals or targets for vaccines to achieve before being field tested

Poliomyleitis caused by *poliovirus*

- Poliomyleitis is a viral disease that can infect the central nervous system and cause lasting disabilities in a small number of infected individuals.
- Polio infection is most common in children but adults are at risk too
  - Franklin Roosevelt developed polio
- Polio was greatly feared.
  - Outbreaks are unpredictable
  - Paralyzed children are a visual reminder
- National Foundation for Infantile Paralysis was formed in 1938 to develop a vaccine.

Key developments

- Virus was isolated in infected subjects 1908
- Identification of three serotypes of polovirus, each serotype has a distinctive surface and a specific antibody works against a specific type.
- Confirmation that neutralizing (blocking) antibodies protect against disease
  - At risk children who received antibodies from polio survivors saw 80% reduction in paralytic poliomyelitis compared to children with gelatin
- Growth of virus in cell culture
  - Allows production of vaccine—germ bits

Vaccine developments

- Inactivated polio vaccine (IPV):
  - Three serotypes grown in cell culture and then killed by formalin
  - Developed by Jonas Salk, injected
  - Can’t cause disease
- Oral polio vaccine (OPV):
  - Three serotypes were weakened by repeated passage in cold non-human cells
  - Replicates in the gut. Very rarely causes disease or mutates to a more virulent form
  - Developed by Sabin, swallowed

