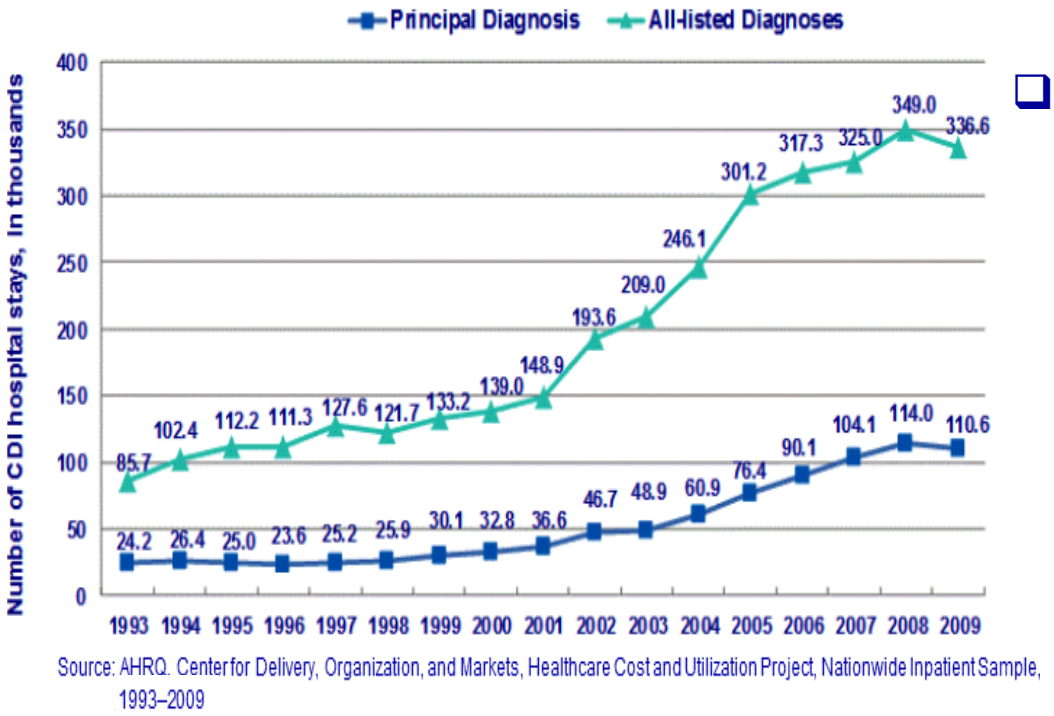
# A NOVEL APPROACH TO A *C. DIFFICILE* TOXOID VACCINE: IMMUNOGENICITY AND PRECLINICAL EFFICACY

Kathrin U. Jansen, PhD on behalf of *C. difficile* team
Senior Vice President
Vaccine Research R&D
Pfizer

20 September 2012

# *Clostridium difficile*-associated Disease
## Unmet Medical Need



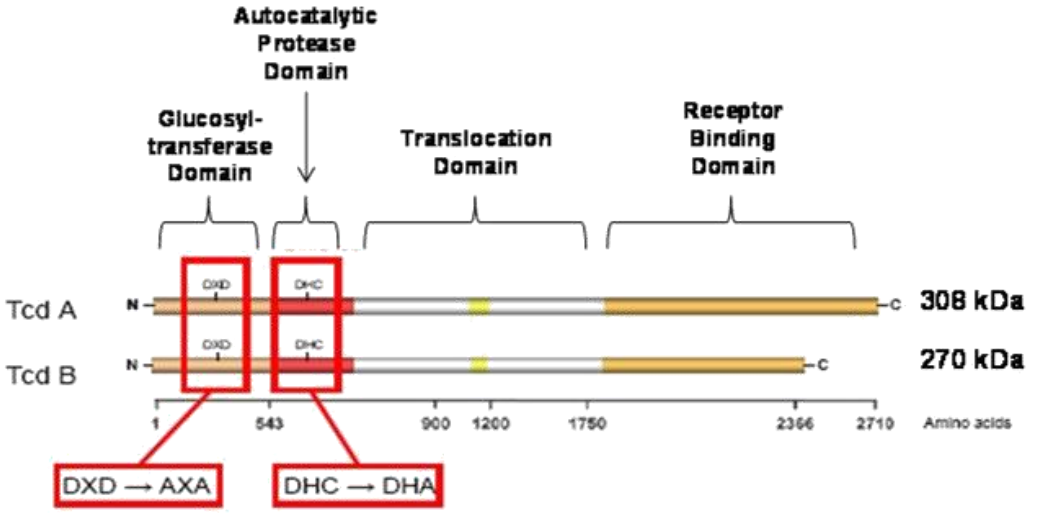

Source: AHRQ. Center for Delivery, Organization, and Markets, Healthcare Cost and Utilization Project, Nationwide Inpatient Sample, 1993–2009

- **Incidence and disease severity have increased over the last decade[1]**
  - ~350,000 cases per year in the US[1]
  - Emergence of hypervirulent strains, increased use of antibiotics and health care exposure
  - High CDAD-attributable mortality reported in European patients[2]
  - High prevalence of C. diff colonization in nursing home residents in Germany[3]
- Magnitude of CDAD rivals MRSA infections[4]
- US annual associated cost of care in multibillion dollar range [5]
- In 2013, hospitals participating in Medicare / Medicaid will be required to report CDAD events to qualify for their 2015 annual payment update[6]
- **No prophylactic vaccine is available for primary or recurrent CDAD**

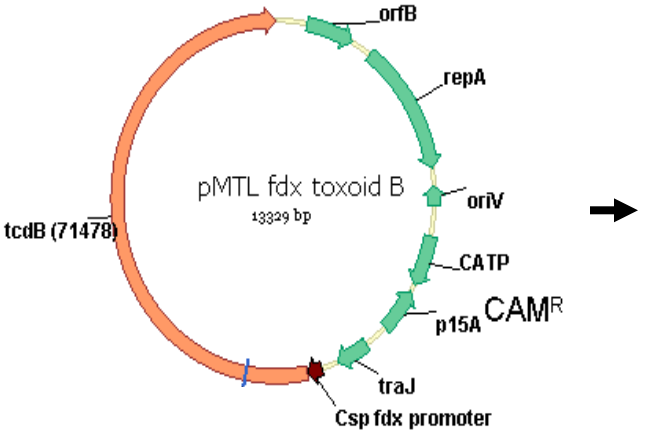
[1] Lucado et al, http://www.hcup-us.ahrq.gov/reports/statbriefs/sb124.jsp
[2] Bauer et al, Lancet 2011;377:63-73
[3] Arvand et al, PLoS ONE 2012;7:e30183
[4] Miller, B. et al, ICHE April 2011
[5] Dubberke, *J Hosp Med. Mar 2012;7 Suppl 3:S1-4*
[6] McDonald et al. MMWR March 9, 2012

