Toxin A and Toxin B Specific Systemic Antibody Levels Correlate with Protection against *C. difficile* Associated Disease in Hamsters

Sanofi Pasteur, R&D Discovery, Cambridge, USA

Natalie Anosova, Ph D

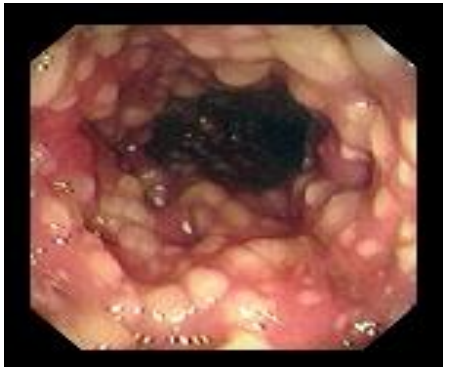
4th International *Clostridium difficile* Symposium 2012

September 20, 2012

SANOFI PASTEUR

Clostridium difficile Associated Disease

- ***C. difficile* infection (CDI) is identified as the leading cause of nosocomial diarrhea and pseudomembranous colitis associated with antibiotic therapy[1]**
- **CDI incidence surpassed methicillin-resistant *Staphylococcus aureus* (MRSA) as the leading cause of hospital-acquired bacterial infection in the US and in Europe[2,3]**
- **CDI is estimated to cause over $7 billion in healthcare costs annually in the US + EU[4]**
- **Emergence and spread of a hyper-virulent strain BI/NAP1/027 (associated with increased morbidity and mortality[5]) since 2003 in North America, and later in Europe, is now becoming endemic[6]**
- **Mortality rates from CDI have more than quadrupled in the US from 1999 to 2004[7] and in the UK from 2004 to 2007[6]**



[1]Rupnik M, Wilcox MH, Gerding DN 2009; 7(7):526-36

[2]Kuijper EJ et al Clin Microbiol Infect 2006;6:2-18

[3]Miller et al Infect Control Hosp Epidemiol 2011;32(4):387-90

[4]O'Brien JA et al Infect Control Hosp Epidemiol 2007;28:1219-27

[5]Miller M et al Clin Infect Dis 2010;15(50):194-201

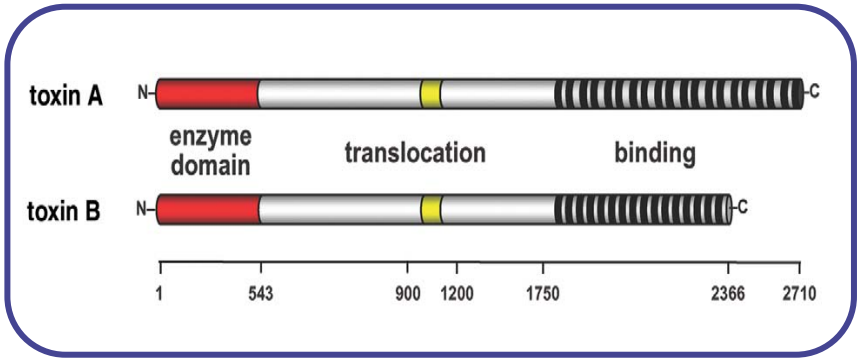
[6]Freeman J et al. Clin Microbiol Rev 2010;23:529-49

[7]Redelings MD et al Emerg Infect Dis 2007;13:1417-9

About *Clostridium difficile*

- **A Gram-positive, spore-forming anaerobic bacillus**
- **Toxin A and B are major virulence factors for *C. difficile* disease**
- **Toxins A and B are similar**
  - **~50% identity at amino acid level**
  - **High molecular weight (~300 kDa)**
  - **Domains**
    - **N-terminal enzymatic (Glucosyltransferase)**
    - **Central hydrophobic region**
    - **C-terminal binding domain (Receptor Binding)**
- **Serum anti-Toxin A and B IgG are associated with protection against recurrent CDI**

Aslam S. et al. *Lancet Infect Dis. 2005; 5:549*



Jank T.,Giesemann T., and Aktories K., *Glycobiology 2007;* vol. 17: 15R–22R

SANOFI PASTEUR

Current treatment options and limitations

- **Current treatment options largely rely on antibiotics[1]**
  - **Metronidazole for mild to moderate CDI**
  - **Vancomycin for severe and recurrent CDI**
  - **Fidaxomicin recently approved and licensed (narrow-spectrum macrolytic antibiotic)[2,3]**
- **Limitations of current treatment options**
  - **High recurrence rate[1,4]**
  - **Increasing treatment failure[5]**
  - **Selection of vancomycin-resistant enterococci[6]**
- **There are no prevention products approved for use**

[1]Cohen SH et al *Infect Control Hosp Epidemiol* 2010;31:431-55; [2]Optimer Pharmaceuticals (Dificid) http://www.dificid.com/
[3]Loule TJ et al *N Eng J Med* 2011;364:422-31;[4]McFarland LV et al *Am J Gastroenterol* 2002;97:1769-75; [5]Kuijper EJ, Wilcox MH *Clin Infect Dis* 2008;47:63-5; [6]Pepin *J Clin Infect Dis* 2008;46:1493-8

