Module 8
Evaluating Immunological
Correlates of Protection

Session 3
Evaluating Correlates of Protection Using Individual, Population, and Titer-specific Approaches

Ivan S.F. Chan, Ph.D.
Merck Research Laboratories

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Individual-Based Correlates of Protection

Identification of Protective Level by Looking at Vaccine Failures

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Individual-Based Correlate of Protection

- Assess antibody titers after immunization or just before infection and determine disease occurrence afterwards
- Identify "protective level" of antibody
  - 100% protection for individuals who have protective levels of antibody
  - Vaccine failures (who develop breakthrough disease) must have lower titers
  - Compare antibody titers in vaccine failures and protected individuals

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Measles Example

Chen *et al*. JID 1990

- A measles outbreak with 112 cases occurred in Boston area in 1985
- 100 cases were in Boston University students
  - 40 were in a 3-tower dormitory complex
- The American Red Cross had held a blood drive at BU during that time
  - Allowing preexposure blood specimens to be obtained from cases and noncases

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Measles Case Definition

- Generalized maculopapular rash of ≥3 days duration
- Fever ≥38.3°C, and
- At least one of the following symptoms:
  - Cough
  - Coryza
  - Conjunctivitis

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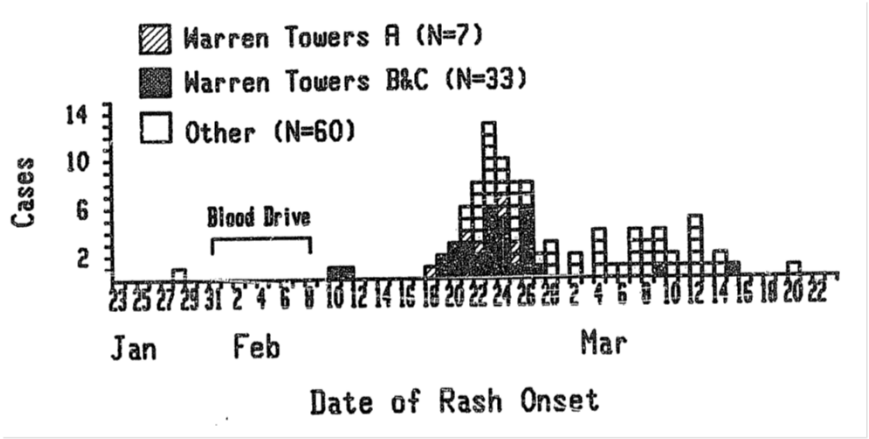
Reported Measles Cases By Date of Rash Onset, Boston University



Chen et al. JID 1990

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Serologic Testing

- Blood samples were available for 80 participants in this study
  - 8 cases and 72 noncases
- Plaque Reduction neutralization (PRN) test
  - 2 or 4-fold dilutions of serum starting at 1:8
  - PRN titer defined as the serum dilution that would reduce the number of plaques by 50%
- EIA

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PRN Titers of Cases and Noncases

Subject(s)	PRN titer: Pre-exposure	PRN titer: Post-exposure	IgM
Cases			
1	<16	35,363	287
2	38	17,723	<160
3	80	39,268	102
4	86	NA	NA
5	86	101,339	<48
6	98	44,661	662
7	118	14,157	509
8	120	13,638	90
Total, %			
Noncases, %‖			
$n = 37$	<1052		
$n = 35$	⩾1052		
Total, %			

Geometric Mean Titer (GMT) = 63 vs 1157 for cases vs noncases (p<.001)

Chen et al. JID 1990

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Protective PRN Titer of 120

- Highest preexposure titer among cases
- 8 out of 9 students with a PRN titer ≤120 had measles
- 0 out of 71 with a PRN titer >120 had measles
- Fisher's exact test: P<.0001
- GMTs in symptomatic noncases (871) significantly lower than asymptomatic students (1549), P<.04

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EIA Results

- No cases had detectable antibody vs. a GMT of 183 for noncases
- 3 out of 72 noncases had undetectable antibody titers
- GMTs in symptomatic noncases (153) not statistically significantly different from asymptomatic students (220), P=.10

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Summary of the Measles Example

- PRN titer of >120 indicates protection
  - Presence of low PRN titers may not offer protection
- PRN assay is more sensitive than EIA
- Protective titer identified by assessing individual-based titer levels among vaccine failures