# Production of Genetically Modified Toxins in the Native Host

## TOXIN STRUCTURE




## GENETICALLY MODIFIED TOXIN EXPRESSION PLASMID



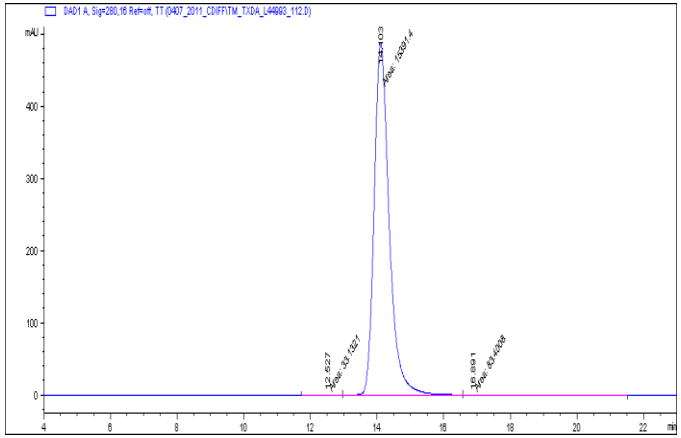
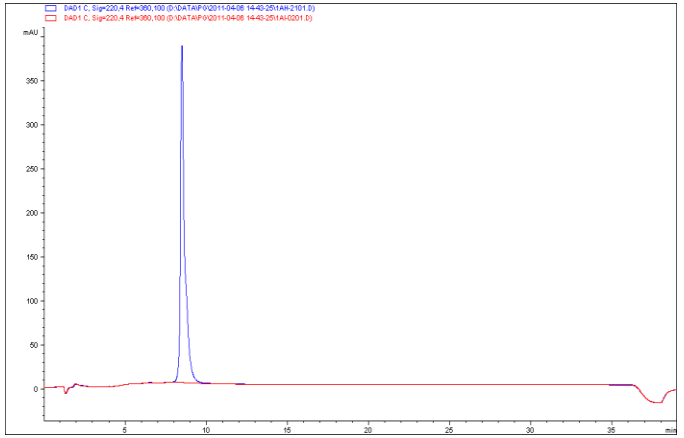
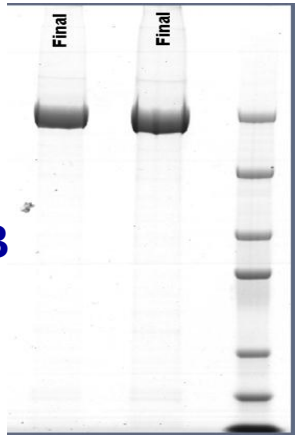
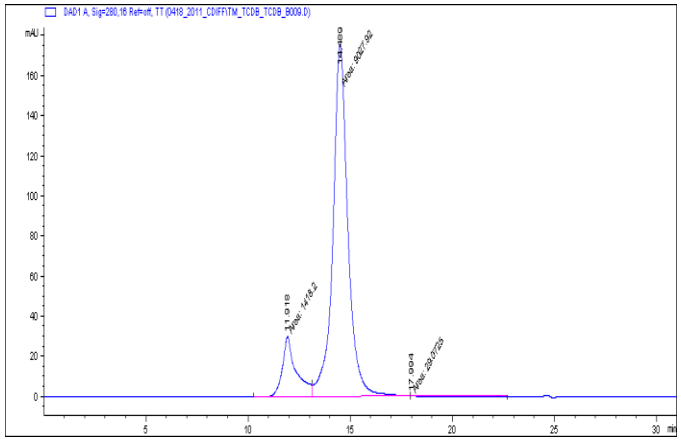
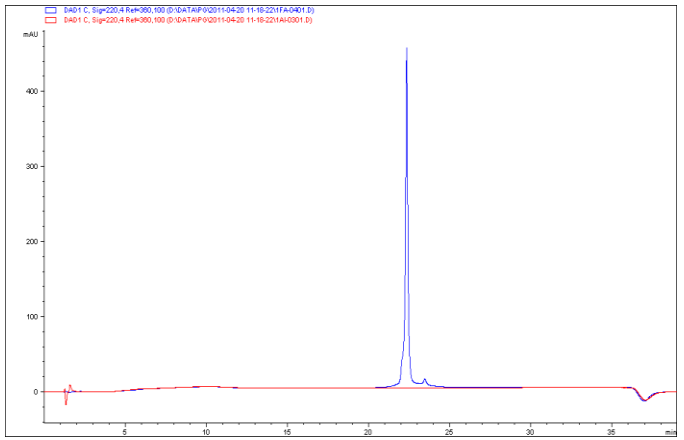

## EXPRESSION IN *C. difficile*


Asporogenic / Atoxigenic



Vaccine Research

# Genetically Modified *C. difficile* Toxins Are of High Purity

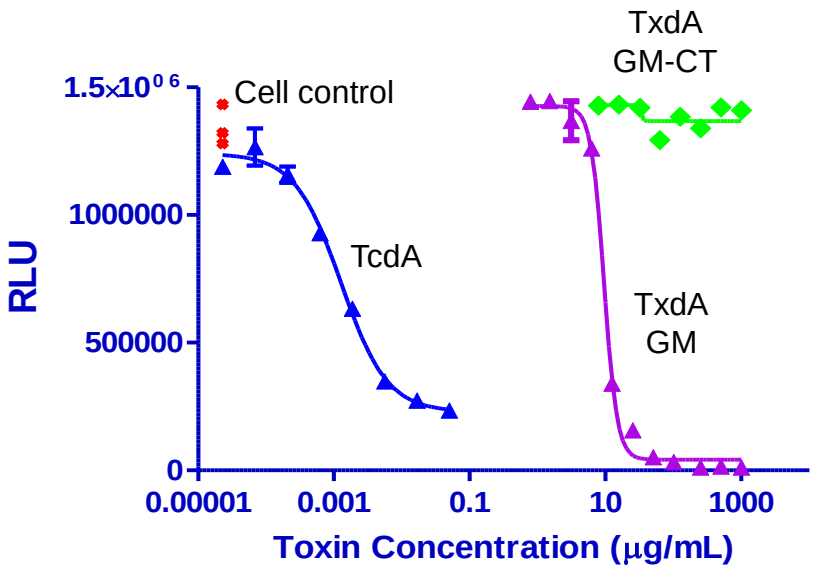
| | SDS-PAGE (reduced) | Analytical SEC | Analytical AEX |
|---|---|---|---|
| **Toxoid A** |  |  |  |
| **Toxoid B** |   |  |  |



