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Controlling the rate of false positives in  
future tests. A Bayesian perspective.

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- Antibodies elicited by therapeutic proteins may significantly alter drug safety and efficacy
- Immunogenicity testing is conducted by a **multi-tiered** approach whereby patient samples are initially screened for the presence of anti-drug antibodies (ADA) in a **screening assay**
- Samples testing positive for the presence of anti-drug antibodies in the screening assay are subsequently analyzed in a **confirmatory assay** which characterizes the specificity of the binding response to the drug.
- The objective is to identify ADA<sup>+</sup> treated patients.
- The question is then  $p(\text{ADA}^+ \mid \text{screening}^+)$

# The background: Screening Cut point

- It's about the performance of the screening test
- The Screening Cut Point (CP) is determined
  - using a +- reduced number of naïve patients, say 100 patients.
  - using kind of ~"95<sup>th</sup> percentile" (parametric or not) of observed values
- The aim is to accept 5% false-positive rate (FPR)
- The false-positive rate is deliberately chosen high because
  - It allows to detect low-affinity positive samples
  - the **sensitivity** of test (  $p(\text{CP}^+ | \text{ADA}^+)$  ) is unknown
- The **prevalence** or risk -  $p(\text{ADA}^+)$  - of immunogenicity is unknown
  - By definition, the drug has not yet been evaluated in human !
  - It is in fact the **objective** of the immunogenicity ADA tests

# The background: The role of the confirmatory test

- To confirm the very objective of the immunogenicity testing, ie to confirm a potential  $ADA^+$   
Confirm  $ADA^+$  given  $CP^+$
- The very objective of the immunogenicity testing procedure is

$$p(ADA^+ | CP^+)$$

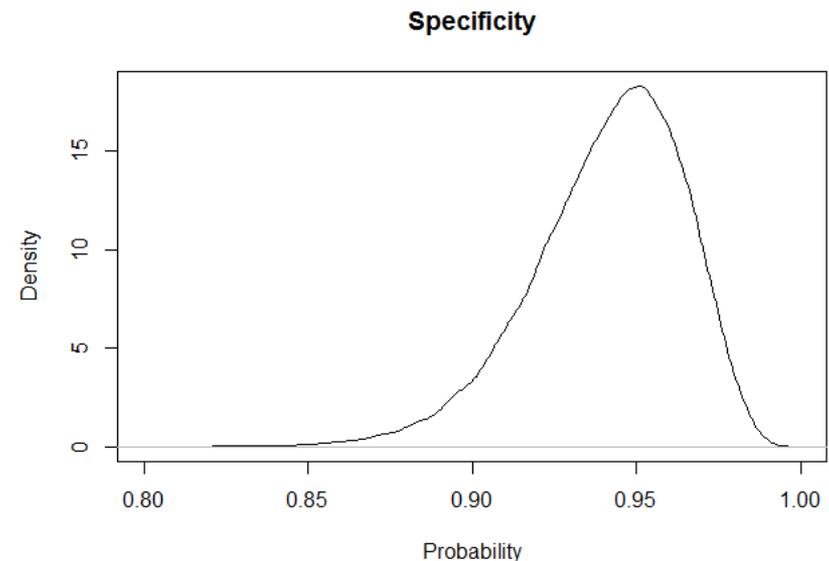
- While the screening cut point of assay is evaluating:

$$p(CP^+ | ADA^-) = \text{False Positive Rate} \sim 5\% \text{ (aim)}$$

$$p(CP^- | ADA^-) = 95\% \text{ Specificity of test}$$

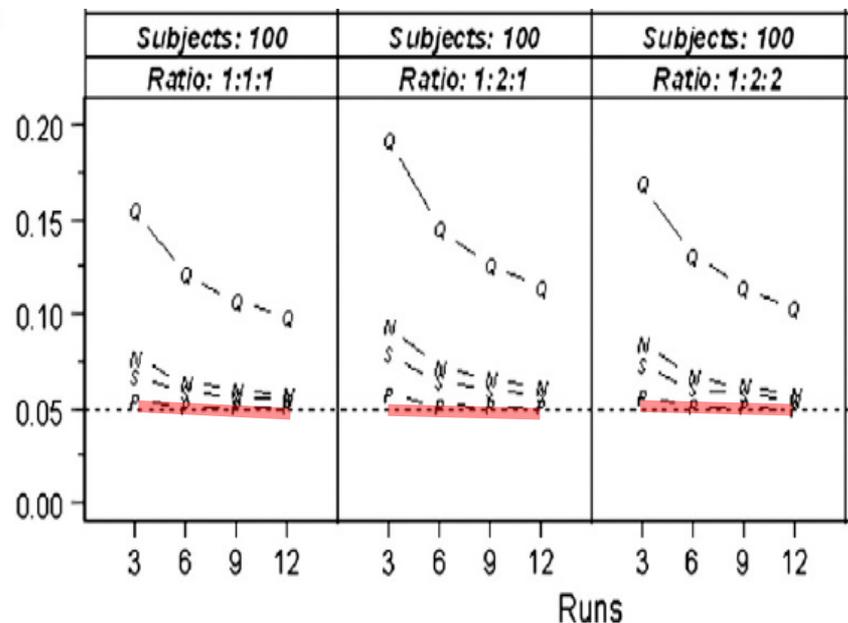
# Specificity of screening let's have a closer look