Sanofi Pasteur's approach to CDI

- **Sanofi Pasteur's *C. difficile* candidate vaccine is being developed for the prevention of primary disease caused by CDI[1]**
- **The target population is adults at risk of CDI, such as those with planned hospitalization, long-term care/nursing home residents, those with co-morbidities that require frequent and/or prolonged antibiotic use[1]**
- **Sanofi Pasteur's *C. difficile* vaccine candidate contains highly purified, formalin-inactivated preparations of Toxins A and B**
  - **Alum-adsorbed, delivered via intramuscular injection**
  - **Has so far proven immunogenic and well-tolerated in adults and elderly**
  - **Currently is in Phase II clinical trials**

[1]**Foglia G. et al *Vaccine* 2012;30:4307-9**

SANOFI PASTEUR

Pre-clinical evaluation of Sanofi Pasteur's *C. difficile* vaccine candidate in hamster model

- **Hamster is naturally susceptible to CDI and is a widely recognized model for *C. difficile* associated disease**

- **Critical questions were addressed in hamster model**
  - **Immunogenicity and efficacy of the adjuvanted toxoid vaccine in active immunization/challenge studies**
  - **Correlation between binding and functional serum toxin-specific antibody responses and protection in active immunization/challenge studies**
  - **Efficacy of immune polyclonal sera against active components of *C. difficile* vaccine in passive protection studies**

Hamster efficacy model

Immunization (IM)

Clindamycin (IP)

Monitor weight loss, disease and survival

Day 0 14 28 34 35

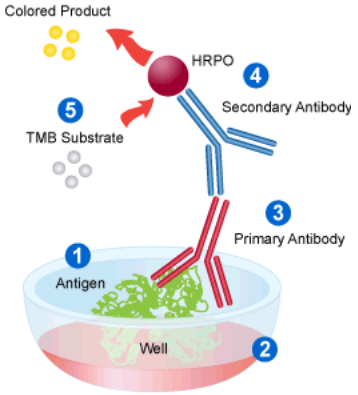
Bleed

C.difficile (IG)