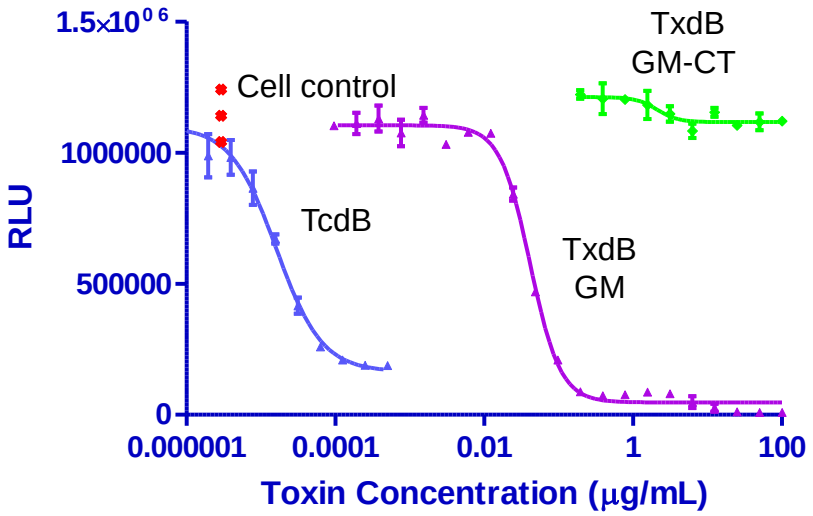
Vaccine Research

# Residual *In Vitro* Cytotoxicity Was Eliminated By Proprietary Chemical Treatment (CT) of Genetically Modified (GM) Toxins

## Toxin A




## Toxin B

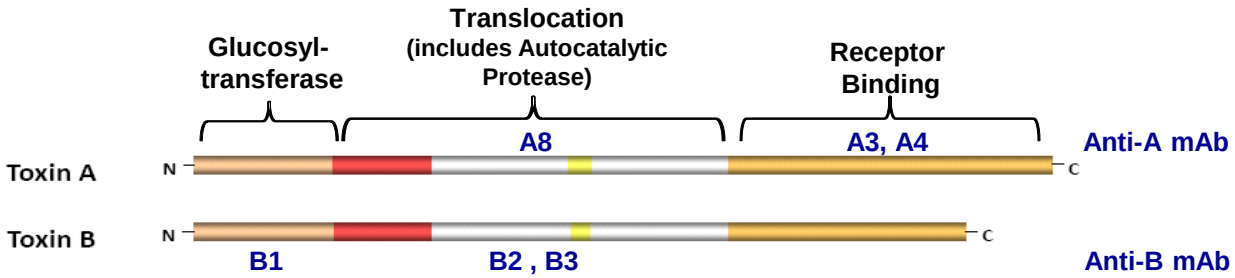



**Posters P94, P6**



Vaccine Research

# Chemical Treatment (CT) of Genetically Modified Toxins Preserves Neutralizing Epitopes



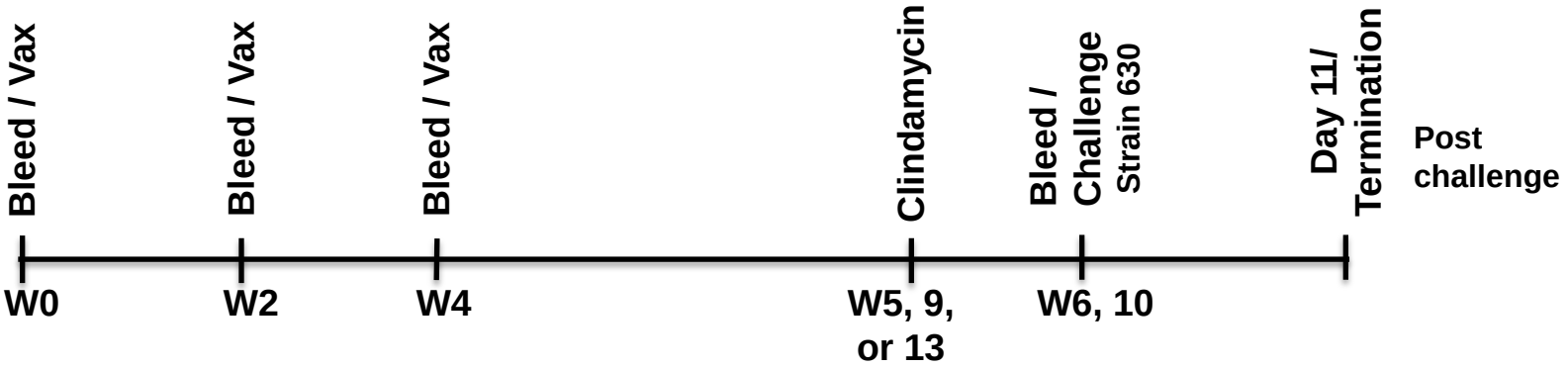

| Sample | Fold-Reduction in Cytotoxicity ($log_{10}$) | % Max Binding Neutralizing mAb | | |
|---|---|---|---|---|
| | | Anti-A mAb A3 | Anti-A mAb A4 | Anti-A mAb A8 |
| TxdA | 4.5 | 100 | 100 | 100 |
| TxdA-Formalin | >6.4 | 53 | 55 | 59 |
| **TxdA-CT** | **>6.4** | **103** | **90** | **94** |
| | | Anti-B mAb B1 | Anti-B mAb B2 | Anti-B mAb B3 |
| TxdB | 4.3 | 100 | 100 | 100 |
| TxdB-Formalin | 8.4 | 67 | 67 | 36 |
| **TxdB-CT** | **8.4** | **87** | **78** | **73** |

