Serology-based and in vitro assays for wP potency testing

Nicholas J. Mantis, Ph.D.
E-mail: nicholas.mantis@health.ny.gov

NEW YORK STATE | Department of Health | Wadsworth Center

Investigative Team

University of Kansas
Dr. Prashant Kumar
Dr. David Volkin
Dr. Sangeeta Joshi

Wadsworth Center
Dr. Yetunde Adewunmi
Dr. Jennifer Doering
Dr. Nicholas Mantis

Antigen Discovery (ADI)
Dr. Joe Campo
Dr. Xiaowu Liang
Dr. Angela Yee

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The Kendrick Assay: Overview

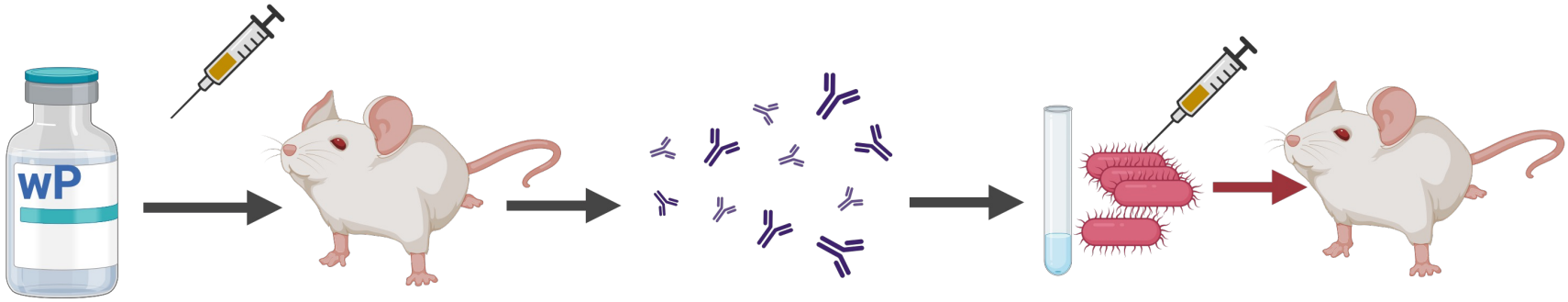
The Kendrick assay or Mouse potency Test (MPT) is the gold standard for wP potency determination and batch release.

However, the test is technically challenging (e.g., time consuming, labor intensive, variable), and of questionable relevance to vaccine-induced immunity in humans. Moreover, the test is incongruent with current animal welfare guidelines due to death as an endpoint.

Ongoing efforts aimed at replacing the Kendrick assay are focused on serology-based assays (PSPT) and *in vitro* (animal free) tests.

The Kendrick Assay: Methodology

Kendrick Test-Pass



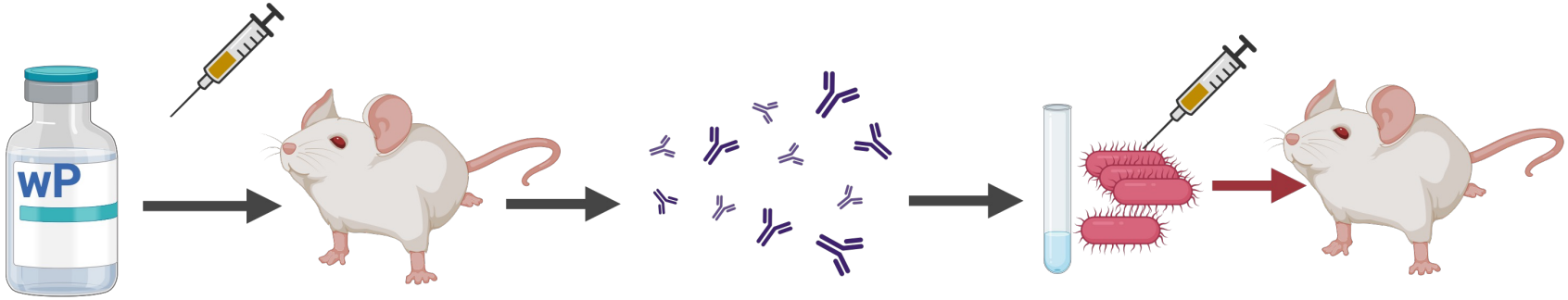
A. Vaccinate **B. Immune response** **C. Challenge** **D. Survival**

The assay involves a single vaccination (intraperitoneal) followed two weeks later by an intracerebral challenge with virulent *B. pertussis*. Survival (live vs. dead) is used for potency determinations.

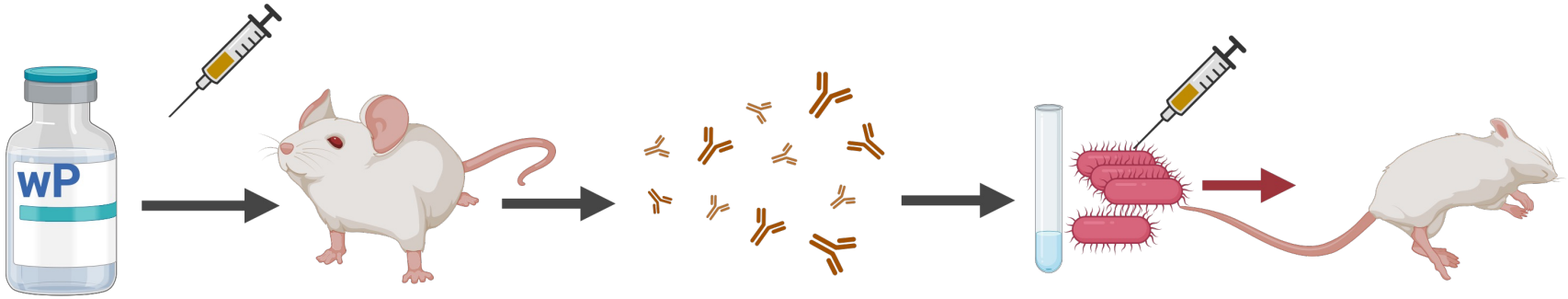
***B. pertussis*-specific antibodies are implicated as the primary immune component associated with protection in the Kendrick assay.**