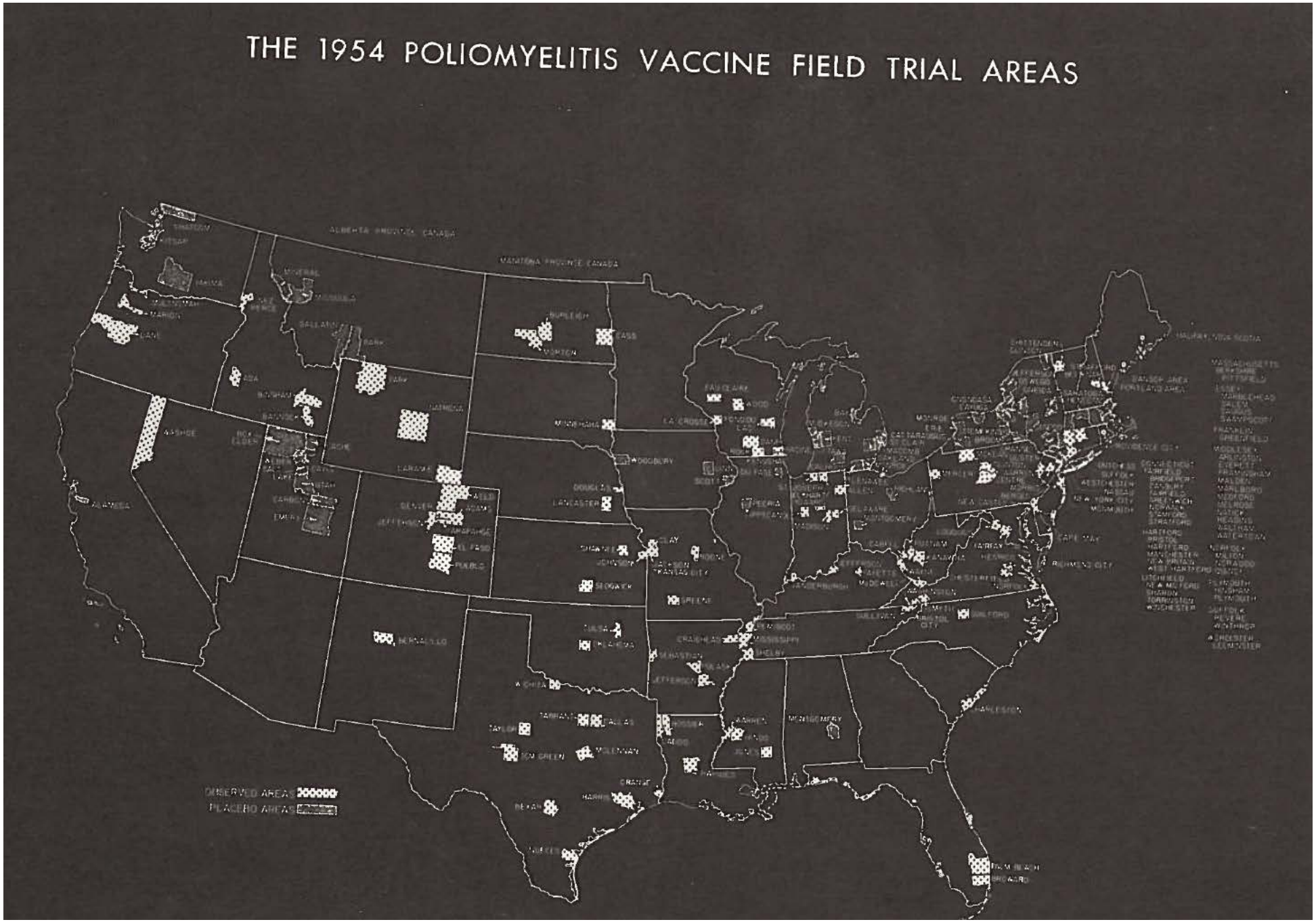
1954 Polio Field Trial of Salk Vaccine

- Salk Vaccine was promising but unproven.
- A field trial was essential. Earlier killed vaccines had some unkilled virus that lead to disease
- Intense publicity about the vaccine. Trial needed to be done in a single season
- Rate of paralytic polio by region was highly variable.

Key Features of Trial

- Two studies
  - Blinded placebo controlled individually randomized study in 84 areas in 11 states. Children in grades 1-3 randomized.
  - Observational trial 127 areas in 33 states. Children in grade 2 vaccinated grades 1 and 3 received nothing. Helped public support
- Conducted in spring and summer of 1954
  - Enrollment took long---vaccinations into mid June
  - Antibodies measured

THE 1954 POLIOMYELITIS VACCINE FIELD TRIAL AREAS



Mother Objects To Son's Girl

Journal and American

WALL ST. SPECIAL

SALK'S VACCINE WORKS!

Official Count

Polio Vaccine Reported 80 to 90% Effective

Table 5

DIAGNOSTIC CLASS BY VACCINATION STATUS OF STUDY CASES PLACEBO AND OBSERVED AREAS

Vaccination Status	Study Population	Total Study Cases		Poliomyelitis						Doubtful Poliomyelitis		Not Poliomyelitis	
				Total		Paralytic		Nonparalytic					
		Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate
All Areas - Total	1,829,916	1,012	55	858	47	682	37	176	10	66	4	88	5
Placebo Areas - Total	749,236	428	57	355	47	267	36	88	12	24	3	49	7
Vaccinated	200,745	81	40	56	28	33	16	23	11	10	5	15	7
Placebo	201,229	162	81	138	69	110	55	28	14	7	3	17	8
Incomplete Vaccinations	8,484	2	24	2	24	2	24	-	-	-	-	-	-
Incomplete Placebo Injections	8,577	6	70	6	70	4	47	2	23	-	-	-	-
Not Inoculated	330,201	177	54	153	46	118	36	35	11	7	2	17	5
Observed Areas - Total	1,080,680	584	54	503	47	415	38	88	8	42	4	39	4
Vaccinated	221,998	75	34	55	25	38	17	17	8	12	5	8	4
Controls	725,173	440	61	391	54	331	46	60	8	24	3	25	3
Incomplete Vaccinations	9,904	4	40	4	40	4	40	-	-	-	-	-	-
Second Grade Not Inoculated	123,605	65	53	53	43	42	34	11	9	6	5	6	5

1-16/55 = .71 Vaccine efficacy

After the 1954 Field Trial

- Cutter incident of Salks inactivated polio vaccine (IPV)
  - One manufacturer didn't properly kill the virus
  - 260 cases were caused: 94 vaccinees, 126 family, 40 community
- Sabin's oral attenuated vaccine (OPV) worked well in Soviet Union
  - Licensed in US 1960
  - Widely used in US 1961-89, simpler & worked better than IPV but
  - Causes paralysis in 1 of 2.9 million vaccinations
- By 2000 US had switched from OPV to IPV

Global Polio Eradication

- Campaign started in 1988, WHO UNICEF & Rotary Foundation, now supported by BMGF & Hutch.
- Afghanistan & Pakistan two remaining countries with endemic polio
  - Challenge: vaccination is a western plot to sterilize
  - Challenge: sham Hep B vaccination campaign used to confirm Osama bin Laden's identity
- Oral polio vaccine (OPV) is highly effective but causes some polio making eradication difficult.
- Plan is to switch from OPV to killed (inactivated) IPV with last wild polio case

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