sanofi

Replacement of *in vivo* Immunogenicity Assay with *in vitro* Antigenicity ELISA for Pertactin Antigen in Acellular Pertussis Combination Vaccines

WCBP 2025

Belma Ljutic, Ph.D.

Quality Control, Sanofi, Toronto

Content

- Sanofi Toronto Vaccine Overview
- History of Pertussis Vaccines
- History of Animal Potency Tests
- Introduction of *in vitro* Potency Assay
- Assay Development in R&D
- Assay Feasibility and Validation in QC
- Product Specification Setting
- Conclusion

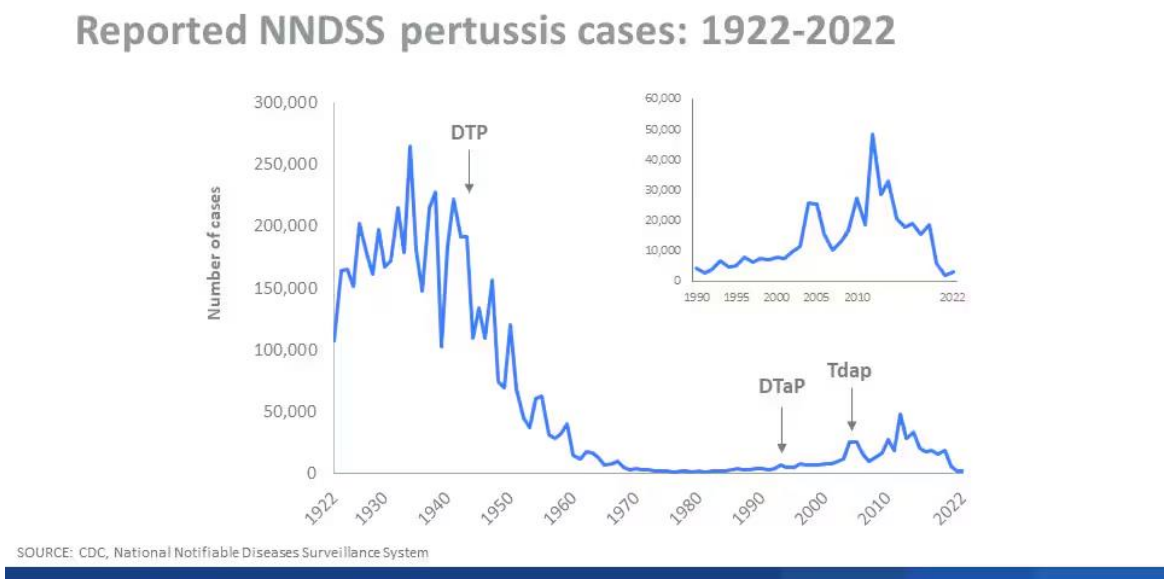
sanofi

Sanofi Vaccines Toronto: Our Products

5-Component Pertussis Pediatric Combos	Hexavalent (DTaP-HB-IPV-Hib) Vaxelis® Pentavalent (DTaP-IPV/Hib) Pentacel® Quadrivalent (DTaP-IPV) Quadracel® Trivalent (DTaP) Triacel® /Daptacel®
5-Component Pertussis Adult Boosters	Quadrivalent (Tdap-IPV) Repevax®/Adacel®-Polio Trivalent (Tdap) Adacel®
Other Combos	Td adsorbed

sanofi

History of Pertussis Vaccines



- Early 20th Century: Pertussis identified as a distinct disease.
- 1926: First whole-cell pertussis vaccine developed.
- 1940s: Combined DTP (Diphtheria, Tetanus, Pertussis) vaccine introduced, significantly reducing disease incidence[1]
- 1990s: Acellular pertussis vaccines (DTaP) developed, causing fewer side effects and becoming the standard[2]
- 2006: Tdap booster recommended for adolescents and adults[2]
- 2011: Vaccination during pregnancy recommended to protect newborns[2]