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Problems with Individual-Based Correlates of Protection

- The level of antibody required to protect a given individual against a particular exposure is likely to vary. Other factors also likely to affect outcome.
- Clear cut threshold may not exist for 100% protection as breakthrough disease may occur in individual with high titers
- Difficult to implement prospective study design as it requires taking blood samples from a large number of participants, particularly when disease incidence rate is low

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Diphtheria Example

(Siber, DBS 1997)

- Diphtheria antitoxin titer of >0.01 AU/ml is presumed to indicate protection in population surveys
  - Produce a negative Schick test (skin test for diphtheria)
- Ipsen studied diphtheria in an outbreak in Copenhagen in 1943-1944
  - 106 patients had been immunized (vaccine failures)

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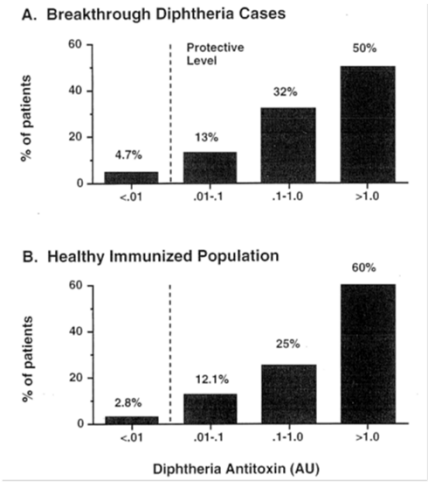
Diphtheria Antitoxin Titers

(Siber, DBS 1997)



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Diphtheria Example

- The antitoxin titers overlaps between breakthrough cases and healthy immunized population
- However, Ipsen noted a strong negative correlation between the level of antitoxin and severity of diphtheria

Antitoxin (AU/ml)	Risk of complications
<0.01	33%
0.1 to 1.0	6.7%
>1.0	5.4%

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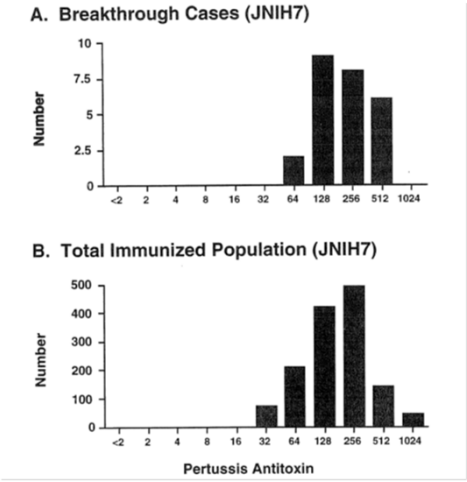
Pertussis Example

Swedish Acellular Pertussis Vaccine Trial in 1985-86

- Vaccine efficacy 80-100% against severe pertussis
- Antitoxin titers overlap
- Conclusion of no correlation between titer and protection



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Varicella Example:
VZV Antibody Response 6 Weeks Postvaccination
(1 Dose) by Varicella Breakthrough Status

Strong correlation between titers and protection based on statistical modeling of the whole titer distribution

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Population-Based Correlates of Protection

Identification of Protective Level by Comparing Antibody Titers of Protected Group and Susceptibles

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Population-Based Correlates of Protection (Siber, DBS 1997)

- Compare antibody levels in the protected group and the susceptible group
- Identify a threshold level achieved by most individuals in the protected group and not reached by most susceptibles
  - Estimate the minimum protective level
- Only require limited serological sampling (e.g., 10% cohort)

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Haemophilus Influenzae b (Hib) Vaccine

- Studies showed PRP polysaccharide vaccine has 88% efficacy in children >18 months of age an no benefit in younger children
- Käythy et al showed post-vaccination anti-PRP level of ≥1.0 µg/ml best discriminated between immunized and control populations aged 18 months or older.
  - This level indicates long-term protection
  - Accepted as criterion for licsencing new Hib vaccines

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Anti-PRP titer Responses

Age	Anti-PRP ≥ 1 µg/ml	
	Immunized	Controls
6-11 mos.	16%	2%
12-17 mos.	44%	5%
18-23 mos.	75%*	15%
24-36 mos.	90%*	18%