- $FPR \sim 1 - \text{Specificity} = p(CP^+ | ADA^-)$
- Using the 95<sup>th</sup> percentile on limited sample size to determine the cut-point doesn't imply that the **specificity** is exactly 95%.
  - The False Positive Rate (FPR) is not truly 5% either.
  - This is an estimate with uncertainty
  - If based on 100 naïve patients then based on 95 negatives and 5 false positives theory is telling us that the specificity is having a  $beta(95 + 1, 5 + 1)$  distribution
- A priori distribution of Specificity =  $beta(N + 1, P + 1)$

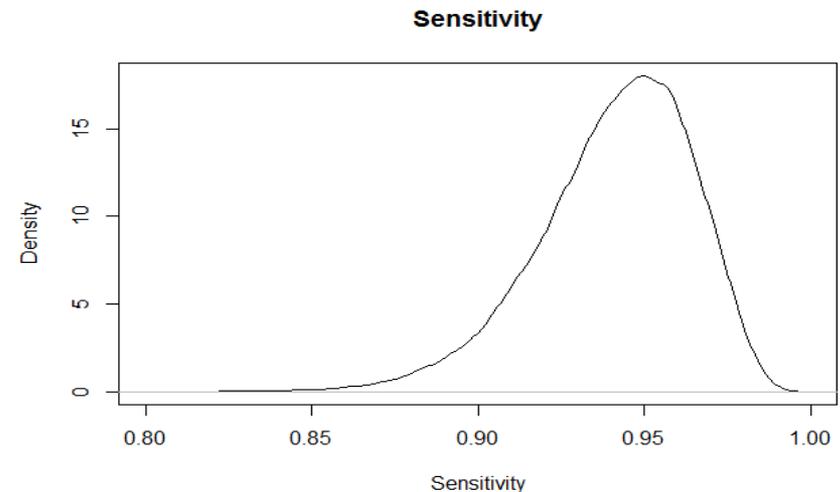


# FPR of screening let's have a closer look

- Specificity =  $p(CP^- | ADA^-)$  , FPR =  $p(CP^+ | ADA^-)$
- As shown by Hoffman and Berger (2011), the  **$\beta$ -expectation Tolerance interval** ensures the FPR to be close to 5% in the future given past data on naïve.
- This also assumes that future samples are drawn from a population similar to naïve patients.
- On average, but an uncertainty remains because of limited sample size (<100)
- A good prior is  $beta(6,96)$



- Sensitivity =  $p(CP^+ | ADA^+)$
- This is unknown at least at the begin of a development
  - A non-informative could a  $beta(1,1)$
  - All values between 0 and 1 are as likely ?
- But a good guess is that most  $ADA^+$  will provide a  $CP^+$  signal otherwise everything is falling apart.
  - $beta(96,6)$
- Better than thinking it's a fixed value.



# Is this new sample a potential ADA<sup>+</sup> ?

- What is the probability that a sample is ADA<sup>+</sup> given the screening results is CP<sup>+</sup> ?

$$\rightarrow p(ADA^+|CP^+)$$

- $$p(ADA^+|CP^+) = \frac{p(CP^+|ADA^+)p(ADA^+)}{p(CP^+|ADA^+)p(ADA^+) + p(CP^+|ADA^-)p(ADA^-)}$$

- $p(CP^+|ADA^+) =$  Sensitivity of test  $\rightarrow beta(96,6)$

- $p(CP^+|ADA^-) =$  1-Specificity of test  $\rightarrow beta(6,96)$

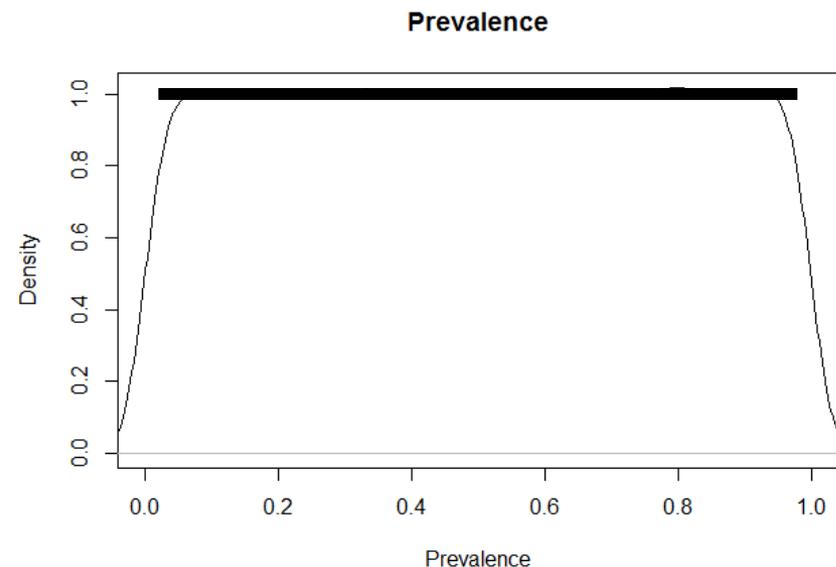
- $p(ADA^+) =$  Prevalence or risk  $\rightarrow unknown\ in\ fact$

- Note that currently potential ADA<sup>+</sup> is based on  $p(CP^+|ADA^+)$

- Prevalence =  $p(ADA^+)$
- Unknown before starting any trial.
- The Prevalence is the objective in fact it's the purpose of the immunogenicity testing approach to evaluate the risk of  $ADA^+$  with the new treatment.