[1]https://www.cdc.gov/pertussis/php/surveillance/index.html
[2]https://www.cdc.gov/pertussis/php/surveillance/pertussis-cases-by-year.html

sanofi

History of Animal Potency Tests for Pertussis Based Vaccines

- **1947**: The mouse intracerebral challenge protection test developed by Kendrick *et al.* to measure vaccine potency[1]
- **1960s-2000s**: Continued use of animal models, primarily mice, to test the potency and safety of pertussis vaccines[1]
- **Modern Approaches**: Efforts to refine and reduce animal testing, focusing on humane endpoints and alternative methods[2]
- ***In vitro* Assay Approaches:** PRN antigenicity ELISA the first to be approved by Health Authorities for testing of pertussis-based vaccines[3]

"From this…"

"…to this?"

sanofi

[1]Cherry, J.D. The History of Pertussis (Whooping Cough); 1906–2015: Facts, Myths, and Misconceptions. *Curr Epidemiol Rep* 2, 120–130 (2015). https://doi.org/10.1007/s40471-015-0041-9
[2]Hendriksen, C.F.M., Steen, B. Refinement of Vaccine Potency Testing with the Use of Humane Endpoints
ILAR Journal, Volume 41, Issue 2, (2000) 105–113. https://doi.org/10.1093/ilar.41.2.105
[3]Szeto, J., et al. "Development of an In Vitro Test Method to Replace an Animal-Based Potency Test for Pertactin Antigen in Multivalent Vaccines." *Vaccines*, **11**: 265. (2023) https://doi.org/10.3390/vaccines11020275

Selection of Relevant Monoclonal Antibodies for Use in *in vitro* Potency Assays

The relevance of anti-PRN monoclonal antibody (mAb) candidates selected for developing an *in vitro* potency assay was evaluated by assessing their:

- Binding affinity to PRN
- Specificity
- Stability indication
- Recognition of conformational epitopes
- Functionality

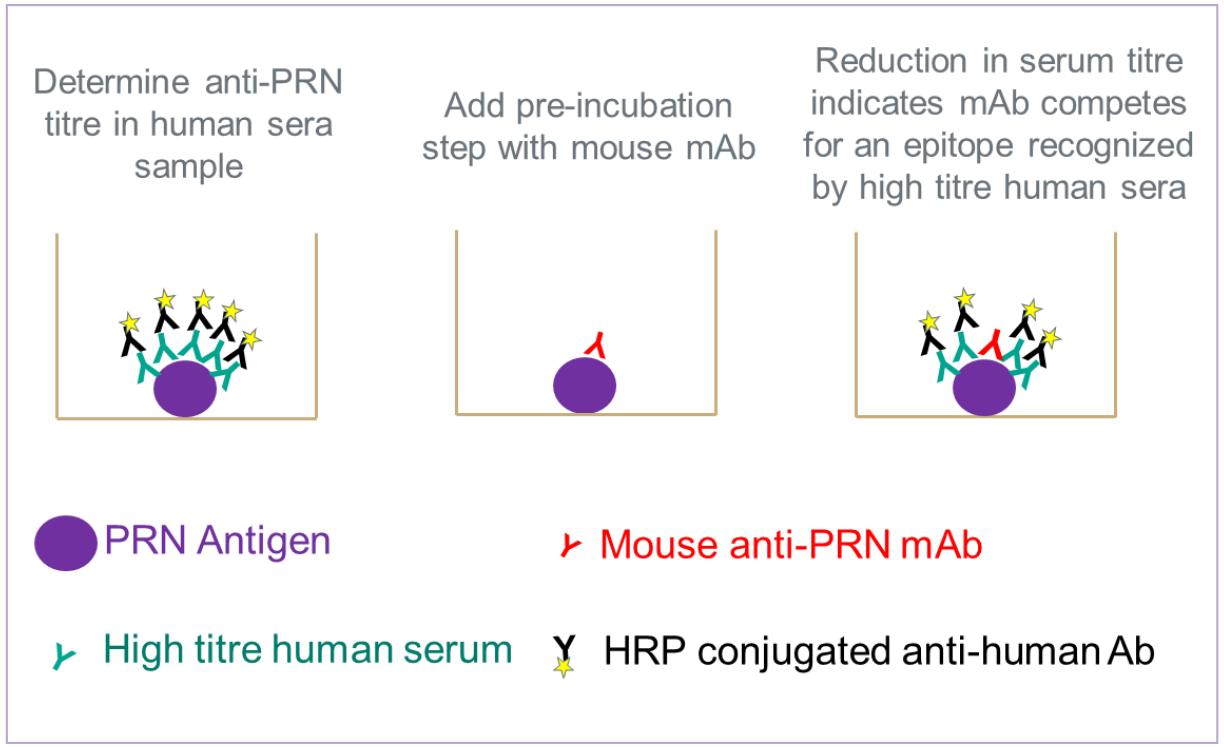
Anti-PRN mAb Clone	Isotype	Affinity (K_D; nM)	Epitope Identification by HDX (Amino Acid Position on PRN) [1]	Epitope Type	mAbs Binding to *B. pertussis* Surface [2]	Relevance to Human Response (CEST)	Relevance to Human Response (SIA)	Inhibition of PRN-Coated Bead Binding to Host Cells [3]
3–1	IgG1	0.143	Not tested	Not tested	<25%	NO	NO	0.7
3–3	IgG1	12.2	588–591	Linear/Continuous	<25%	NO	NO	0.1
3–4	IgG1	0.23	420–436, 455–464, 477–492, and 505–517	Conformational	>25%	YES	YES	0.7
3–5	IgG1	0.51	234–244	Likely conformational (formed by linear sequence)	>25%	YES	YES	0.4
3–16	IgG1	6.68	36–46 and 56–67	Conformational	>25%	YES	YES	0.1
3–21	IgG1	16.5	35–78 and109–116	Conformational	>25%	YES	YES	0.4