Indirect ELISA

Toxin A

Toxin B

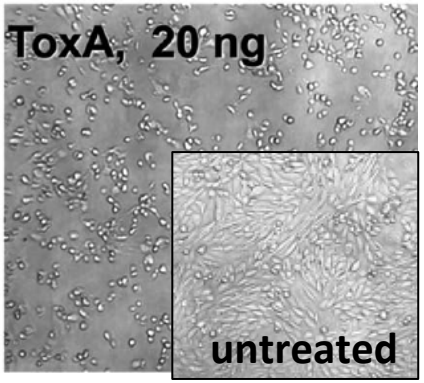


http://www.leinco.com/indirect_elisa

IMR-90 Neutralization Assay

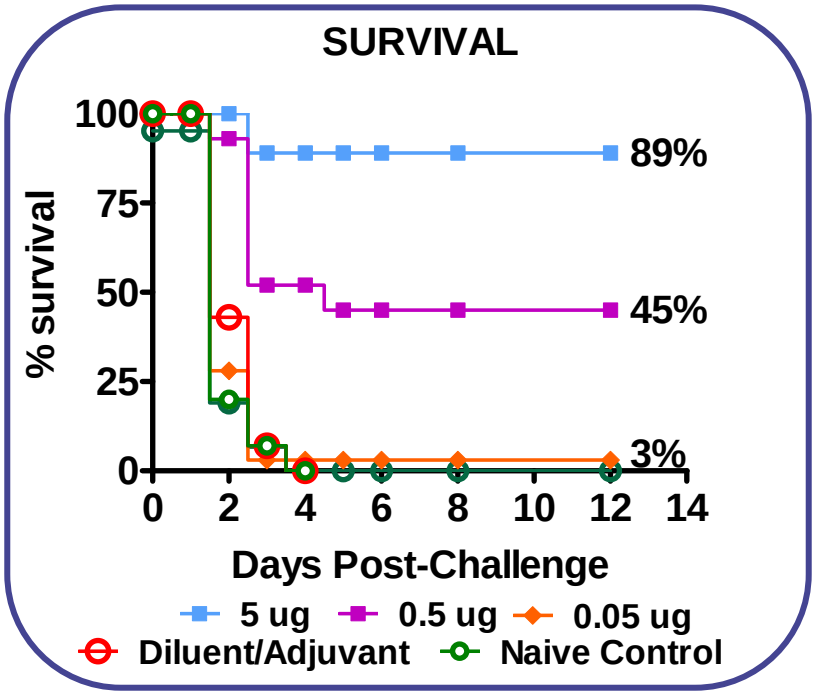
Toxin A

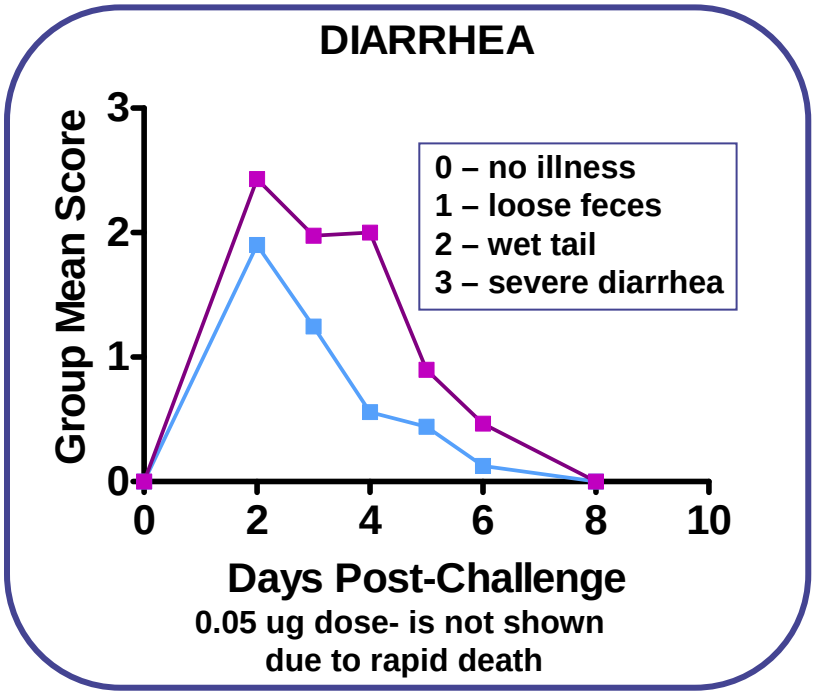
Toxin B



SJ. Demarest et al *mAbs* 2:2, 1-9, 2010

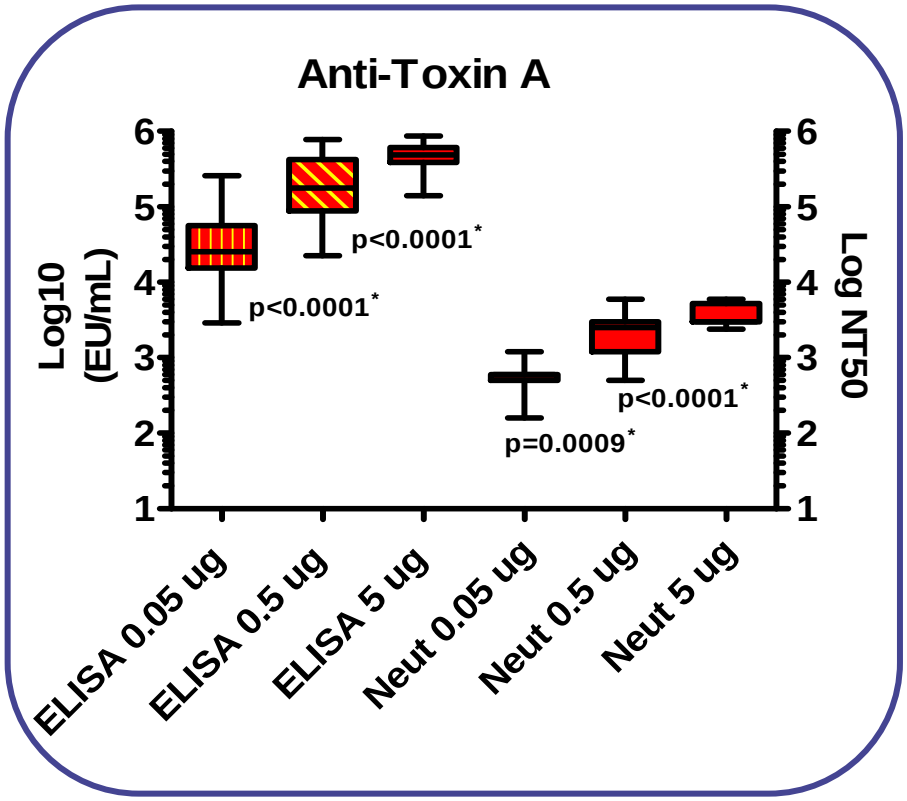
Immunization with the toxoid vaccine conferred protection against *C. difficile* challenge in a dose-dependent manner