* Protected population

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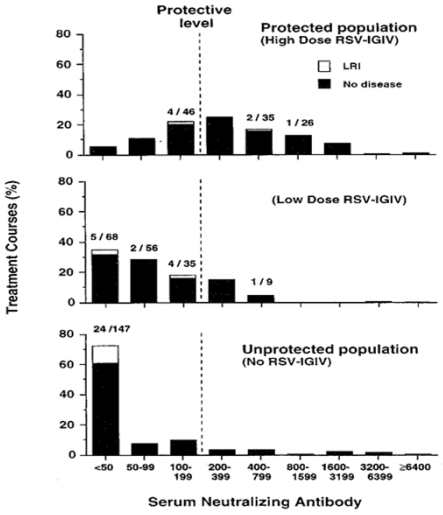
Respiratory Syncytial Virus (RSV)

- RSV Lower respiratory infection (LRI) accessed during RSV immune globulin infusion
- LRI reduction of 63% in high-dose group and 27% in low-dose group relative to control
- Potential protective level is 200



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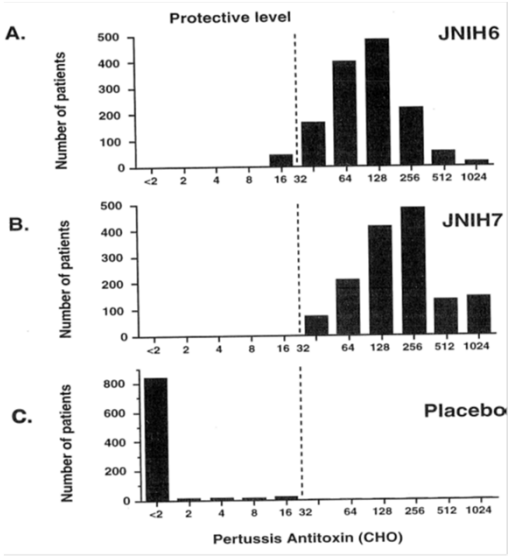
Pertussis Vaccine Example

Swedish Acellular Pertussis Vaccine Trial in 1985-86

- Titers clearly separated between vaccine and placebo groups

(Siber, DBS 1997)



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Concerns with Population-Based Method

- The definition of protection is somewhat arbitrary and not rigorous
- The level of protection changes abruptly as implied by the protective level
  - Perhaps more likely to vary as a continuous function of antibody level

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Titer-Specific Correlates of Protection

Siber, DBS 1997; Jódar et al, Vaccine 2003;
Siber et al, Vaccine 2007

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Titer-Specific Method

- Model the risk of disease as a continuous function of antibody titer
  - Logistic regression is often used
  - Other models have also been used
  - A step function may be used to establish a protection level
- More rigorous than the population-based method

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Titer-Specific Method

- Obtain antibody titers in all individuals who develop disease in vaccinated and control groups
- Obtain antibody distribution in the entire population
  - Can be estimated using a random sample of study population
- Calculate titer-specific rate of disease:

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Titer-Specific Method

- Fit a statistical model to evaluate the relationship between titer and disease risk
- For example, a logistic regression:

X = antibody titer level

- A step function can be used to estimate the protective level

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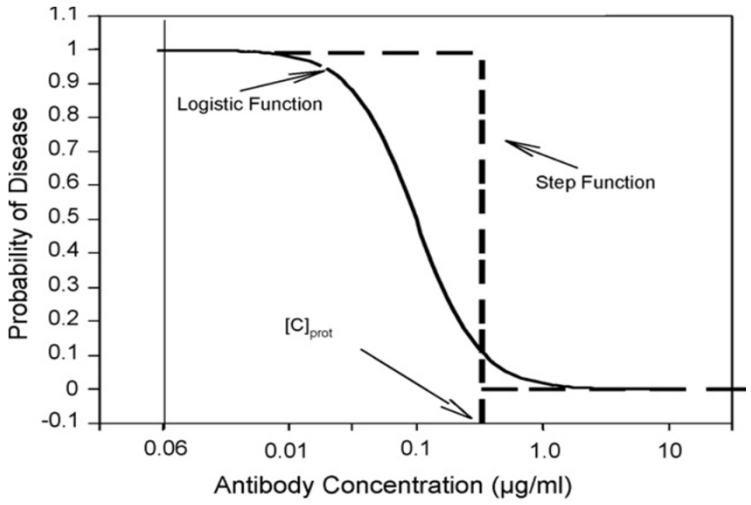
A Logistic Regression Model
(Siber et al 2007)