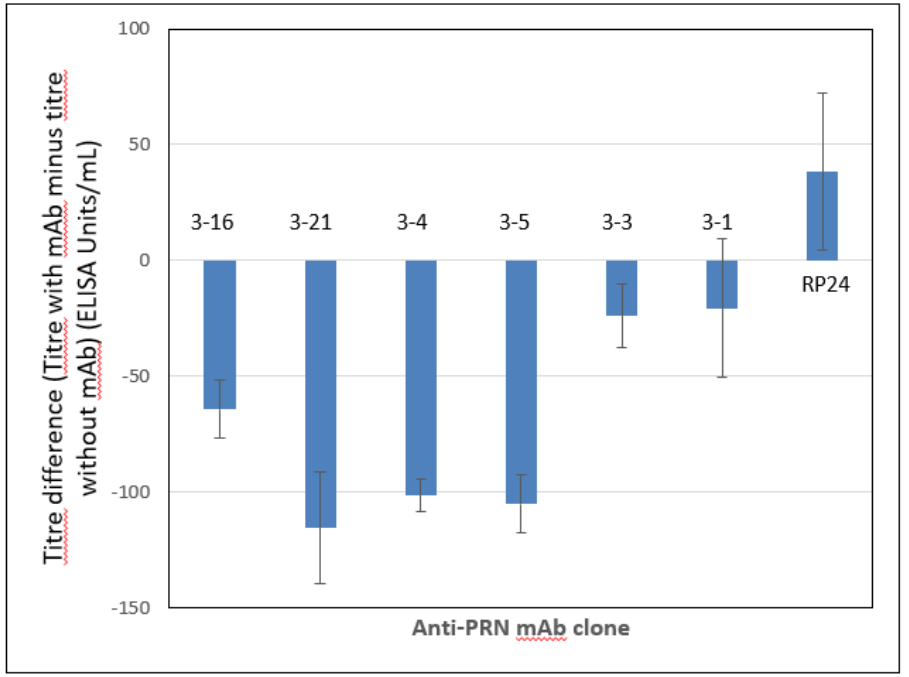
[1] Data from Zhu *et al*. Biotech. J. 2021 17, e2100358. [2] Data from He *et al*. J.Pharm Sci. 2020, 109: 1002-1007, showing the percentage of positively stained bacteria as determined by flow cytometry. [3] Data from same reference showing relative bead-binding prevention capacity.