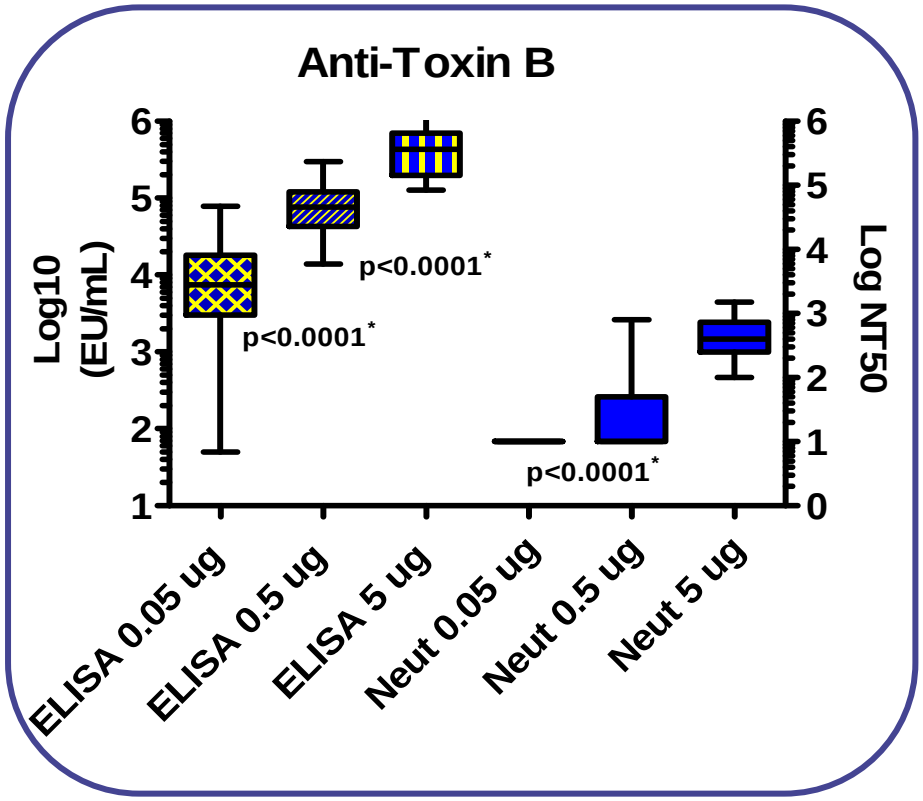




- **Animals immunized with decreasing doses of the vaccine were protected against death and disease in a dose-dependent manner from challenge**
  - Animals challenged with *C. difficile* vegetative culture of the VPI10463 strain
  - Endpoint of the model ≥30% weight loss or morbidity

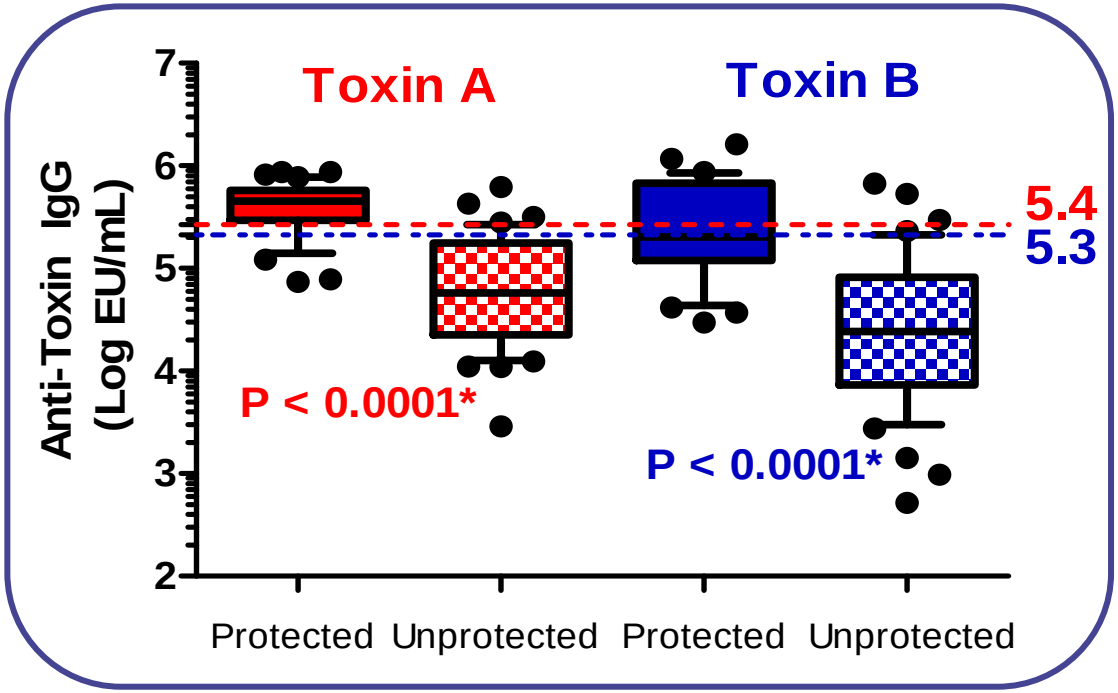
Immunization with the toxoid vaccine elicited serum Toxin A and B binding and neutralizing responses in a dose-dependent manner