- A good prior for

$$p(ADA^+) \sim \text{beta}(1,1)$$



# Is this first sample a potential ADA<sup>+</sup> ?

- Assume first patient, measure is > CP ( $CP^+$ )

- fixed specificity/sensitivity → 0.95 and 0.95
- Unknown prevalence → say 0.5

- $$p(ADA^+ | CP^+) = \frac{p(CP^+ | ADA^+)p(ADA^+)}{p(CP^+ | ADA^+)p(ADA^+) + p(CP^+ | ADA^-)p(ADA^-)}$$

- $$p(ADA^+ | CP^+) = \frac{0.95 \cdot 0.5}{0.95 \cdot 0.5 + 0.05 \cdot 0.5} = 0.95$$

- This seems to imply that  $p(CP^+ | ADA^+) = p(ADA^+ | CP^+) !$

- Maybe the underlying idea behind the FPR choice.

- This is only true when prevalence  $p(ADA^+)$  is unknown !

# Is this 101th sample a potential $ADA^+$ ?

- Assume 100 patients already tested, 2/100 have been confirmed as  $ADA^+$ , 98/100 as  $ADA^-$
- The 101th patient is  $> CP$  ( $CP^+$ )
  - fixed specificity/sensitivity → 0.95 and 0.95
  - A priori prevalence → estimated as 0.02

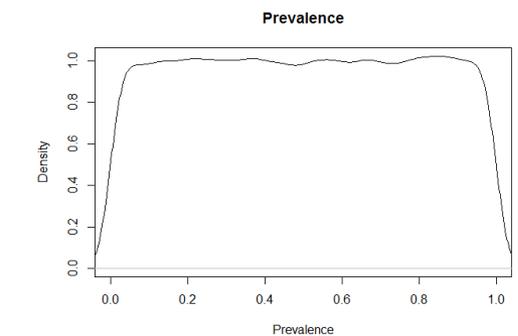
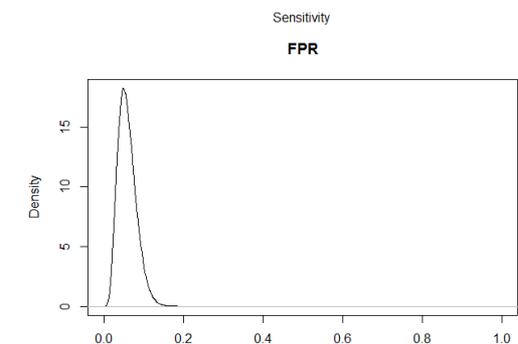
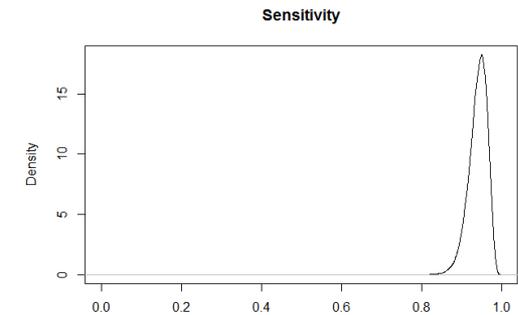
$$\blacksquare p(ADA^+|CP^+) = \frac{p(CP^+|ADA^+)p(ADA^+)}{p(CP^+|ADA^+)p(ADA^+) + p(CP^+|ADA^-)p(ADA^-)}$$

$$\blacksquare p(ADA^+|CP^+) = \frac{0.95 \times 0.02}{0.95 \times 0.02 + 0.05 \times 0.98} = 0.28$$