Table from Szeto et al. 2023. Vaccines. 11(2):275

sanofi

Example of Functional Assay to Assess anti-PRN mAb Relevance: Human Serum Inhibition Assay

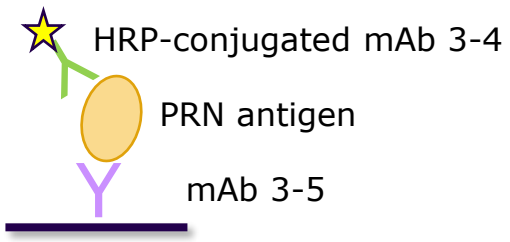


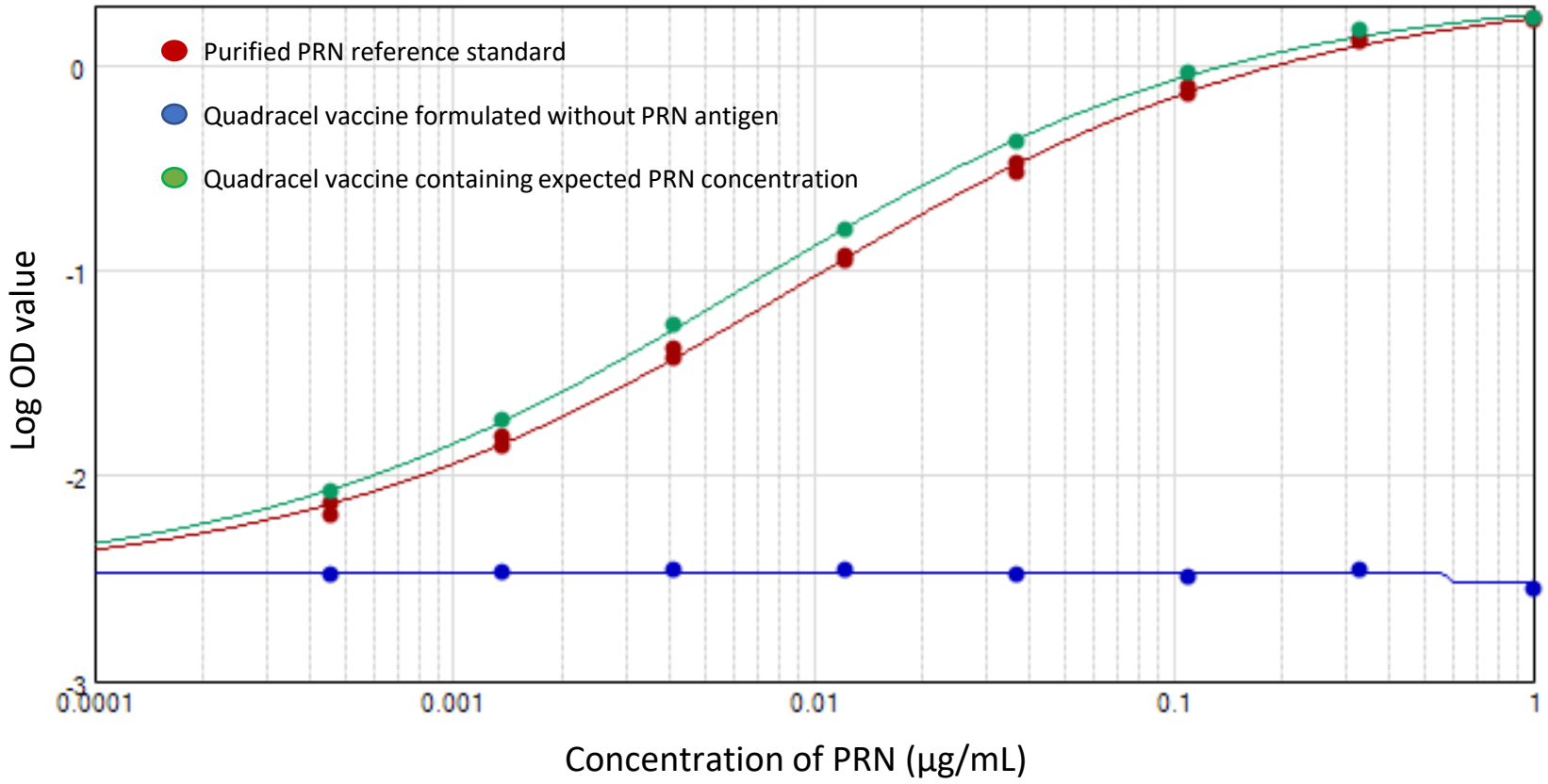


sanofi

Specificity of PRN *in vitro* Antigenicity ELISA

Specificity of ELISA confirmed by testing mock DTaP-IPV vaccine formulated without PRN