The Kendrick Assay: Pass vs Fail

Kendrick Test-Pass



Kendrick Test-Fail

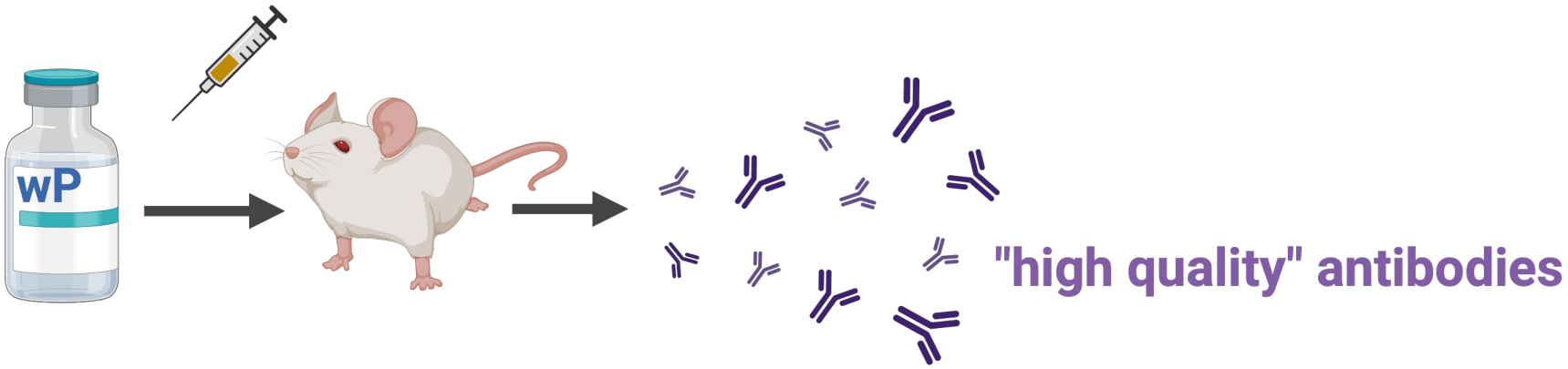


Mice that succumb to *B. pertussis* challenge have wP-specific antibodies, so why did they fail in the Kendrick assay? Differences in antibody quantity? Or quality?

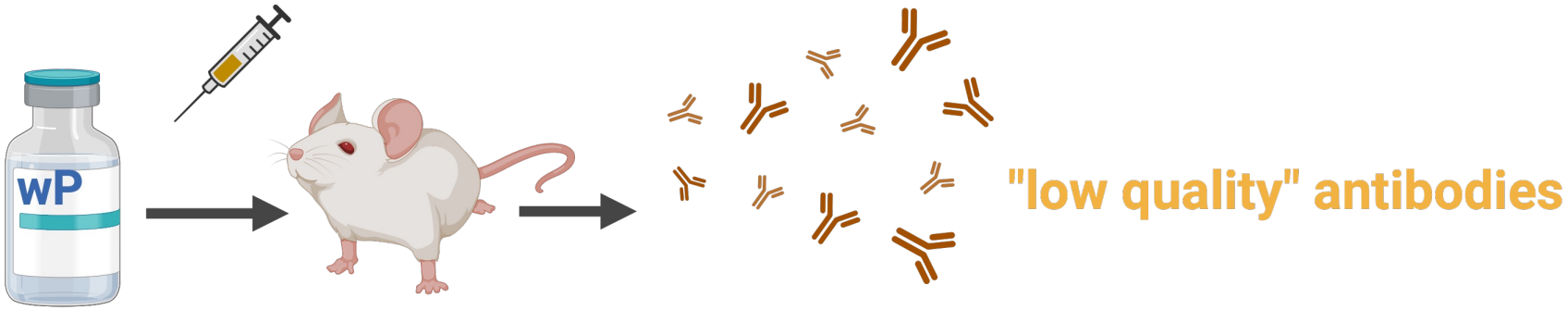
Immunology underlying the Kendrick

We postulate that differences in the quality of the antibodies elicited by potent versus subpotent vaccines determines the outcome of the Kendrick assay.

Kendrick Test-Pass



Kendrick Test-Fail



Research Objectives

Efforts to replace the Kendrick assay will depend on our ability to distinguish between high- and low-quality antibody responses elicited by wP.