**Average of two experiments**

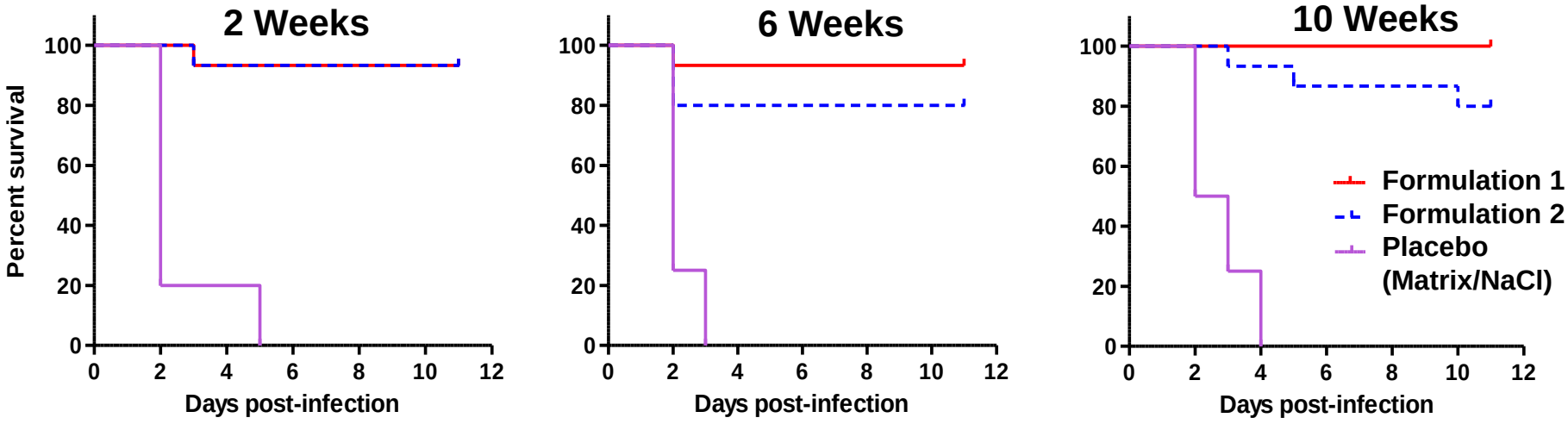


Vaccine Research

# *C. difficile* Toxoid Vaccine Candidate Protects Hamsters (n=15/group) from *C. difficile* Challenge




## Challenge Post Last Vaccination




**Formulation 1 = 93% (14/15)**
**Formulation 2 = 93% (14/15)**

**Formulation 1 = 93% (14/15)**
**Formulation 2 = 80% (12/15)**

**Formulation 1 = 100% (15/15)**
**Formulation 2 = 80% (12/15)**

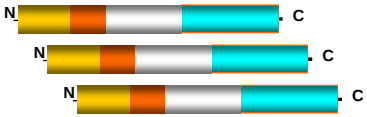


Vaccine Research

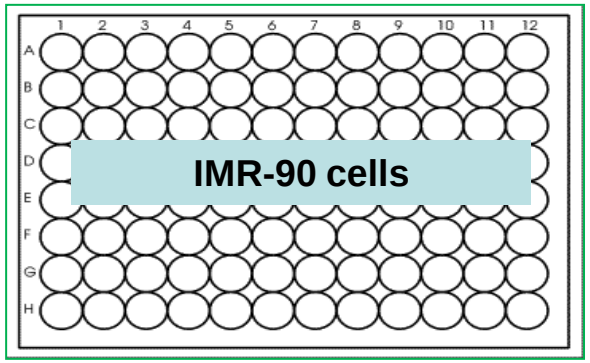
# *Principle of C. difficile* Toxin Neutralization Assay (Posters P94, P6)