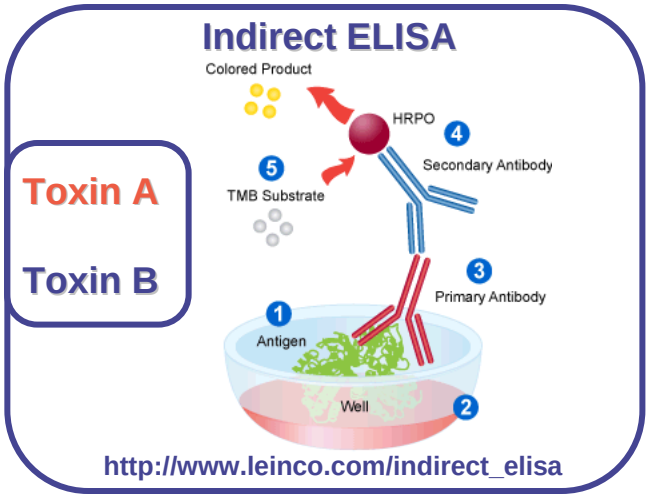




- **Pre-challenge Toxin A and B antibody responses in the animal groups that received descending doses of the vaccine showed a dose-dependent pattern**
  - **Pre-challenge antisera were tested in ELISA and IMR-90 neutralization assay using highly purified native *C. difficile* Toxins A and B**

ELISA toxin A and B specific serum IgG levels help to predict survival outcome in primary challenge model

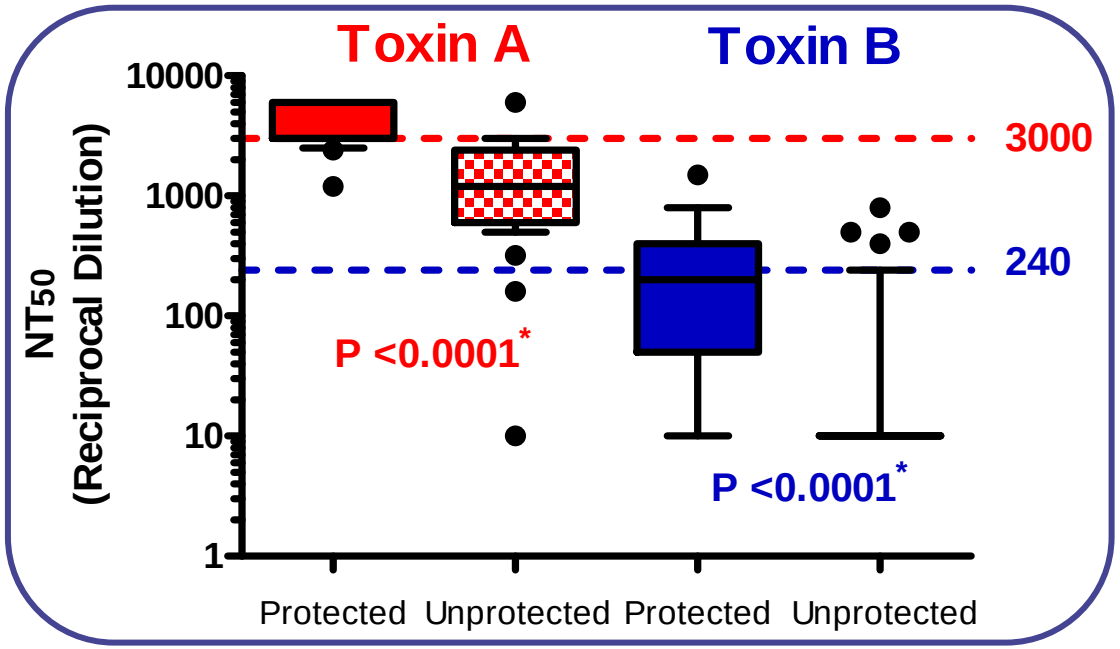


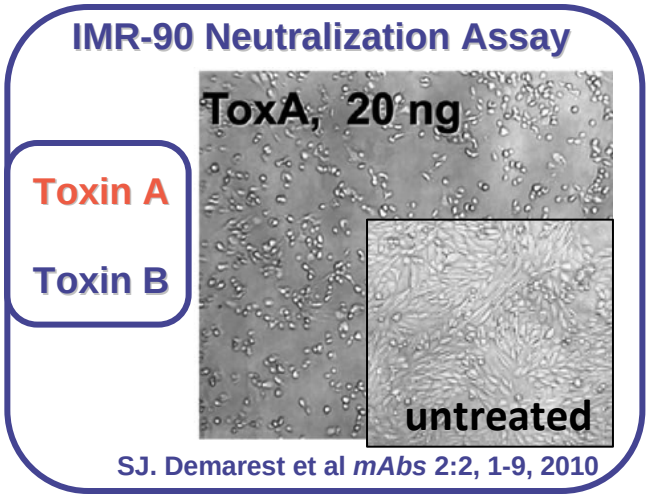


- Median Toxin A- and B-specific IgG titers in the group of surviving animals were statistically higher compared to the group that died
- The ELISA's predictability of ***lack of protection*** was higher than predictability of ***protection***
- An 'unprotective' titer cutoff was established: 90% of animals with anti-toxin A titers ≤ 5.4 log10 EU/mL or anti-toxin B titers ≤ 5.3 log10 EU/mL succumbed to challenge