1. **Determine what constitutes “high quality” antibodies elicited by wP**
2. **Determine the difference between “high quality” and “low quality” antibodies and relationship between potent and subpotent wP**
3. **Use information from Aim 2 to develop a serology-based and/or in vitro assays to discriminate between potent and subpotent wP**
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5. **Implement assay within vaccine manufacturing space**

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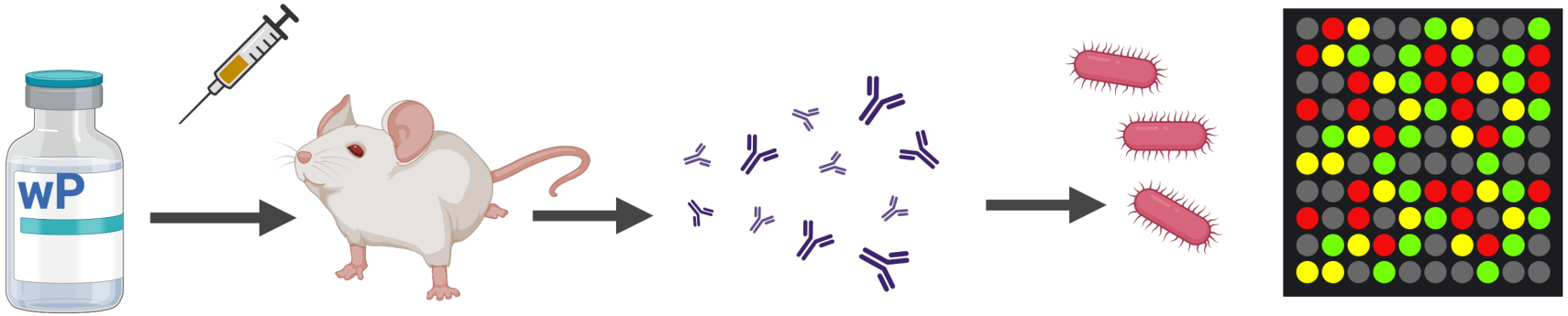
Identify *B. pertussis* antigens recognized wP immune serum

Kendrick Test-Pass



We employed a limited *B. pertussis* Tahoma I proteome array to identify antigens recognized by immune sera from DTwP vaccinated mice. In other words, can we establish an immune profile associated with ”high quality” antibody responses.

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Identification of a wP immune profile in mice

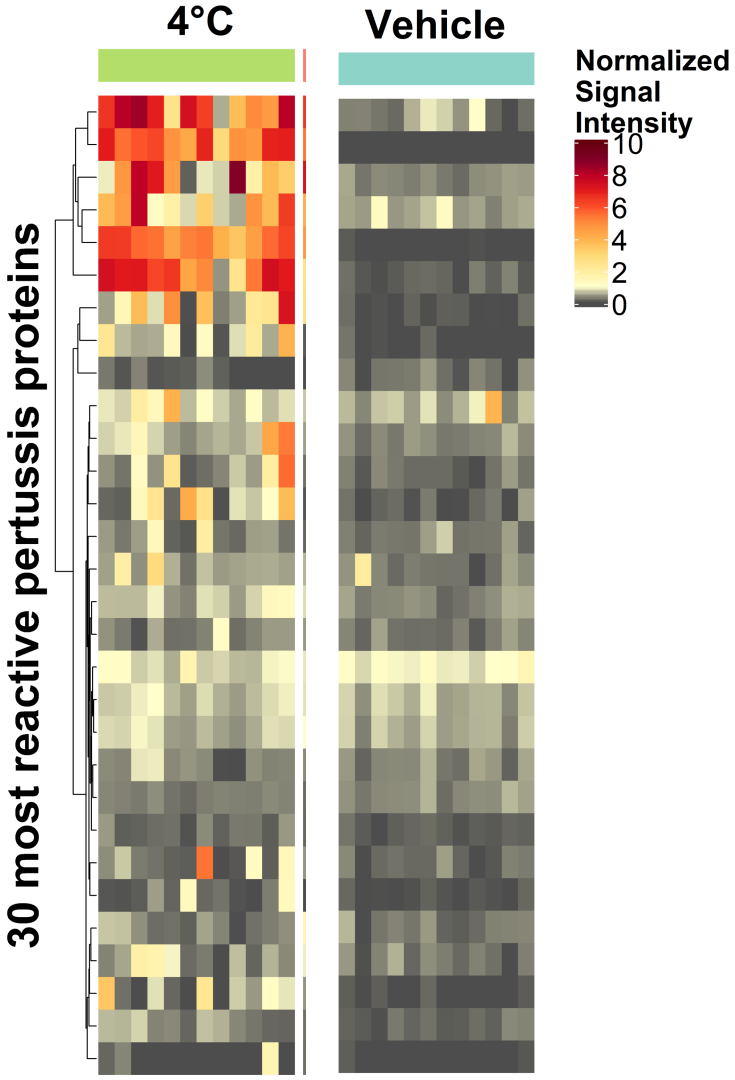

Table 1. *B. pertussis* antigens recognized by mouse wP immune sera

Bp id[a,b]	UniProt[c]	Description	Loc[d]
3342	Q7VU04	Peptidoglycan-associated lipoprotein (Pal)	O
2992	Q7VUT2	Putative lipoprotein (Pcp)	O
0943	Q7VZG6	Outer membrane protein A (OmpA)	O
0840	Q04064	Outer membrane porin protein precursor (Omp)	O
3755	Q7VT02	Putative Outer membrane protein (OmpW)	O
1721	Q7VXN0	Putative peptidase	O
2667	Q7VVJ2	Adhesin (FhaS)	na
2235	Q79GR8	Putative type III secretion protein (BscC)	O
1767	Q79GU5	Autotransporter phg	O
1112	Q7VZ27	Bordetella intermediate protein A (BipA)	O
3655	Q7VT95	Penicillin-binding protein 1A	I
3366	Q7VTY1	Putative phage tail protein	na
1201	Q79GX8	Tracheal colonization factor precursor	O
2497	Q7VVY4	Zinc protease	na
2077	Q7W9J8	Efflux system outer membrane component	O
1251	Q7VYQ9	Putative toxin	S
3494	Q45340	Serum resistance protein (BrkA)	S
1382	Q7VYG0	Flagellar hook-associated protein 1	S
1378	Q7VYG4	Flagellar basal-body rod protein FlgG	I,O
2851	Q7VV52	Outer membrane porin protein precursor	O
0760	P0DKX7	Bifunctional hemolysin-adenylate cyclase precursor	S
1884	P35077	Filamentous hemagglutinin transporter protein FhaC	O
0184	Q7W0F3	Putative exported protein	I
1879	P12255	Filamentous hemagglutinin	O
2315	Q79GN7	Autotransporter Vag8	O
1401	Q7VYE7	Flagellar assembly protein FliH	C
2566	Q7VVS1	Exported endonulease	na
2434	Q7VW38	Probable periplasmic serine endoprotease DegP-like	P
0456	Q7VSG8	Heme receptor	O
1189	Q7VYW4	Lipoprotein	na