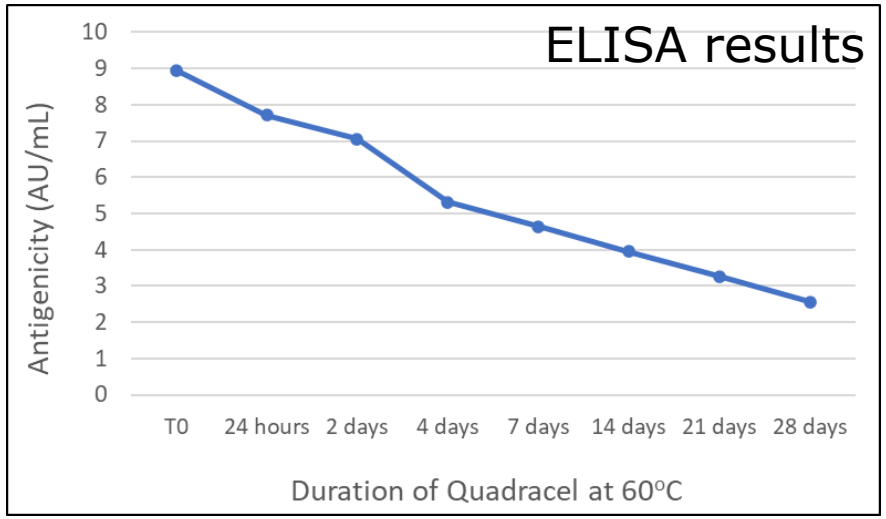


sanofi

PRN Mouse Immunogenicity Assay versus PRN Antigenicity ELISA

The *in vitro* assay is superior at detecting changes to PRN in Heat-Treated Quadracel samples

	PRN Mouse Immunogenicity Study 1		PRN Mouse Immunogenicity Study 2		PRN Antigenicity ELISA Results
Treatment	GMU fold [1]	# of Mouse Responders Meeting Criteria [2]	GMU Fold [1]	# of Mouse Responders Meeting Criteria [2]	Antigenicity (AU/mL)
Time zero	2.5	Yes	5.1	Yes	8.95
24 h, 60 °C	5.5	Yes	3.4	Yes	7.72
2 days, 60 °C	5.4	Yes	3.5	Yes	7.05
4 days, 60 °C	2.6	Yes	4.3	Yes	5.32
7 days, 60 °C	6.9	Yes	4.6	Yes	4.64
14 days, 60 °C	4.5	Yes	8.2	Yes	3.96
21 days, 60 °C	4.8	Yes	3.2	Yes	3.26
28 days, 60 °C	5.1	Yes	2.6	Yes	2.56



1. Geometric mean unitage (antibody titer) is shown as a fold increase over the minimum passing GMU acceptance criteria. Any value equal to, or greater than 1, indicates the GMU has met the acceptance criteria.
2 Indicates whether the number of responding mice met/exceeded the minimum acceptance criteria.

sanofi

Analytical Transfer to Quality Control Following Method Development

- **Feasibility study was performed in QC Immunochemistry lab** by trained analysts to assess assay suitability and generate data to determine assay validity control for routine use
  - A **four-parameter logistic (4-PL) curve** fit is applied to the reference standard, test sample and positive control
  - An **equivalence test** is then used to assess parallelism between the reference standard and test sample/positive control
  - The **Parallel Line Analysis module** (PLA) determines the antigenicity result of each test sample and positive control
  - **Assay validity limits** are applied to blank OD, R^2 and slope of Reference standard, antigenicity of positive control, B ratio and D ratio for parallelism assessment, and CV% for replicates.