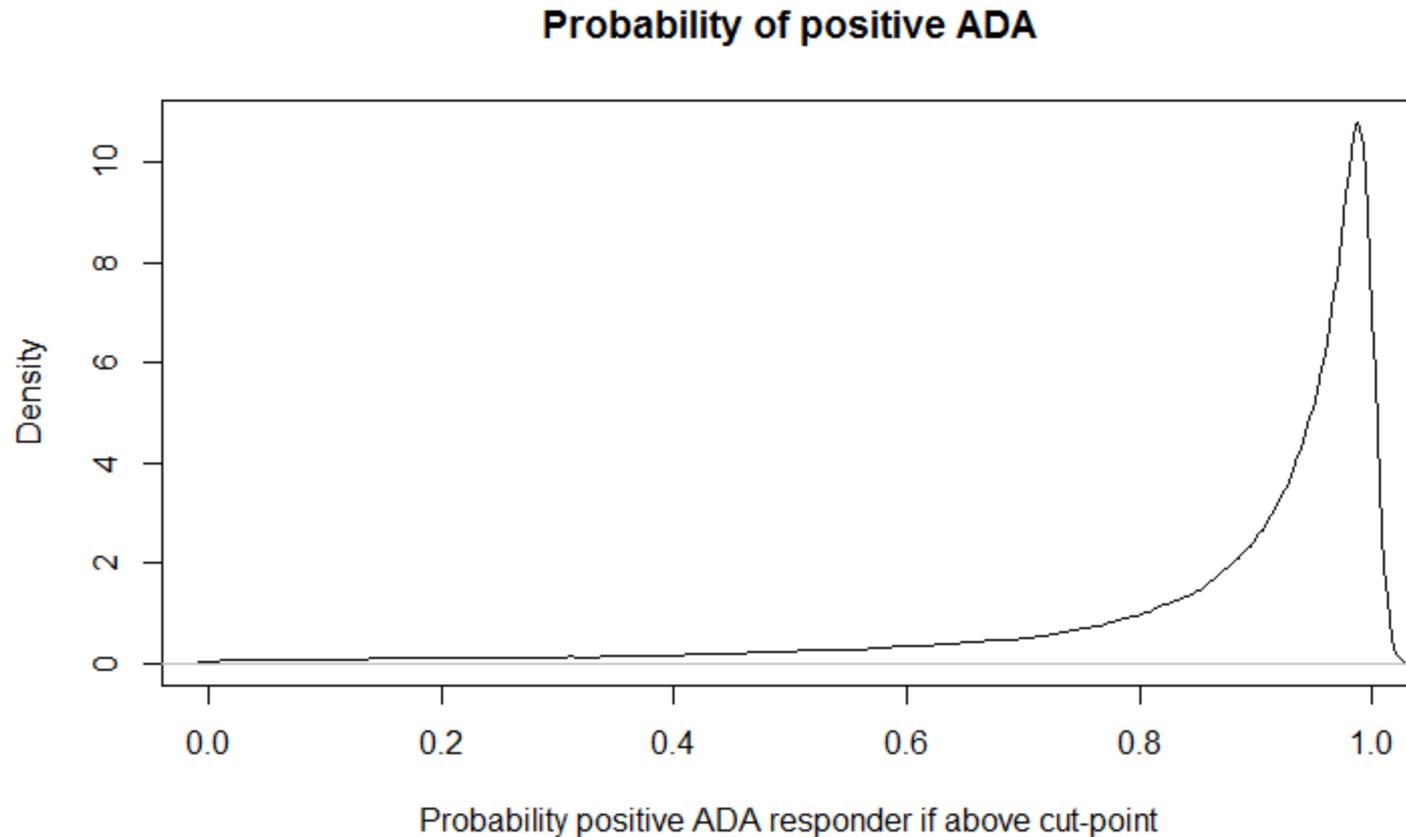
- There is little chance to be confirmed as  $ADA^+$
- Now  $p(ADA^+|CP^+) \ll p(CP^+|ADA^+)$

# But all are guesses with uncertainty

- At the beginning one can assume:
- $p(CP^+|ADA^+) = \text{Sensitivity of test}$   
→  $beta(96,6)$
- $p(CP^+|ADA^-) = 1 - \text{Specificity of test}$   
= FPR  
→  $beta(6,96)$
- $p(ADA^+) = \text{Prevalence or risk}$   
= Unknown  
→  $beta(1,1)$



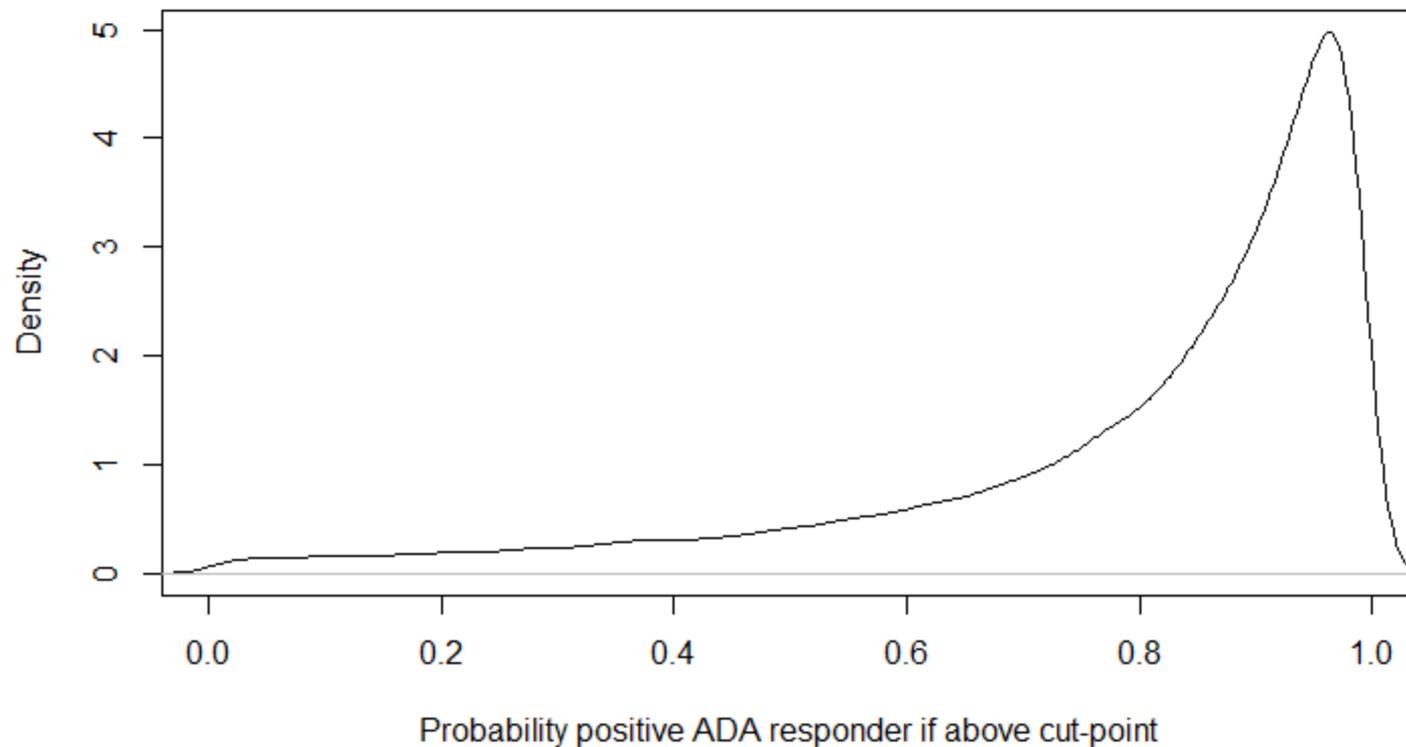
**FPR ~ 0.05    Sensitivity ~ 0.95**  
**unknown prevalence, before starting**