[a], **bold** indicates proteins identified by Gregg et al (2023) as being essential for survival in vitro and in vivo in a baboon model for *B. pertussis* colonization identified by convalescent serum or NPW [27]; [b], underline indicates *B. pertussis* antigens identified by Gregg et al (2023) detected in convalescent baboon serum or nasal pharyngeal washes (NPW) [27]; [c], UniProt accession [42]; [d], abbreviations indicate known or proposed cellular location of protein: O, outer membrane; I, inner membrane; P, periplasm; C, cytoplasm; S, secreted; na, not available

We identified a total of 34 reactive antigens with ≥10% seroprevalence with the top 8 antigens being known membrane associated proteins.

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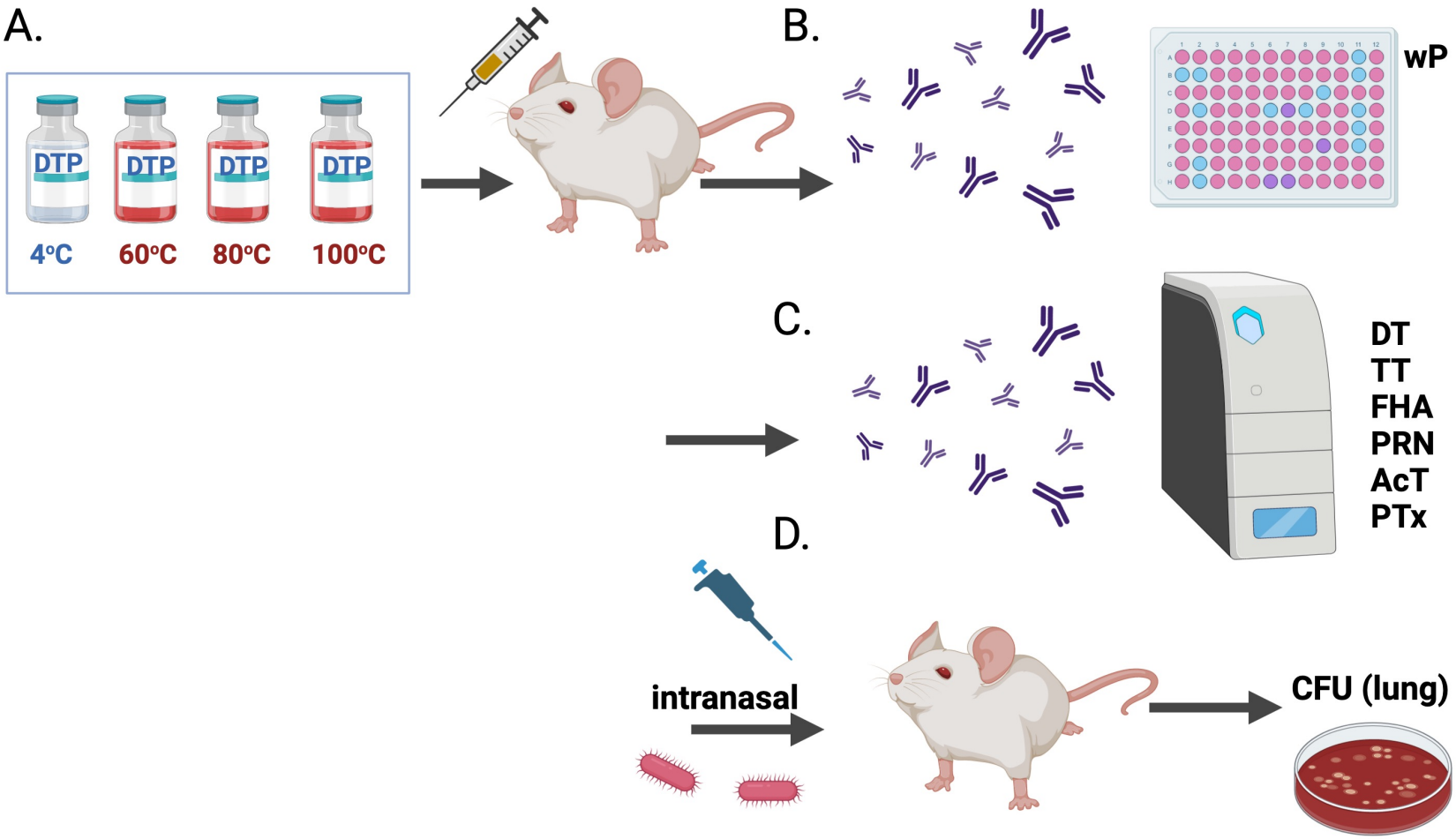
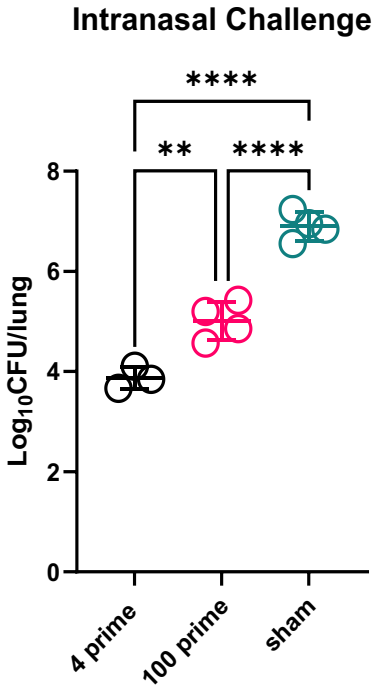
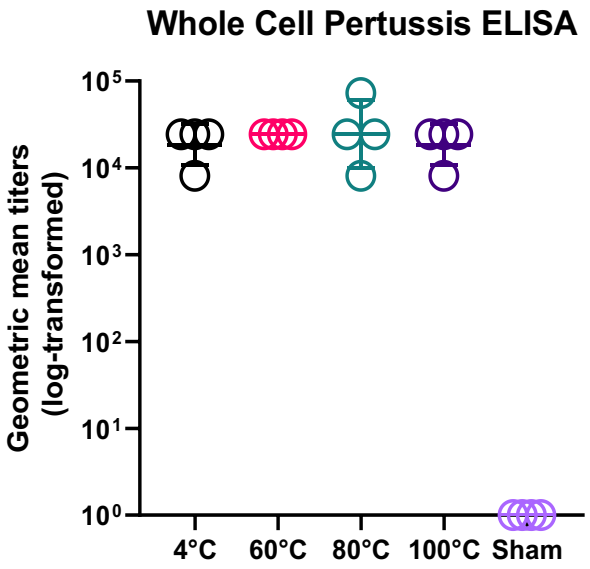
Characterization of “altered” DTwP vaccine preparations