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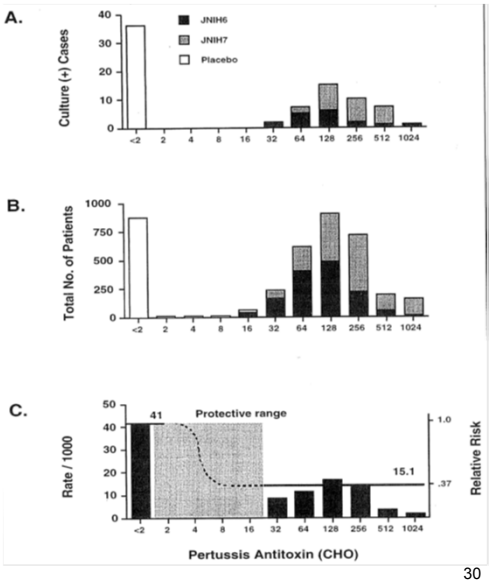
Pertussis Vaccine Example

Swedish Acellular Pertussis Vaccine Trial in 1985-86

- Titers >16 are associated with a substantially lower risk

(Siber, DBS 1997)



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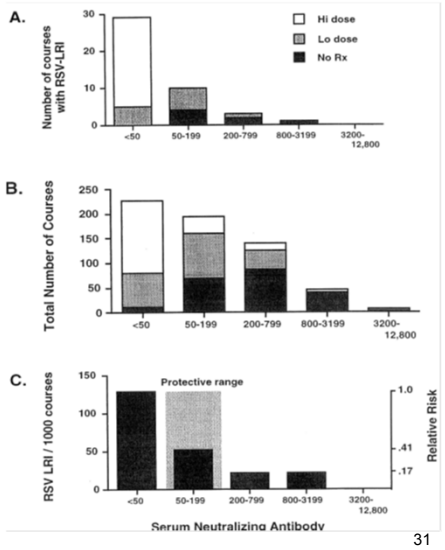
RSV Example

- RSV LRI risk decreases as antibody titer increases
- Titer of ≥200 is associated with a relative risk of 0.17 (83% efficacy)

(Siber, DBS 1997)



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Pneumococcal Vaccine Example

Developing Serological Criteria for Licensing New Vaccines

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Background

- A 7-valent pneumococcal vaccine (Prevnar®) was approved in 2000 for immunization of infants and toddlers for prevention of invasive pneumococcal disease (IPD) caused by *Streptococcus pneumoniae*
- IPD includes bacteremia (bloodstream infection) and meningitis (infection of the membranes surrounding the brain and spinal cord)
- The seven serotypes (strains) of *S. pneumoniae* included in the vaccine are 4, 6B, 9V, 14, 18C, 19F, and 23F
- Vaccine given as 4-dose series at 2, 4, 6 and 12-15 months of age

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Efficacy of Prevnar®

Three placebo controlled efficacy trials

Study	Evaluable N	Case Split (Vaccine/Control)	Vaccine Efficacy (VE, 95% CI)
NCKP*	21,935	1/39	97.4% (82.7,99.9)
American Indian	5,792	2/8	76.8% (-9.4, 95.1)
South Africa	37,107	1/10	90.0% (29.7, 99.8)

*Northern California Kaiser Permanente

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Need for Additional Vaccines

- Prevnar does not contain additional serotypes, such as 1 and 5, that are an important cause of IPD in South America, Africa, and Asia
- New vaccines containing 9, 11, 13 and 15 serotypes are being developed
- Prevnar 13™ was approved in US in 2010 based on immunogenicity bridging
  - Additional serotypes: 1, 3, 5, 6A, 7F, and 19A

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Proposed Licensure Criteria for New Pneumococcal Vaccines

- Placebo-controlled trial not ethical
- Noninferiority of new vaccines to Prevnar must be established
  - Efficacy trial not feasible due to high efficacy of Prevnar
  - Immunogenicity trial is possible but clear serological criteria need to be established
- Discussion at FDA Advisory Committee in 2001
- World Health Organization (WHO) formed a working group in 2002 to recommend serological criteria that will predict protective efficacy