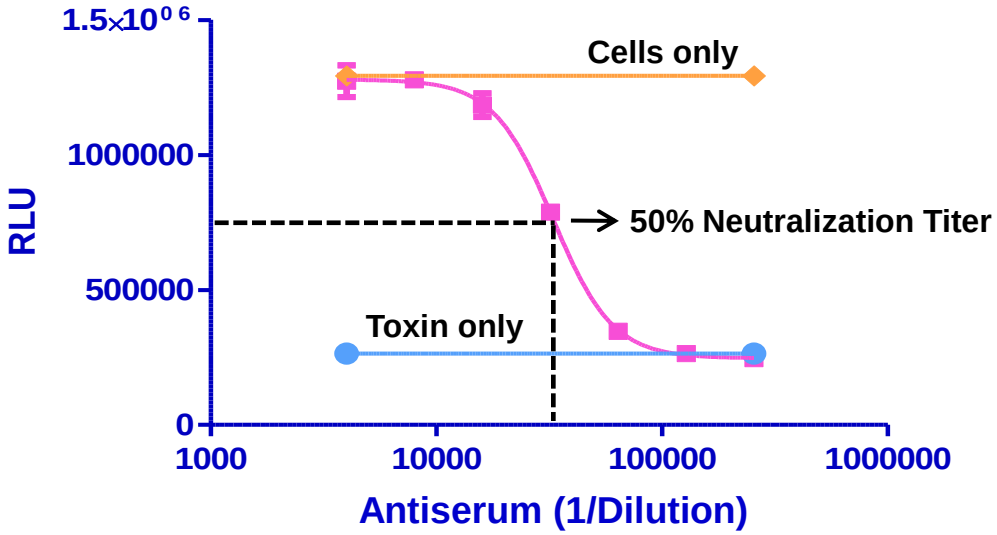
Antibodies

+




Toxin



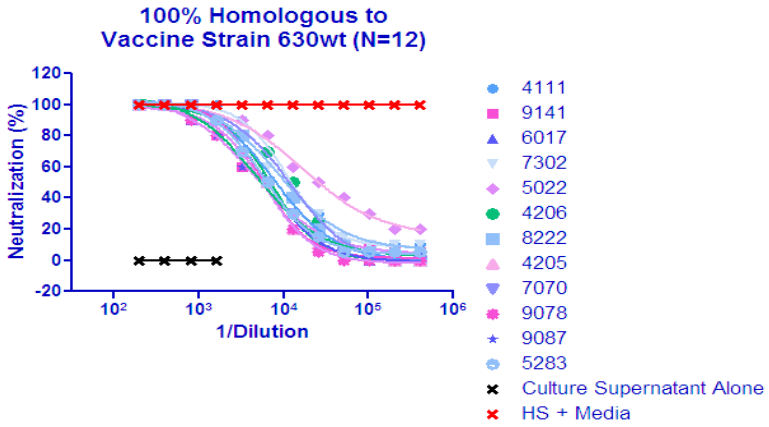



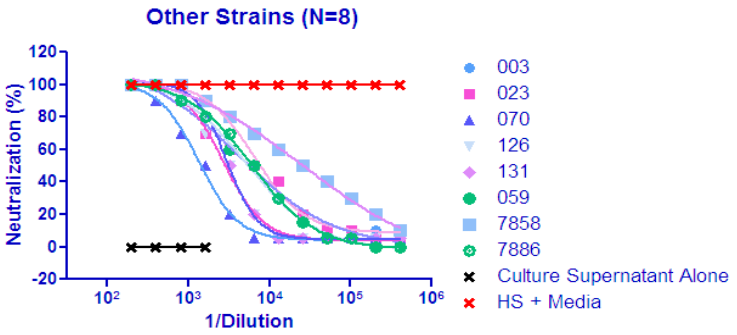

- 72h, 37ºC
- Add CellTiter-Glo®
- Read Luminescence

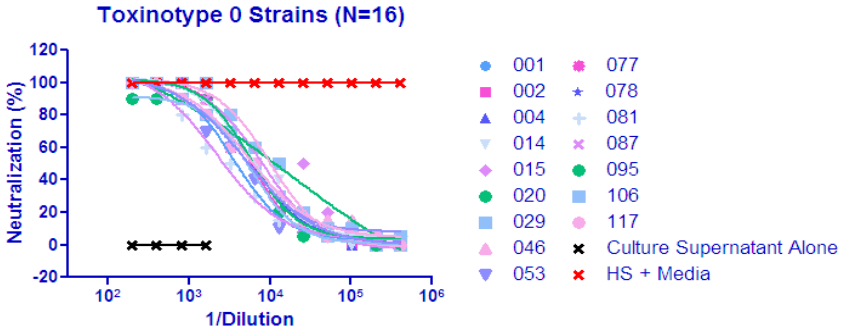


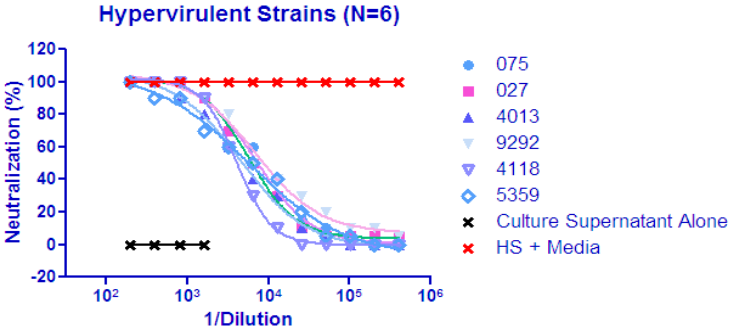
Vaccine Research

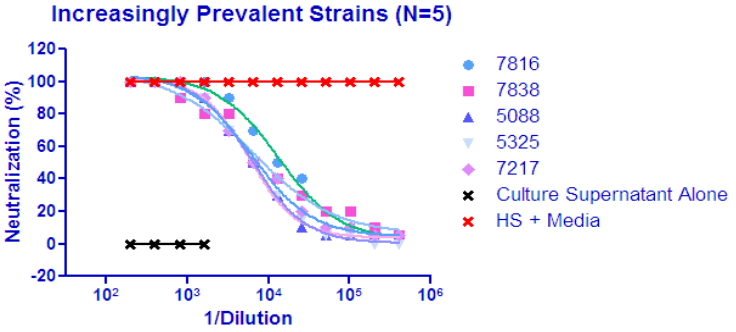
# Vaccine Induces Toxin A-Neutralizing Abs to Clinically Relevant *C. difficile* Strains Including Hypervirulent Strains (*n* =47)