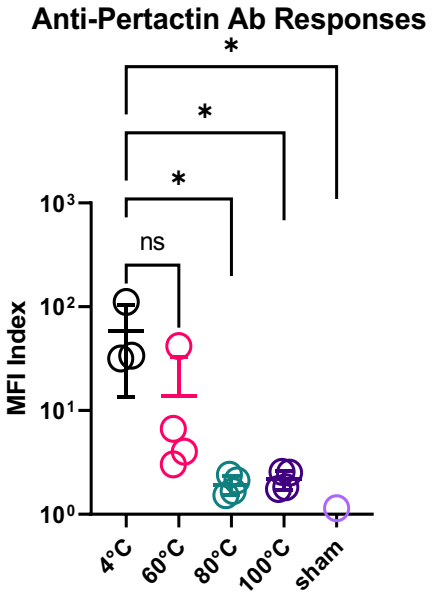
We subject DTwP vaccine preparations to accelerated (thermal) decay and evaluated potency in mouse model of *B. pertussis* intranasal challenge. The same samples were assayed on the proteome array.



Characterization of “altered” DTwP vaccine preparations

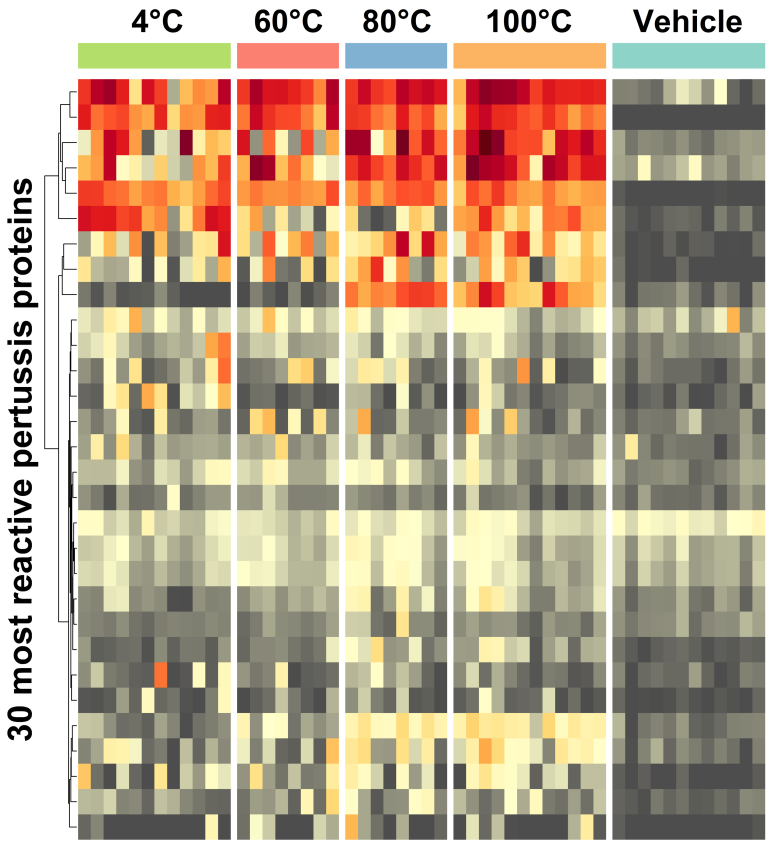


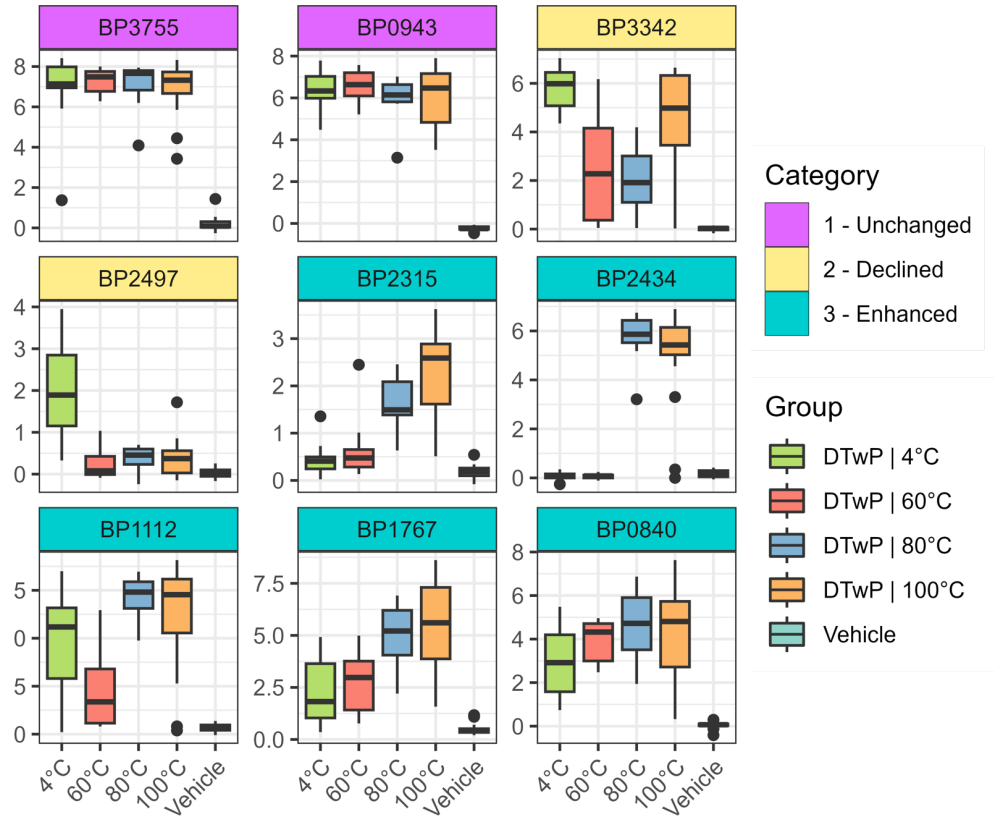




Altered wP (100°C) was less effective at promoting bacterial colonization in a mouse model of intranasal challenge, even though total *B. pertussis* IgG levels are unaffected. Antibody titers against pertactin (PRN) declined in a temperature-dependent manner. Thus, antigen-specific rather than whole cell specific antibodies may be better indicators of potency.