**This is the intended performance: ~ 5% chance to be a FPR**

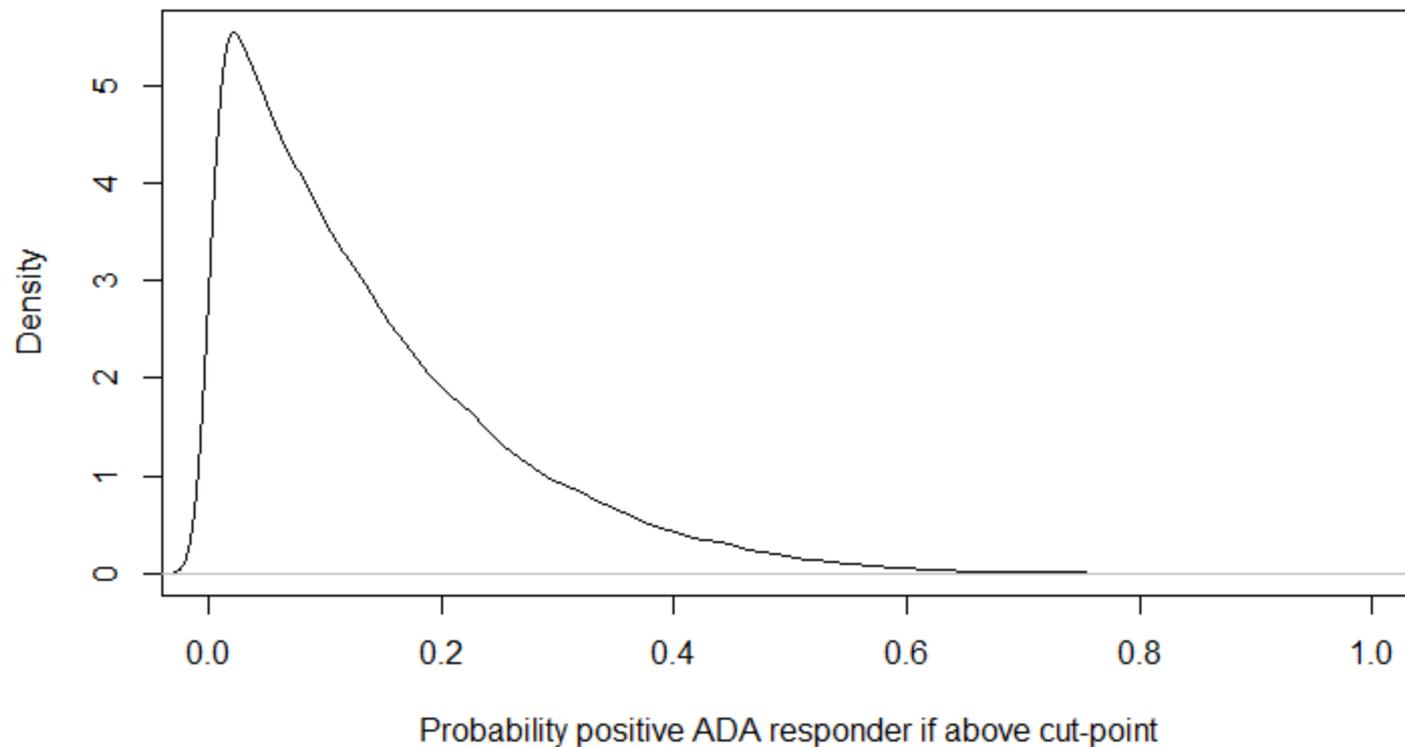
**FPR ~ 0.05    Sensitivity ~ 0.95**  
**1/0 patient confirmed negative**

**Probability of positive ADA**



**FPR ~ 0.05    Sensitivity ~ 0.95**  
**100/0 patients confirmed negative**

**Probability of positive ADA**



- The lower the **prevalence** the higher the probability a potential ADA+ to be a False Positive.
- When the prevalence appears to be low –hopefully- confirmatory tests are busy testing samples likely to be negative ADA-

## Solution ?

- Should the decision to go in confirmatory test be based on  
 $p(ADA^+|CP^+)$
- Should the Specificity or 1-FPR be adapted to observed prevalence?