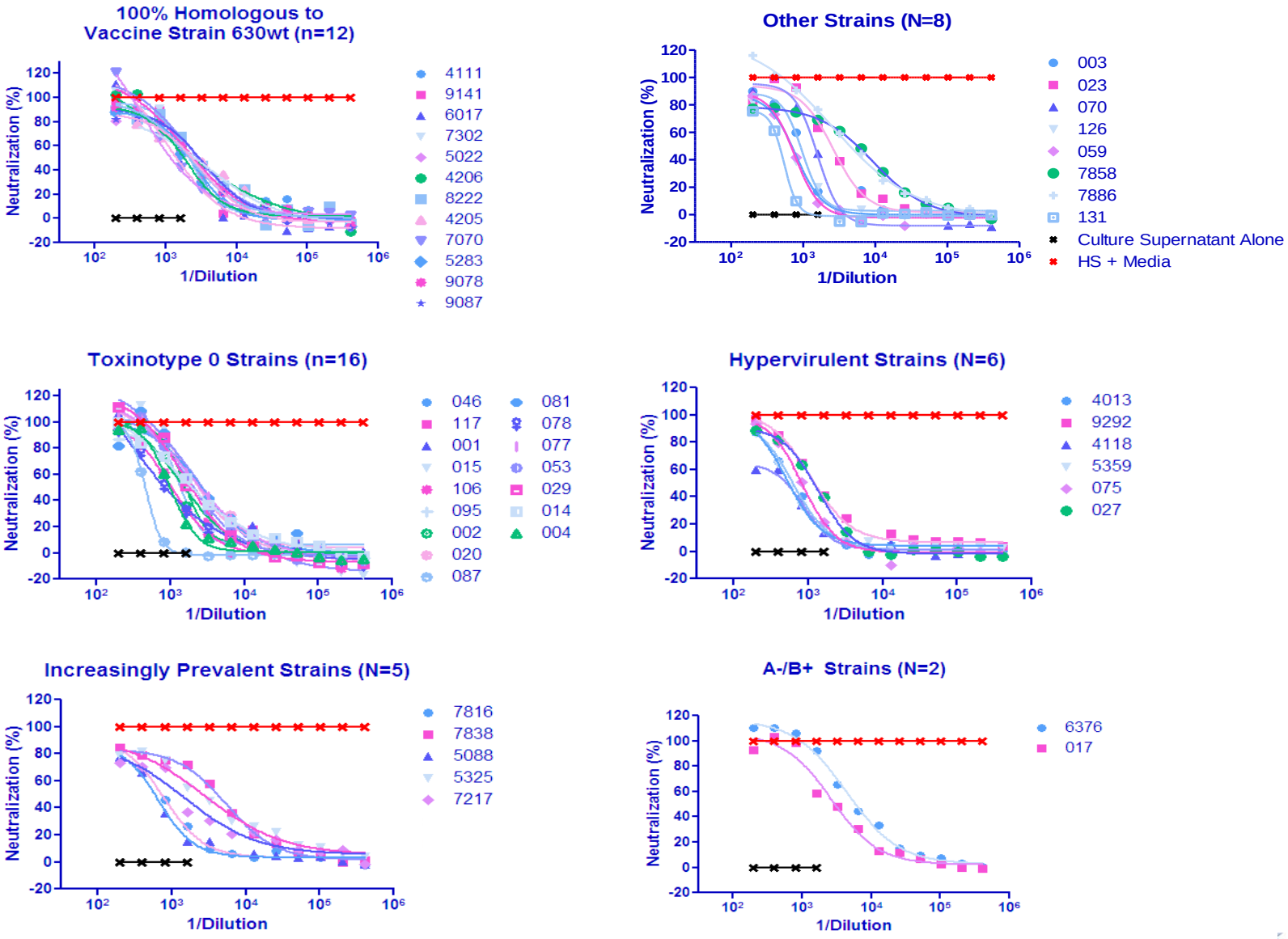




x Positive control (100% Neutralization)
x Negative control (Toxin only; No Neutralization)

# Vaccine Induces Toxin B-Neutralizing Abs to Clinically Relevant *C. difficile* Strains Including Hypervirulent Strains (*n*= 49)



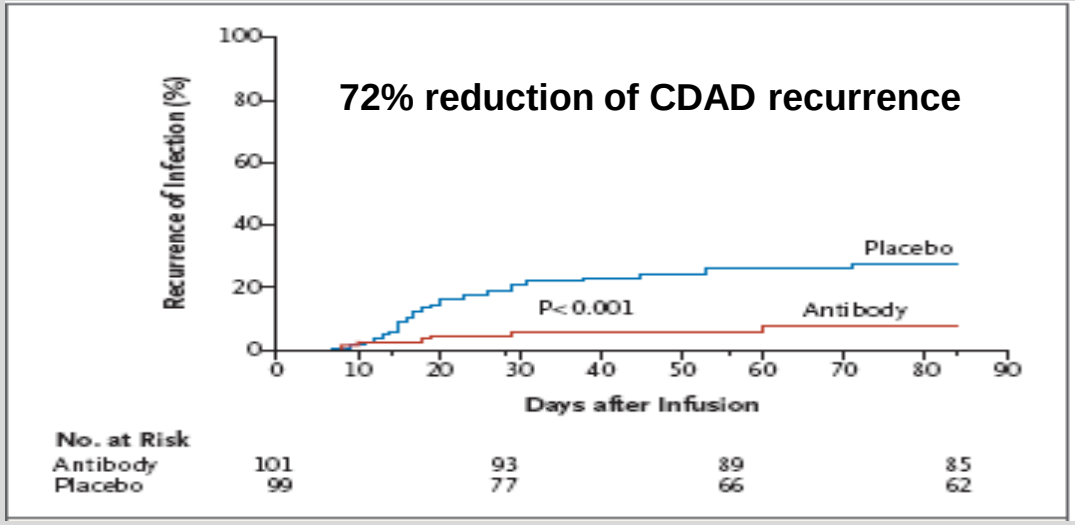

x Positive control (100% Neutralization)

x Negative control (Toxin only; No Neutralization)