Immune profiles associated with potent and subpotent wP





We vaccinated mice with altered DTwP preparations (heat stress), collected sera 30 days later and compared proteome profiles. The majority of the responses were unchanged. However, reactivity with several antigens (BP3342, BP2497). Hence, there are distinct differences between the potent and subpotent vaccine preparations.

Proteome analysis is consistent with differences in antibody “quality” elicited by altered wP vaccines

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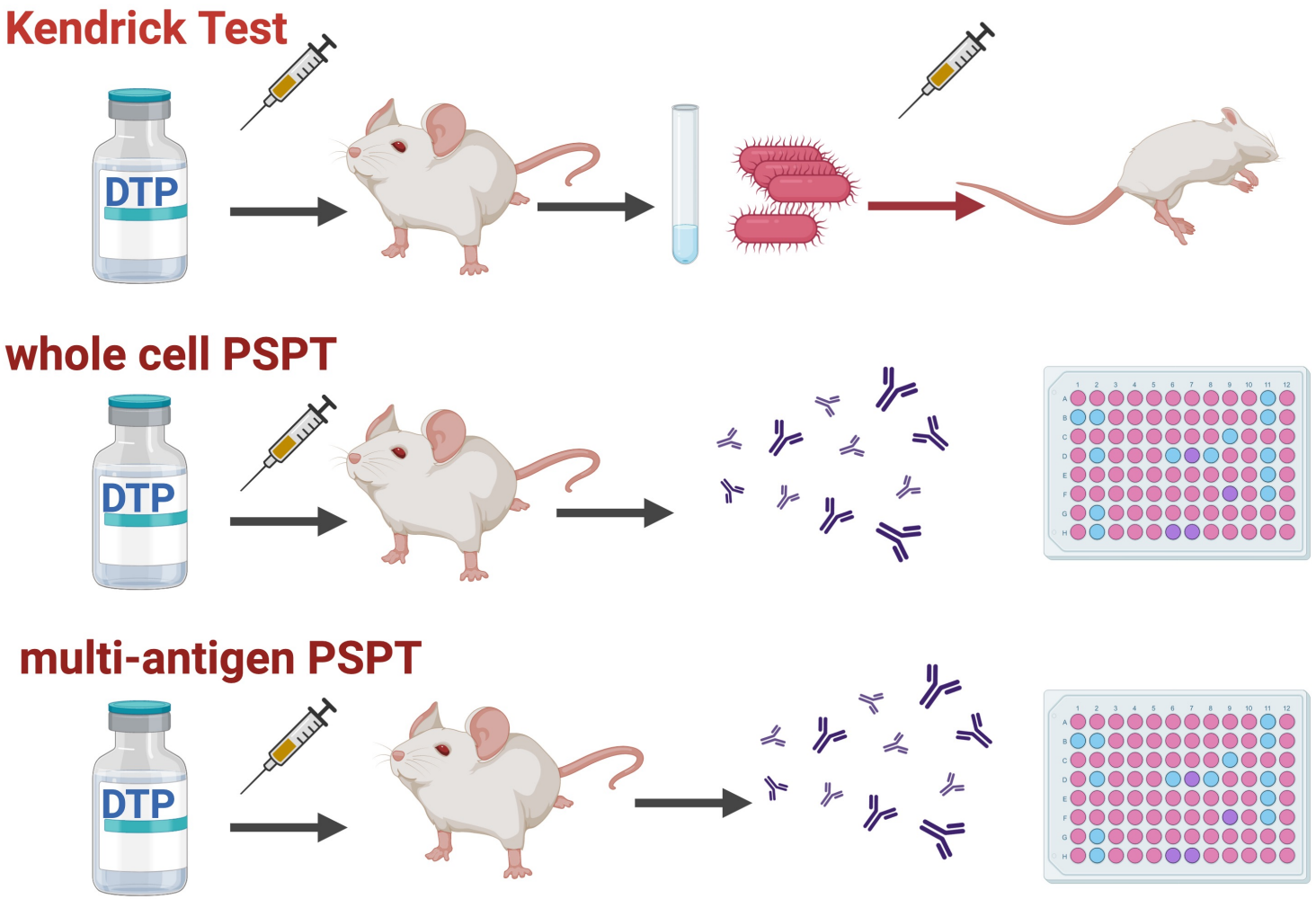
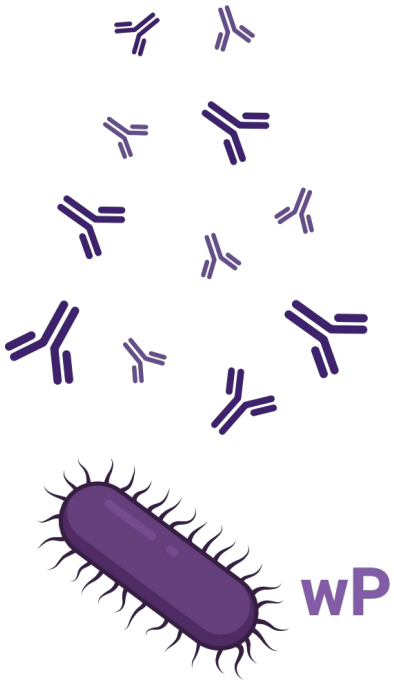
Development of an antigen-specific serology assay