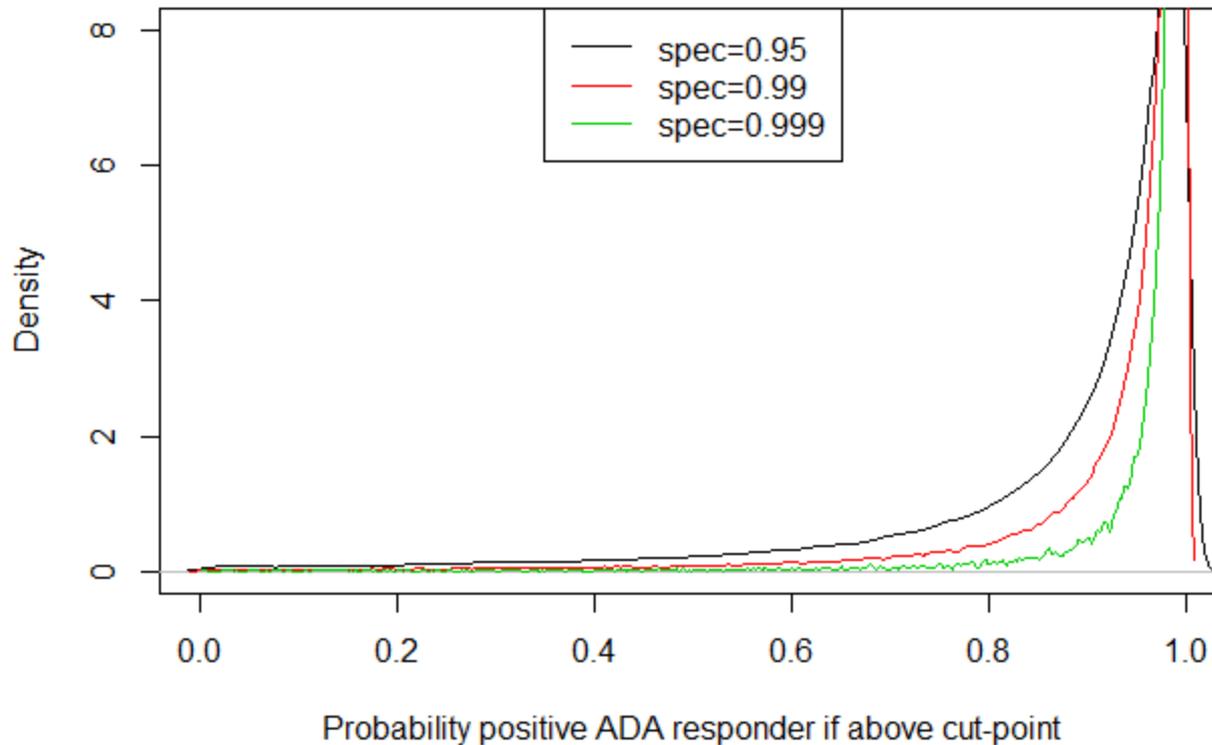
$$p(CP^+|ADA^-) = 0.05 \rightarrow 0.01 \rightarrow 0.001 ?$$