Vaccine Research

# Proof of Concept That Toxin Neutralizing Antibodies Are Sufficient For Protection

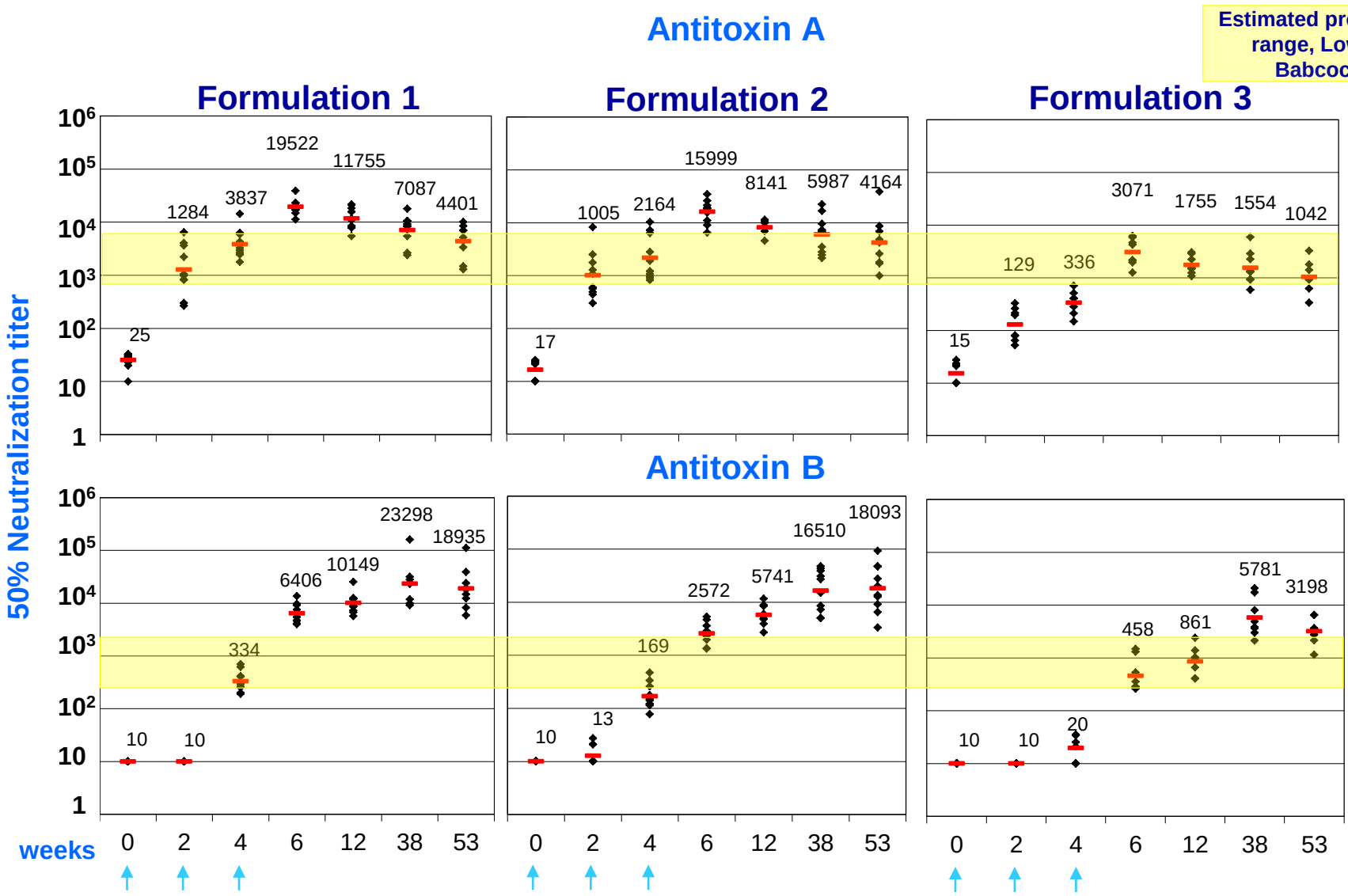



Lowy et al, NEJM 2010



Vaccine Research

# Vaccine Candidate Formulations Differ by Speed of Response But All Achieve Sustained Antitoxin Neutralization Responses in Cynomolgus Monkeys (N= 8-10/ Group)

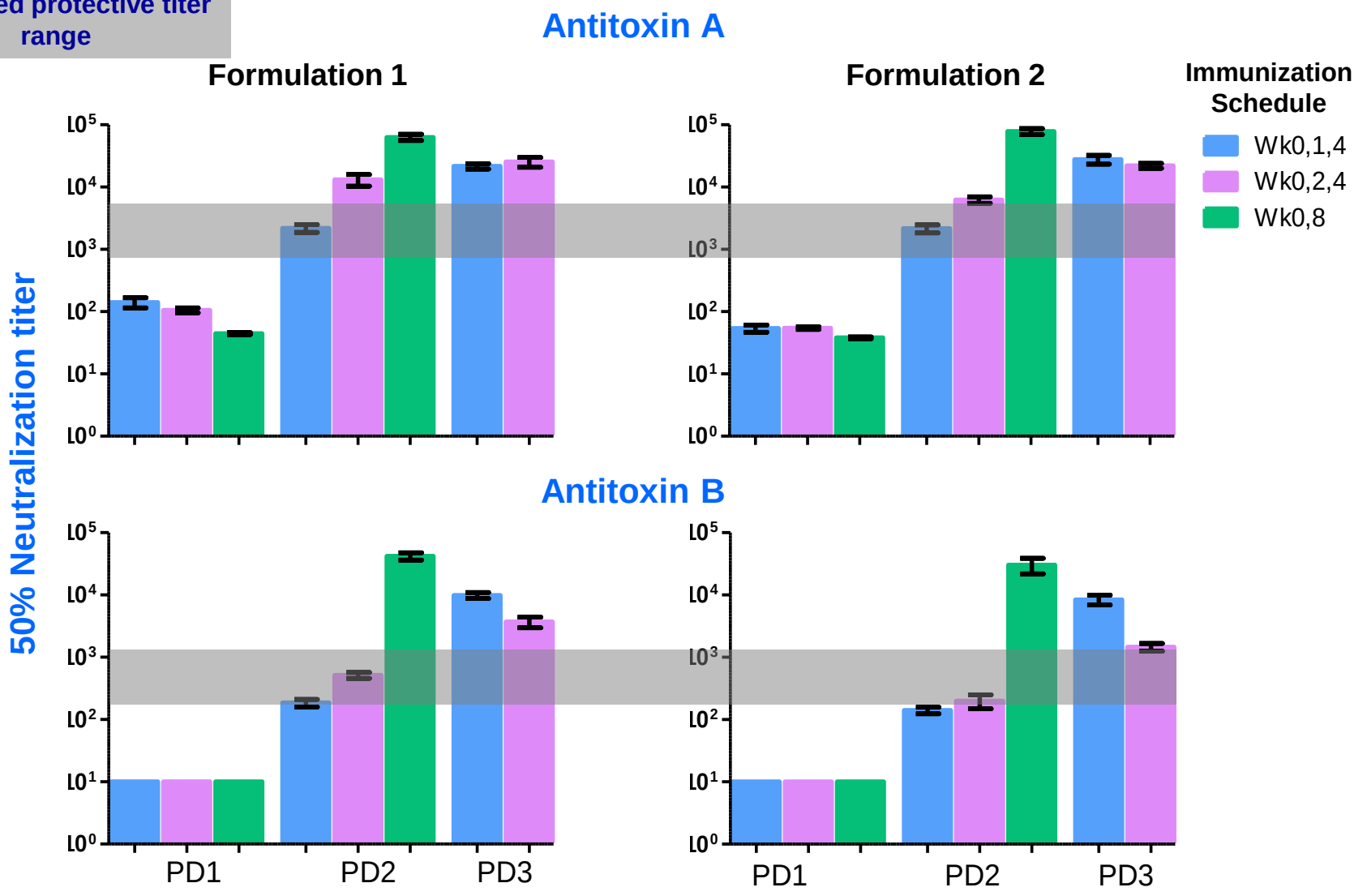





Vaccine Research

# Classical Immunization Schedule Induces Superior Antitoxin Neutralization Responses PD2 Compared to Accelerated Schedule (Cynos N = 15/group)

**Estimated protective titer range**




**PD1 = 1 week postdose 1**
**PD2,3 = 2 weeks postdose 2,3 respectively**



Vaccine Research

# Conclusion

## Pfizer's bivalent *C. difficile* toxoid vaccine candidate

- **Induces neutralizing antibodies in hamsters and non-human primates**
  - Rapid induction
  - High magnitude
  - Durability
- **Protects hamsters from lethal challenge**
- **Broad neutralizing coverage against toxins obtained from >95% of clinically relevant disease isolates (US, Canada, EU)**



14

# Acknowledgements


**Core Program Team**
Mini Sidhu
Louise Pedneault
Barbara Detjen
Hadi Falahatpisheh
Bruce A. Green
Michael Hagen
Lynda Kulp
Denise Pretzer
Michael Pride
Mark Ruppen
Susan Sciotto-Brown
Donna Valter

**Research**
Ozgur Akcan
Annaliesa Anderson
Lubomira Andrew
Don Arrigo
Leena Bagle
Ayele Bandele
Ping Cai
Marjolaine Carriere
Liuquan Chang
Kshitiz Chaudhary
Sherry Chi
David Cooper
Annie Cruz
Deb Dilts
Robert Donald
Jing Run Fan
Vern Fahie
Pam Fink