We envision a multi-antigen ELISA assay with *B. pertussis* antigens selected from the proteome array described in Aim 2. In other words, devise an assay that measures antibodies associated with protection rather than total antibodies.

Development of an *in vitro* release assay

"high quality" Abs
or MAbs

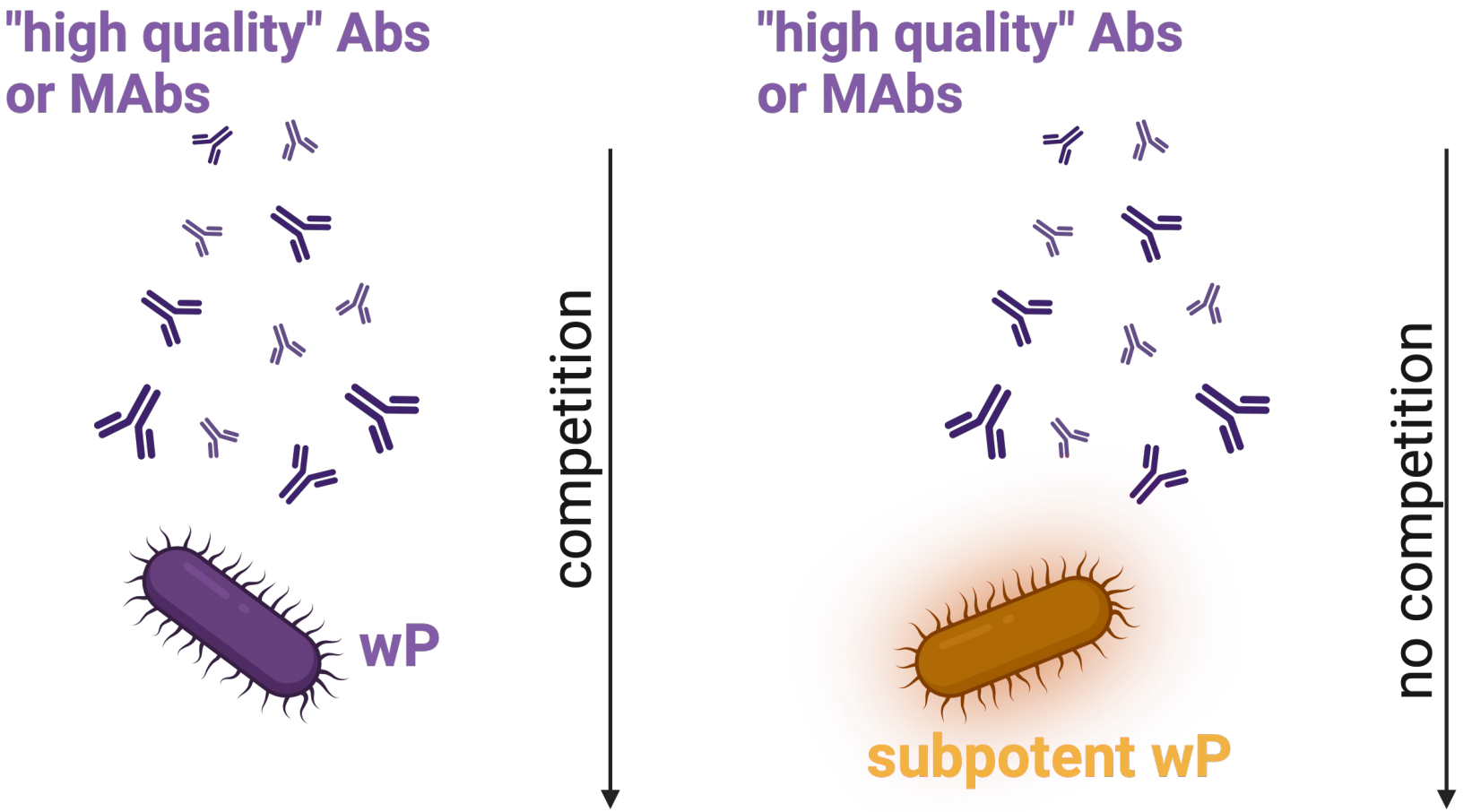


wP

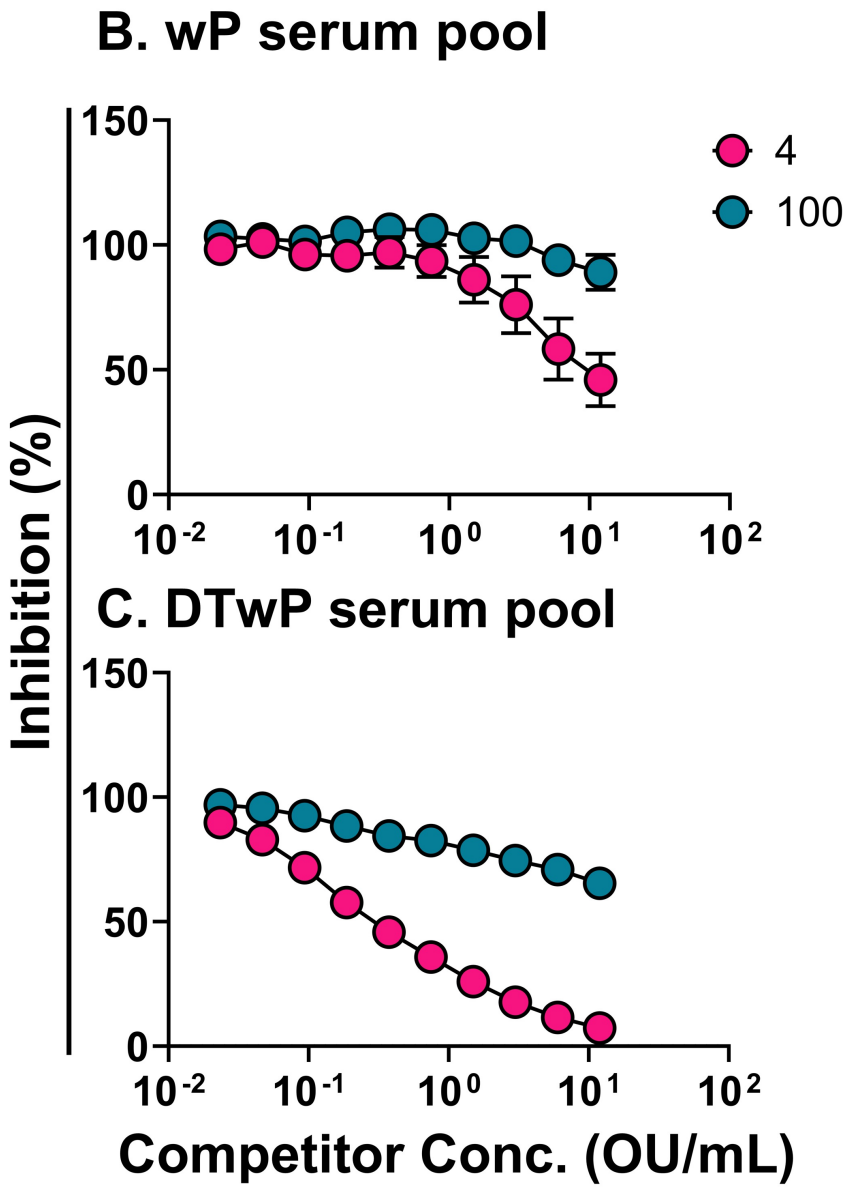
competition

B.pertussis multi-antigen
ELISA

Development of an in vitro batch release competition assat



Development of Pertussis Competition ELISA (PetCoE)



Research Objectives

1. Identify the targets of “high quality” antibodies elicited by potent wP vaccination

2. Determine the difference between “high quality” and “low quality” antibodies elicited by potent and subpotent wP vaccination

3. Use information from Aims 1 and 2 to develop a serology-based or animal-free assay to discriminate between potent and subpotent wP

4. **Validate assay developed in Aim 3 against Kendrick assay**

5. **Implement assay within vaccine manufacturing space**

Conclusions

1. **We have identified a preliminary wP "immune profile" in mice that includes known outer membrane proteins and virulence factors.**

2. **Antibody responses elicited by control and altered wP vaccine preparations are quantitatively different.**

3. **Our preliminary studies suggest that an antigen-specific, serology-based assay may discriminate between potent and subpotent wP.**

4. **Additional work alludes to the possibility of an animal-free assay for wP potency determination.**

Reference/Citation

Antibody Profiles Elicited by Potent and Subpotent Whole Cell Pertussis Vaccines in Mice

Yetunde Adewunmi, Jennifer Doering, Prashant Kumar, Jozelyn V. Pablo, Andy A. Teng, Vu Huynh, Kathryn Secrist, David B. Volkin, Sangeeta B. Joshi, Joseph J. Campo, Nicholas J. Mantis

Microbiology Spectrum, *in press*

bioRxiv 2024.10.22.619719; doi: https://doi.org/10.1101/2024.10.22.619719

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