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Serology Assay – IgG ELISA

- IgG ELISA measures the IgG antibody
- Shown to correlate with opsonophagoctic (functional) activity (OPA) measured by Opsonophagoctic assay
- Post dose-3 antibody response correlates with booster response
- ELISA assay has been validated and standardized across multiple laboratories
- Serology data available from efficacy trials to assess correlates of protection

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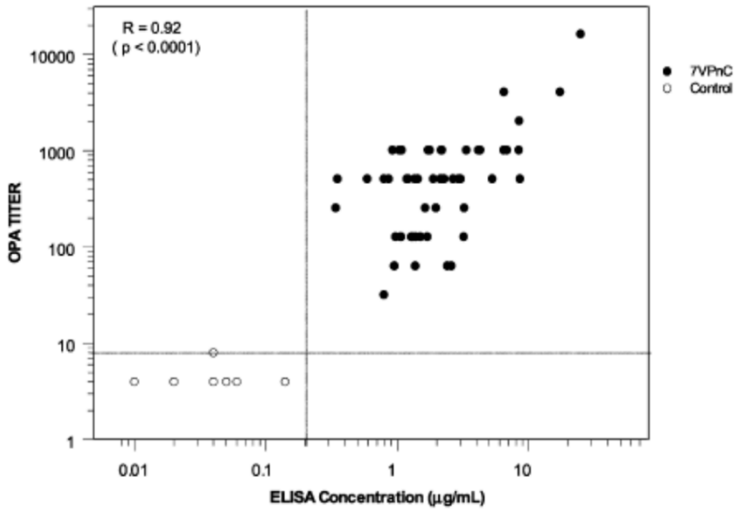
Correlation between ELISA and OPA Titer



Jódar et al, Vaccine 2003

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Correlation between Primary and Booster Responses

Table 1
Correlation between post dose 3 and post dose 4 ELISA antibody

Serotypes	Pearson correlation[a] $N = 269–271$	P-value
4	0.441	<0.001
6B	0.526	<0.001
9V	0.439	<0.001
14	0.341	<0.001
18C	0.498	<0.001
19F	0.306	<0.001
23F	0.545	<0.001

Data source: Wyeth Vaccine Research. Data on file: Manufacturing bridging study of 7-valent pneumococcal conjugate vaccine. D118-P16.

[a] Correlation based on post dose 3 and post dose 4 Ab concentrations on log-scale.

Jódar et al, Vaccine 2003

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Statistical Framework

- Assume a theoretical underlying logistic regression model between antibody tiers and risk of IPD
- Assume a step-function to define a specific protective level
- Assume the relationship is the same for all serotypes
  - Allowing pooling of efficacy and immunogenicity data
- Assume antibody at 4 weeks post dose 3 (primary series) is important in predicting long-term protection

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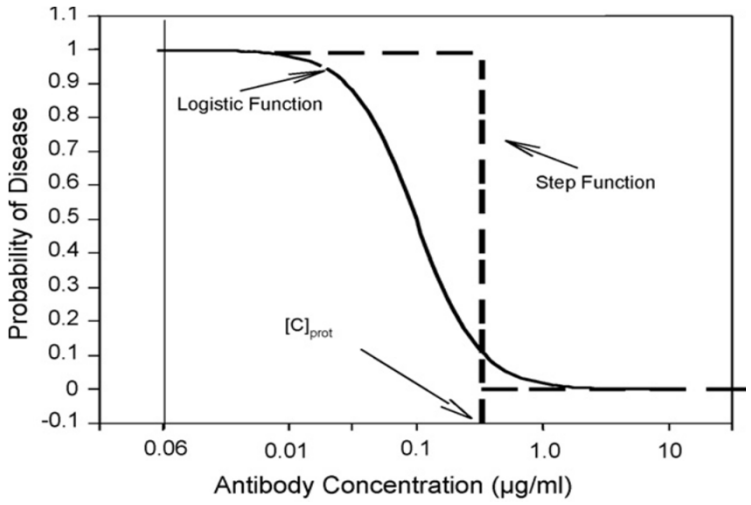
The Logistic Regression Model

(Siber et al 2003)



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Step Function Model and Vaccine Efficacy (VE)