SANOFI PASTEUR

Toxin A and B neutralizing titers in serum help to predict survival outcome in primary challenge model

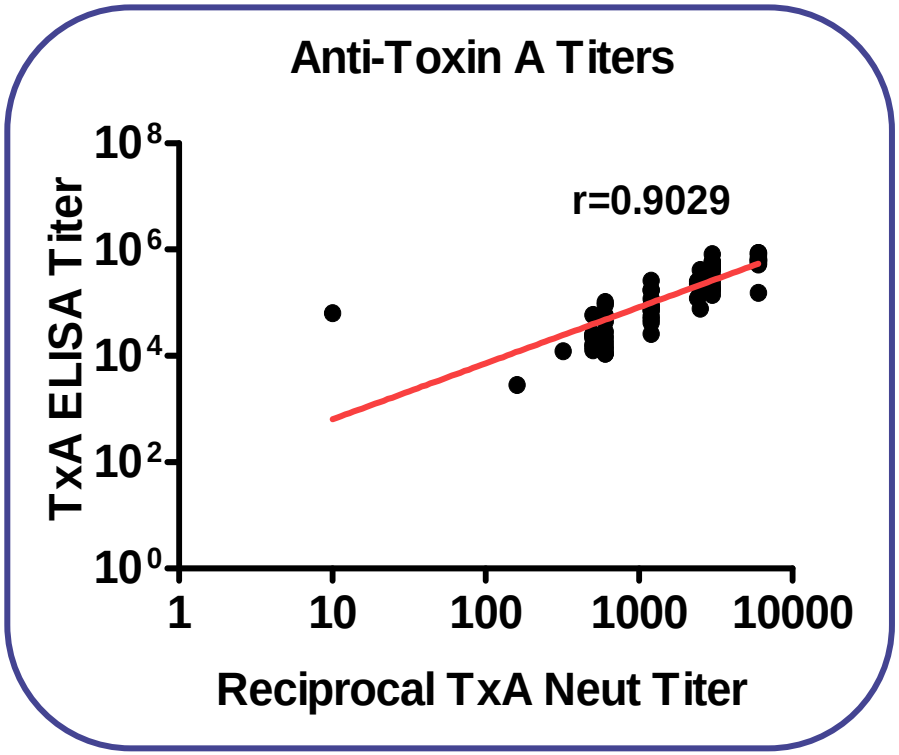


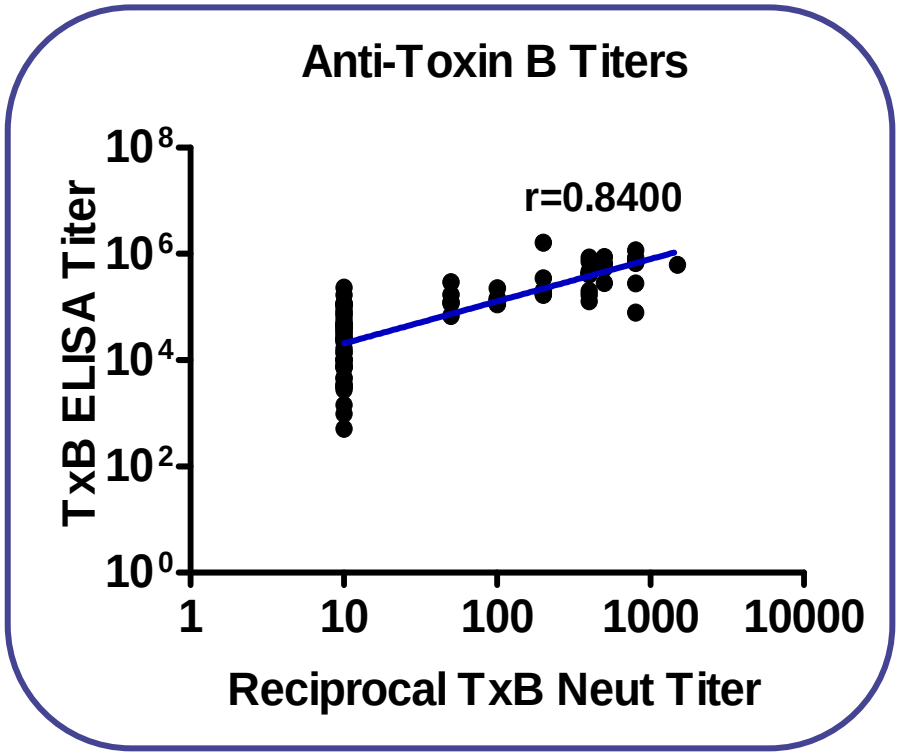


- Median Toxin A- and B-neutralizing titers in the group of surviving animals were statistically higher compared to the group that died
- Similar to ELISA, the neutralization assay's predictability of ***lack of protection*** was higher than predictability of ***protection***
- An 'unprotective' titer cutoff was established: 90% of animals with anti-toxin A titers ≤3000 or anti-toxin B titers ≤240 succumbed to challenge