**Specificity = 0.95 - 0.99 - 0.999**

**Assuming sensitivity is 0.95**

**0/0 patients confirmed negative**

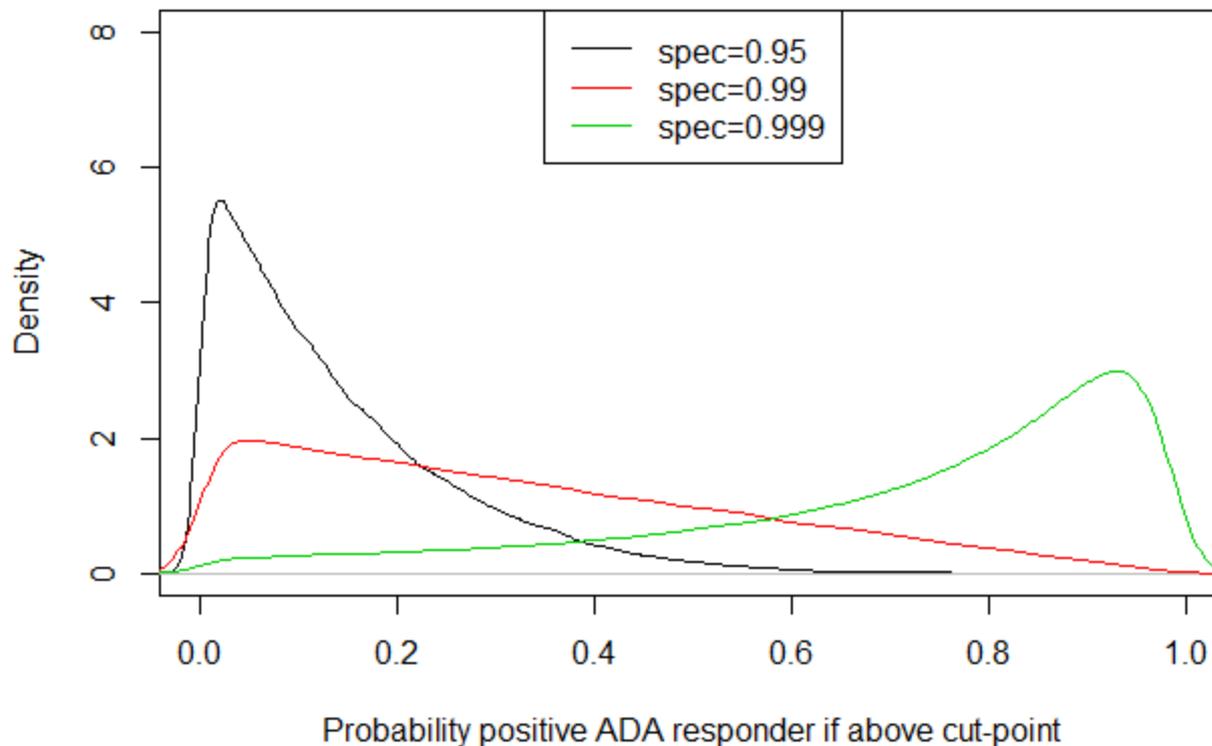
**Probability of positive ADA**



**Specificity = 0.95 - 0.99 - 0.999**

**Assuming sensitivity is 0.95 (ie 95/5)  
100/100 patients confirmed negative**

**Probability of positive ADA**



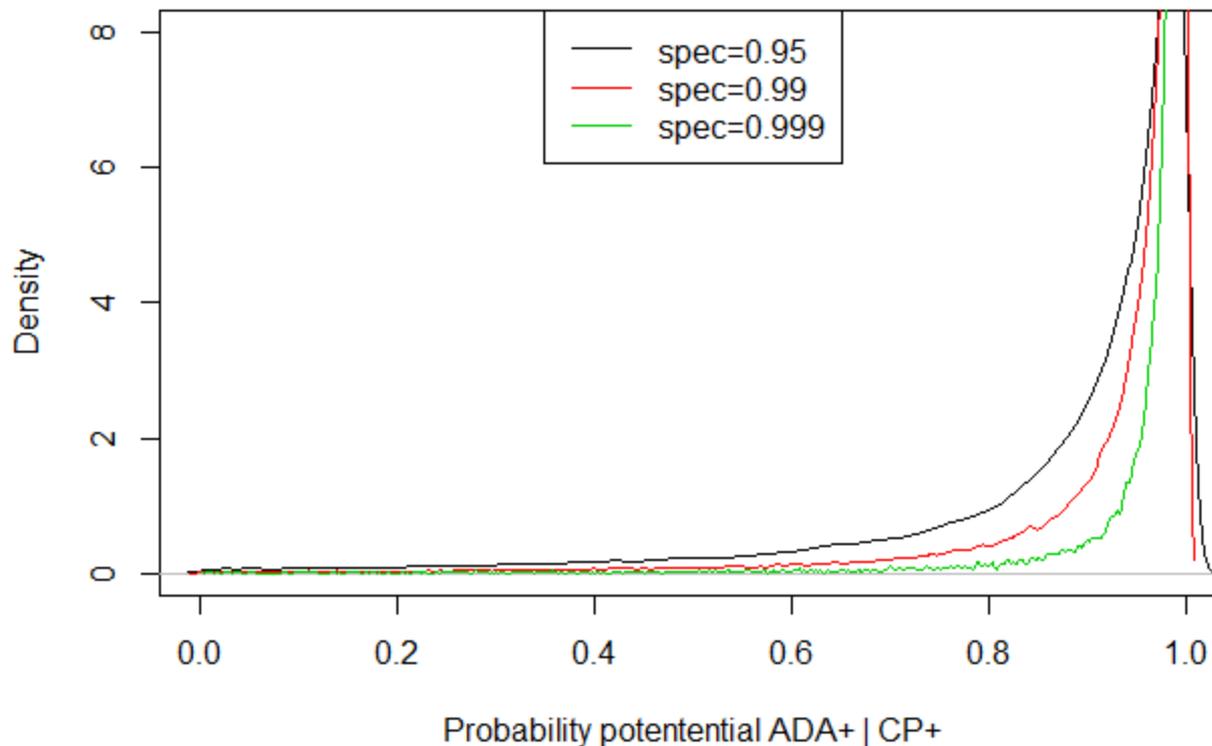
- When the risk of  $p(ADA^+)$  is unknown, then the FPR is about the 5% aimed
- When the risk of  $p(ADA^+)$  is known / estimated to be **low**, then the  $p(ADA^+ | CP^+)$  is becoming low and the rate of false positive is becoming very large.
- Increasing progressively the Specificity with estimated prevalence  $p(CP^+ | ADA^-) = 0.05 \rightarrow 0.01 \rightarrow 0.001$  allows to keep the  $p(ADA^+ | CP^+)$  close to the original intended levels.
- → When the risk of  $p(ADA^+)$  is known / estimated to be **medium**, then what is happening with  $p(ADA^+ | CP^+)$ ?