$[C]_{prot}$ = protective level of antibody

p_v = % subjects with antibody levels < $[C]_{prot}$ in the vaccinated group

p_c = % subjects with antibody levels < $[C]_{prot}$ in the control group

a = Prob of IPD when antibody < $[C]_{prot}$

b = Prob of IPD when antibody ≥ $[C]_{prot}$

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Step Function Model and Vaccine Efficacy (VE)

P(IPD in vaccines) = a p_v + b (1 – p_v)

P(IPD in controls) = a p_c + b (1 – p_c)

$$VE = 1 - \frac{P(\text{IPD in vaccines})}{P(\text{IPD in controls})}$$

$$= \frac{(a - b)(p_c - p_v)}{b + p_c(a - b)}$$

$$\approx 1 - \frac{p_v}{p_c} \quad \text{if } b \text{ is close to } 0$$

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Step Function Model and Vaccine Efficacy (VE)

- If the prob of IPD when antibody ≥ $[C]_{prot}$ is very small (*b* is close to zero):

  Relative risk of IPD = Relative risk of having antibody titer < $[C]_{prot}$

- When VE is known, $[C]_{prot}$ may be determined directly from the reverse cumulative distribution curves (RCDC) of the antibody titers
  - If antibody titers in placebo group is very low, it can be ignored in determining $[C]_{prot}$

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Step Function Model and Vaccine Efficacy (VE)

- Variability in $[C]_{prot}$ estimate depends on the variability of VE estimate and serology data
  - Dominated by VE variability if serology sample size is large
  - CI for $[C]_{prot}$ determined using CI for VE

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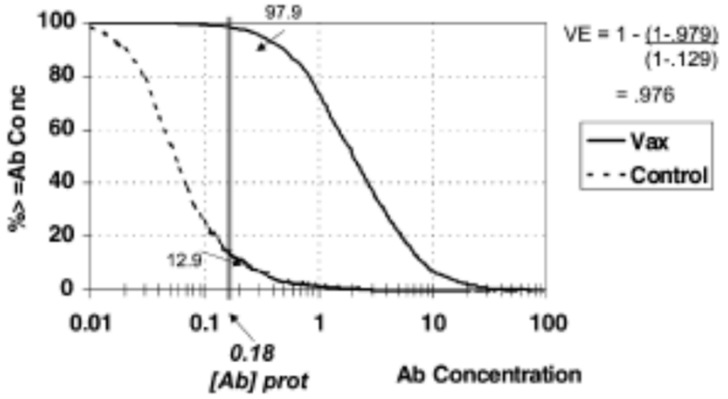
Determining Protective Level Based on RCDC



Ignoring Ab levels in controls obtains [Ab] prot = .20 µg/ml

Jódar et al, Vaccine 2003

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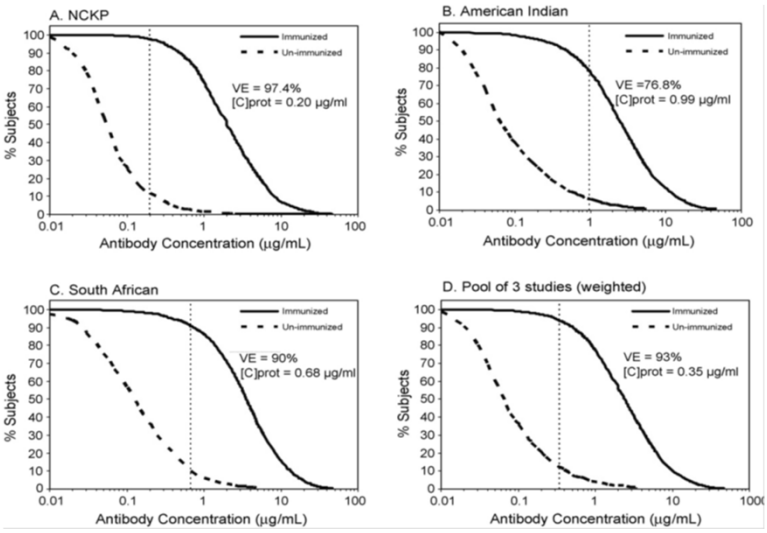
Protective Level for IPD

(Siber et al, Vaccine 2007)



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Estimated Protective Pneumococcal Antibody Level