SANOFI PASTEUR

Concordance observed between ELISA and Neutralizing titers for each component of vaccine

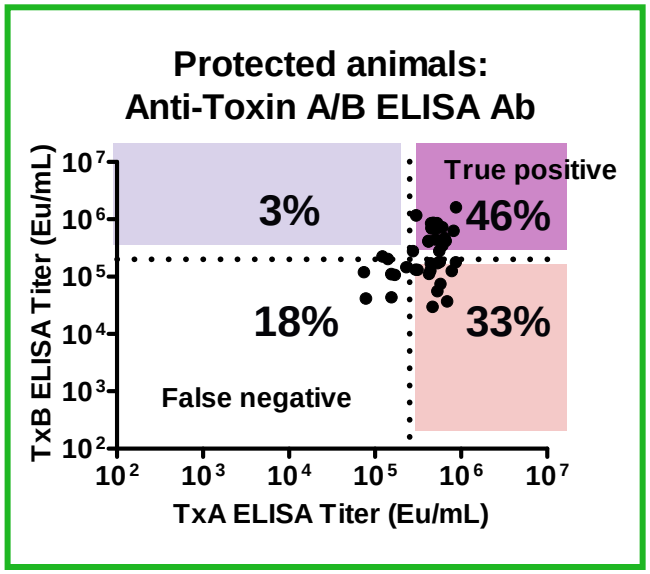


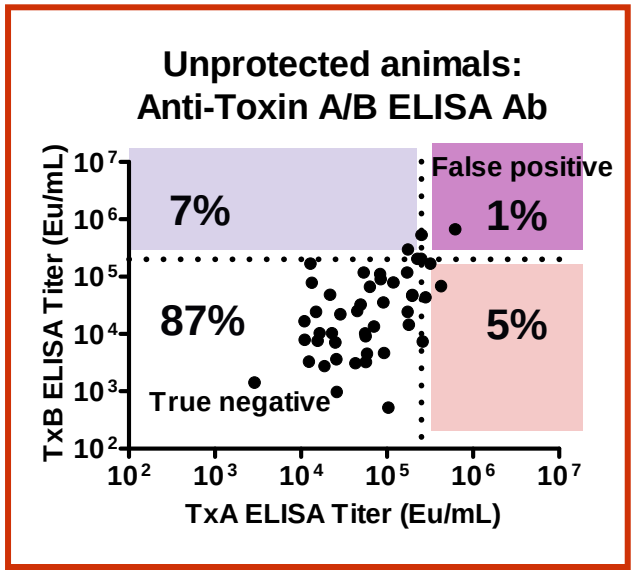


- Statistically significant correlation between the toxin-specific titers measured by ELISA and toxin neutralizing titers measured by IMR-90 cell-based assay was seen for both toxin A and toxin B responses by nonparametric Spearman test
- Data confirmed that the toxin A and B binding serum antibodies correlated with functional (toxin neutralizing) activity

SANOFI PASTEUR

Responses to both toxins were the best predictors of lack of protection in hamster efficacy model