**Specificity = 0.95 - 0.99 - 0.999**

**Assuming sensitivity is 0.95**

**0/0 patients confirmed negative**

**Probability of potential ADA**

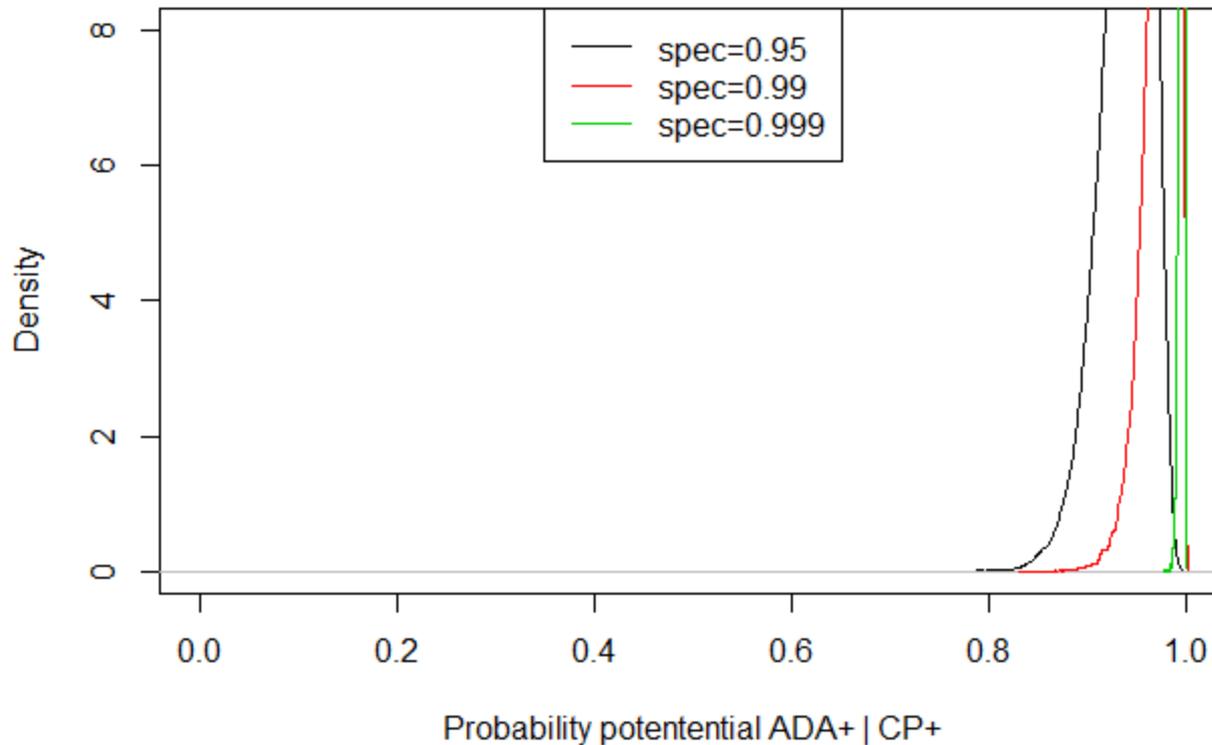


**Specificity = 0.95 - 0.99 - 0.999**

**Assuming sensitivity is 0.95 (ie 95/5)**

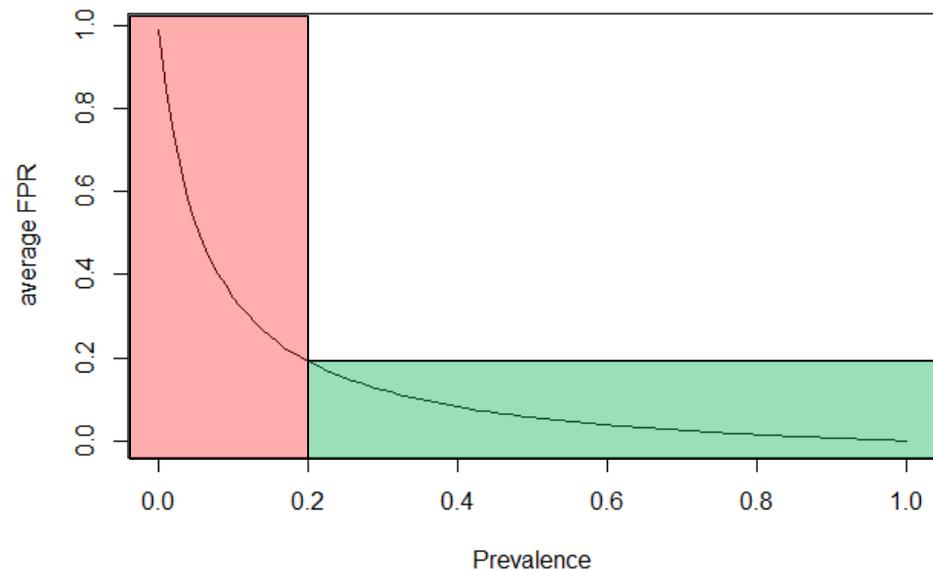
**50/100 patients confirmed negative**

**Probability of potential ADA**



# What's does that means?

- When the risk of  $p(ADA^+)$  is **>20%**,
  - the FPR is remaining around the intended 5%.
  - Using  $p(CP^+|ADA^+)$  -as currently done- instead of  $p(ADA^+|CP^+)$  will give about the same outcome.
- When  $p(ADA^+)$  is smaller than 20%, then it's recommended to shift to the adequate decision rule:  $p(ADA^+|CP^+)$



- The intended decision rule is in fact  $p(ADA^+|CP^+)$
- When **Prevalence** is unknown and **response** of ADA+ is unknown, the current CP decision rule  $p(CP^+|ADA^+) \sim p(ADA^+|CP^+)$ 
  - This is good news
- When information about **Prevalence** is available, then

$p(CP^+|ADA^+) \sim p(ADA^+|CP^+)$  when  $p(ADA^+)$  is  $>20\%$

$p(ADA^+|CP^+)$  is preferred when  $p(ADA^+)$  is  $<20\%$

- Using the Prediction interval or  $\beta$ -expectation interval for the CP determination is recommended to achieve intended FPR
  - 
  - $\beta$ -expectation interval is based on  $E[p(CP^+|ADA^+) | data] \geq 5\%$

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**THANK YOU**