Study	Observed VE	Estimated $[C]_{prot}$ (µg/ml)	95% CI
NCKP	97.4%	0.20	(0.03, 0.67)
American Indian	76.8%	1.00	(0.25, >50.0)
South Africa	90%	0.68	(0.03, 6.0)
Pooled* (weighted)	93%	0.35	(0.11, 0.85)

Weighted by the number of subjects in the trial

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WHO Recommendations for Licensure of New Pneumococcal Vaccines

- Noninferiority study of immunogenicity is acceptable
- The percent of subjects achieving an ELISA antibody titer by of ≥0.35 µg/ml is a useful benchmark (can be used as primary endpoint)
- More emphasis on assessing the OPA titers as they reflect the functionality of antibody, especially for new serotypes not included in the original vaccine
  - Explore the relationship between ELISA and OPA

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Evaluating Protective level using Receiver Operating Characteristic Curve (ROC)

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Evaluating Protective level using Receiver Operating Characteristic Curve (ROC)

- Consider disease status as gold standard and assay value as diagnostic test

  Test positive = assay value < protective level
- Evaluate the sensitivity and specificity of the test for a variety of cutoff value
- Choose the optimal cutoff value to be the protective level based on ROC
  - Balance sensitivity and specificity

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Sensitivity and Specificity

Sensitivity = TP/(TP+FN) Specificity = TN/(FP+TN)

Test (Y)	Disease Status (Z)		Total
	Present	Absent	
Positive	True + (TP)	False + (FP)	TP + FP
Negative	False – (FN)	True – (TN)	FN + TN
Total	TP + FN	FP + TN	

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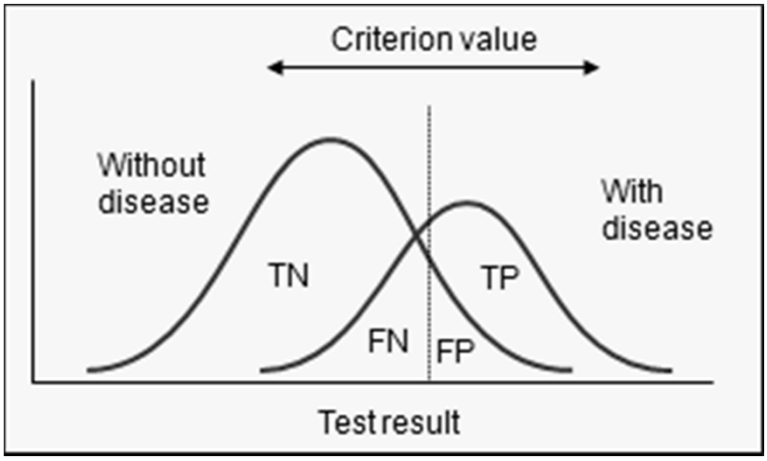
Balance Between Sensitivity and Specificity



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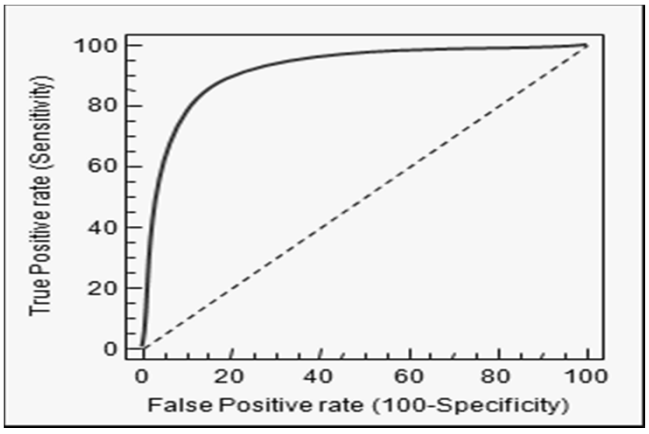
Receiver Operating Characteristic Curve

- Choose the cutoff value that has high sensitivity and specificity



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Further thoughts on ROC Methods

(Li, Parnes and Chan, JBS 2013)

Identify the cutoff threshold

- By maximizing the correlation between Disease (Z) and immune responses (Y)
- By minimizing the misclassification rate:

$$MR = \pi \times fn + (1 - \pi) \times fp$$

π is the disease prevalence

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