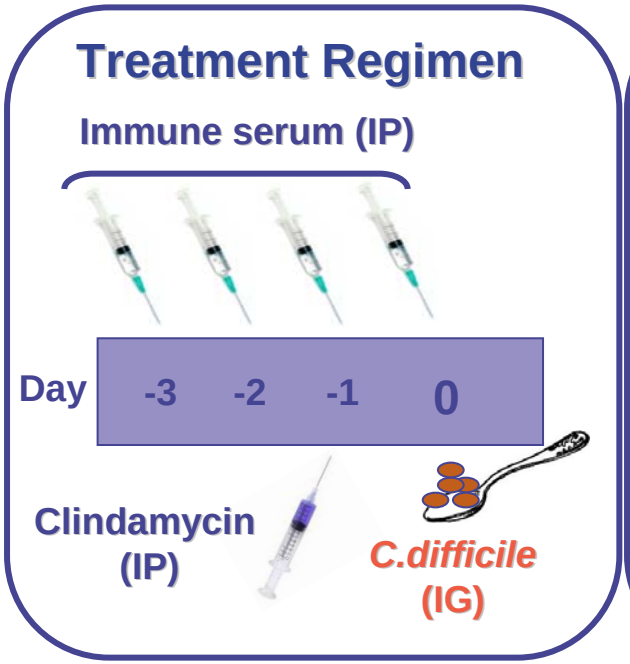


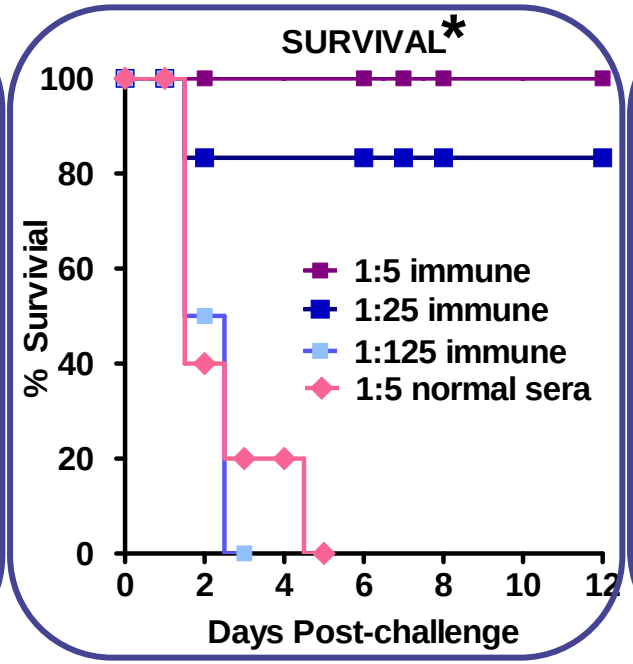
- *True positive* – test indicates protection and animals protected
- *False positive* – test indicates protection, but animals do not survive
- *True negative* – test indicates lack of protection and animals do not survive
- *False negative* – test indicates lack of protection, but animals survive

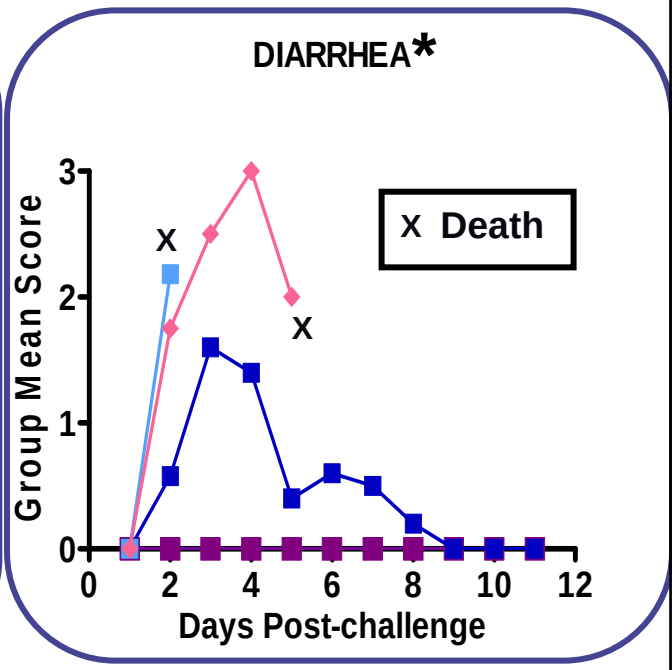
- With established titer cutoff, TcdA and TcdB ELISAs combined had high prediction value of lack of protection (*True negative* 87%) with low *false positive* rate (1%)
  - *False positive* rates on the basis of either anti-TcdA (5%) or anti-TcdB (7%) only titers were slightly higher
- Assays had lower predictability of protection (*True positive* 46%) on the basis of both titers with higher *false negative* rate of 18%
- Assays had even lower predictability of protection on basis of either anti-TcdA or anti-TcdB only titer (33% and 3%, respectively)
  - *True positive* rate on basis of anti-TcdA/B, anti-TcdA only and anti-TcdB only titers combined was 82%

SANOFI PASTEUR

Passive immunization with the anti-Toxoid A/B serum conferred protection against *C. difficile* challenge







- **Hamsters treated with one regimen of immune, but not normal, sera were protected against morbidity and mortality in a dose-dependent manner from *C. difficile* spore challenge (630* or VPI10463 strains)**
  - **Immune serum pool was generated in hamsters by immunization with active components of the Sanofi Pasteur vaccine candidate**
  - **Dose-dependent protection was observed for the challenge against both strains tested**

Summary of findings

- **Sanofi Pasteur's *C. difficile* vaccine candidate delivered by intramuscular immunization elicited high Toxin A and B specific serum IgG response in hamsters**
- **The vaccine was efficacious in both active and passive protection models in hamsters**
  - **Critical role of toxin-specific circulating antibodies in protection was demonstrated**
- **Two *in vitro* readouts were identified as immune correlates of protection, toxin ELISA and IMR-90 neutralization assay**
  - **Demonstrated correlation between serum toxin-specific antibody levels and efficacy**
  - **Observed concordance between ELISA and neutralization assays for both Toxin A and B responses**
  - **Insufficient responses to both toxins were the best predictors of lack of protection suggesting importance of both components in vaccine**
  - **The assays had very low *false positive* and higher *false negative* rates**
    - **The *false negative* indications suggest that parameters other than toxin-specific Ab (such as duration of exposure, normal gut flora, response to non-toxin components of bacterium *etc*) may play auxiliary role in protection**

Acknowledgements

Sanofi Pasteur

(Cambridge, MA & Swiftwater, PA)

Anna Moralez Brown

Nana Liu

Lu Li

Leah Cole

Jinrong Zhang

Charlotte Houston

Amy Lundberg

Hersh Mehta

Harold Kleanthous

Patricia Pietrobon

Thank you