**Research (cont)**
Michael Flint
Sharmistha Ghosh
Jeannette Gordon
Alexey Gribenko
Patty (Huijing) Gu
Li Hao
Arthur Illenberger
Deanne Illenberger
Kwang il Jeong
Han-Qing Jiang
Erik J. Johnson
Oleg Jouravlev
Narender Kalyan
David Keeney
Fidenatou Kerim-Taylor
Lakshmi Khandke
Srinivas Kodali
Eun Hee Koh
Cheryl Kotash
Lubomir Kovac
Ksenia Krylova
Philip Lee
Peter Li
Yongdong Liu
Scott Lomberk
Jason Lotvin
Peimin Lu
Kathrin Mason
Yury Matsuka
Antony Mathews
Melissa McCulloch

**Research (cont)**
Shakuntala Megati
Terri Mininni
Soraya Moghazeh
Justin Moran
Stanley Mullen
Ellen Murphy
Lorna Nunez
Jennifer Obregon
Lida Oum
Manoj Pal
Sujata Patel-Brown
Lynn Phelan
Paulina Plewa
Dorothy Plummer
Earl Pursell
Vidia Roopchand
Ramon Rodriguez
Elena Severina
Jian She
Willie Sun
Yekaterina Timofeyeva
Gow-Jen-Gordon Tsai
Nancy Twomlow
Eugene Vidunas
Michele Weaver
Susan Witko
Cherry Wu
Karen Xu
Cindy Yang
Hailey Yuan
Yongmao Yu

**Research (cont)**
Irene Yurgelonis
Lily Zhang
Ping Zhao
Zack Zheng
Gary Zlotnick

**Bioprocess**
Bruce Bishop
Robert Bilbrey
Deborah Bock
Frank Brello
Tara Carter
Leeann Chrisco
Rob Curtiss
Larry Determan
Andy Espenschied
Nathan Jenkins
Angela Kantor
Gregg R.D. Kelly
Dustin Kerr
Kathy Kofsky
Robert Kottmeier
William (Tom) Mueller
Lori Norton
Michael Pearson
Teresa Roberts
Michael Smith
Bruce Thompson
Bernard Violand
Carol Weiss
George Lee
Scott Lennon

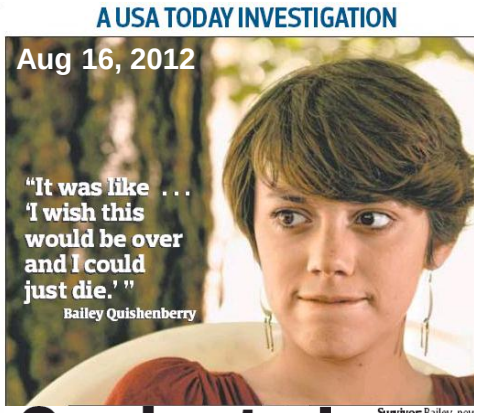

Vaccine Research

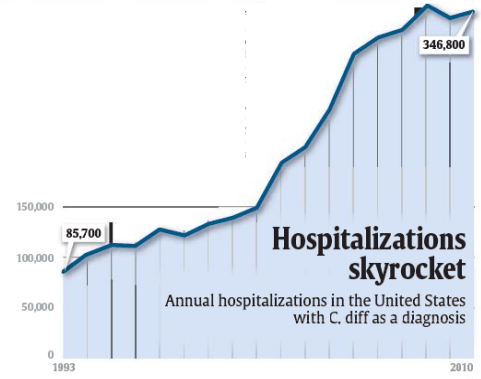
# Backups



Vaccine Research

# The Faces of C. diff Victims




Charlee Ratliff, 8 months old when she died in May 2010. She was diagnosed with C. diff while recovering from surgery to repair a hole in her heart.

## Infections killing thousands at U.S. hospitals

U.S. slow to adopt plans to curb bacteria

**A USA TODAY investigation shows that C. diff is far more prevalent than federal reports suggest. The bacteria is linked in hospital records to more than 30,000 deaths a year in the United States— about twice federal estimates and rivaling the 32,000 killed in traffic accidents. It strikes about a half-million Americans a year. A new C. diff reporting rule for U.S. hospitals isn't scheduled to take effect until 2013.**

Carol Dye of Manchester lost her father, James Louis Dye (age 78), in November of 2007 to multiple C. difficile infections. "C. diff was absolutely horrible," Carol Dye, 58, said. "He was in a lot of pain. He was alive, but he wasn't living. He had no life. It still makes me cry."

Richard Gerald Croke Jr., died at 48 in August 2008. He was diagnosed with C. diff after successful treatments for esophageal cancer.

**Other countries are racing ahead of the U.S. in battling the bacteria. In England, the government requires hospitals to report all C. diff cases, underpinning a regulatory campaign that has slashed infections more than 50% since 2008.**

# Defining Protective 50% Neutralization Titer Range Target from Medarex Data

Protective Range of Medarex's mAb in Humans[1]

***10 – 100 µg/mL (10,000 – 100,000 ng/mL) of each mAb in serum***

↓

**Pfizer and Medarex neut assays report $IC_{50}$ values using identical assay conditions***
**For Medarex's mAb, the reported $IC_{50}$ are [2] as follows:**
**TcdA mAb: 15 ng/mL**
**TcdB mAb: 45 ng/mL**

↓

**Convert Medarex protective mAb concentration to 50% neut titers in toxin neut assay as follows:**

**50% neut titer = mAb concentration ÷ mAb $IC_{50}$**

## Antitoxin A

**Lower titer : 10,000 ng/mL ÷ 15 ng/mL = 667**
**Upper titer : 100,000 ng/mL ÷ 15 ng/mL = 6,667**
***Thus, 50% neut titer protective range: 667 to 6,667***

## Antitoxin B

**Lower titer : 10,000 ng/mL ÷ 45 ng/mL = 222**
**Upper titer : 100,000 ng/mL ÷ 45 ng/mL = 2,222**
***Thus, 50% neut titer protective range: 222 to 2,222***

*** The neut assays at both labs were performed on IMR90 cells using *C. difficile* toxins at 4 - 8 ng/mL for A and 0.04 – 0.08 ng/mL for B.**


[1] Lowy et al. 2010. NEJM
[2] Babcock et al. 2006 Infect. Immun.






Vaccine Research