Antibody-based methodology to assess the stability of whole cell pertussis vaccines (PSPT 2022)



Introduction

Albany, New York, USA

Microbial Pathogenesis Immunology and Mucosal Immunology Vaccinology

Salmonella, cholera, borreliosis, toxins, SARS-CoV-2

Dr. Jennifer Doering Dr. Yetunde Adewunmi

Investigators

Motivation

How do you assess the potency and stability of whole cell Pertussis (wP) within context of pentavalent and hexavalent vaccines?



In collaboration with Drs. David Volkin and Sangeeta Joshi (University of Kansas) with support from BMGF

Kendrick Assay for wP Potency and Stability



The Kendrick Assay is designed to assess potency, but we have been unable to identify its applications to stability studies. The Kendrick Assay is also associated with other shortcomings.

Alternatives to Kendrick Test

Biologicals (1994) 22, 233-242

Development of Pertussis Serological Potency Test

Serological assessment of antibody response induced by whole cell vaccine as an alternative to mouse protection in an intracerebral challenge model

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Whole-cell pertussis vaccine potency assays: the Kendrick test and alternative assays

Expert Rev. Vaccines 13(10), 1175-1182 (2014)

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Adapting PSPT



Multivalent Nature of DTwP



"Enhanced" PSPT at Wadsworth Center



DTP Dose Response (Day 30)



We chose **1.2 OU** for all vaccination studies going forward.

Antigen-specific Ab Titers (Day 30)



Intranasal challenge with *B. pertussis* (Day 30)



DTP vaccination results in ~4 log reduction in *B. pertussis* 18323 CFUs (day 4)

Summary of "Enhanced" PSPT



The "enhanced" PSPT (for research purposes only) provides serological dose response with whole cell ELISA, titers to different DTP antigens, and can be combined with intranasal *B. pertussis* (18323) challenge to assess relative degrees of clearance.

Further Simplifying wP Potency Assays



<u>Pertussis Competition ELISA (PetCoE)</u>



Concept Underlying PetCoE



Vaccine potency reflects the biochemical integrity of the antigen, adjuvant and its given formulation. With time, potency declines due to multiple factors, including loss of antigen integrity.

Decline in Vaccine Integrity Yields Low Quality Abs



Proof of Concept (Ricin toxin vaccine; RiVax™)



Doering J et al.. Estimating Vaccine Potency Using Antibody-Based Competition Assays. Methods Mol Biol. 2022;2410:693-705. doi: 10.1007/978-1-0716-1884-4_37. PMID: 34914076.

Concept: Use Antibodies to Probe Antigen Integrity

Monoclonal antibodies (MAbs) or a reference serum panel could be used to interrogate the conformational integrity of D, T and wP within DTP.







Probing RiVax Integrity by Competition ELISA with PB10



<u>RiVax-Alhydrogel</u> in liquid was incubated at 25°C for indicated time points then subjected to competition ELISA with PB10. Decline in "integrity" results in reduced potency, as reflected in lethal dose challenge.

Applications of Competitive ELISA to DTP

PetCoE Enhanced PSPT Juik 4oC % **Competiion** DTP DTP 100oC Threshold DT 4°C 100°C Dilution (DTP) ТΤ FHA PRN AcT VIIIIIII ΡΤχ vi)Dun; CFU (lung)

Competition ELISA with DTP Antisera (Mouse)



DTP at 4°C or 100°C was used as competitor with DTP antiserum for binding to ELISA plates coated with DTP. Boiled DTP was less effective competitor, suggesting a loss in integrity.

Thermal stress (accelerated decay) of DTP



B. pertussis challenge of DTP (4°C, 100°C) Vaccinated Mice



Mice that were vaccinated with boiled DTP were less able to clear B. pertussis from lungs, as compared to DTP control mice.

The reduction in CFUs correlates with loss of vaccine integrity revealed by PetCo.



Serological analysis reveals different antibody profiles between DTP 4oC and 100°C. Sharp decline in TT and PRN titers, but little change in FHA, PT, ACT or LPS.

Differential Stability of DTwP



The differential stability of different DTP antigens raises questions about weak link in the chain regarding formulation optimization.

Conclusions (Preliminary)



PetCoE



Interrogating DTP upstream may provide insight into vaccine potency and stability.

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