sanofi

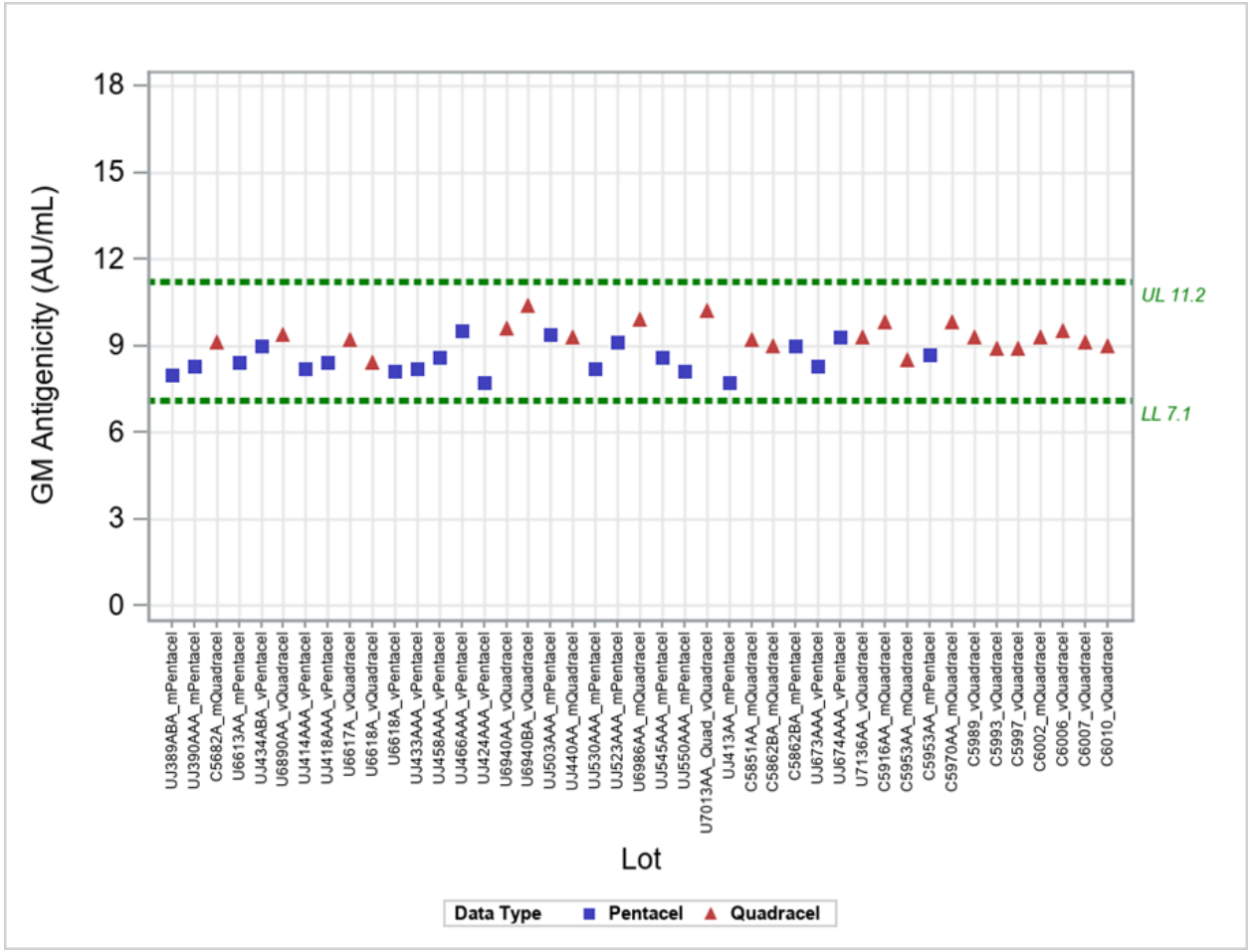
Validation of PRN Antigenicity ELISA

Test Method Validation Parameter	Acceptable limit	Results
Accuracy	The average recovery of PRN antigenicity for each concentration level of mock Quadracel samples must be between 80-120%	Average recovery = **90%**
Precision **(Intermediate Precision and Repeatability)**	The %CV for repeatability and intermediate precision must be less than or equal to 15% (%CV≤15%) for each of Mock vQuadracel samples	Repeatability: %CV: **5-7%** Intermediate precision: %CV= **9-11%**
Specificity	Mock Quadracel sample which lacks PRN antigen must not generate a valid result (i.e., PRN antigenicity of 0 AU/mL). Mock Quadracel sample must generate a valid result for PRN antigenicity and must be between 80-120% of the expected value.	PRN was not detected in mock vQuadracel sample without PRN PRN was detected in mock vQuadracel sample with **90%** recovery
Linearity	The coefficient of determination (R^2) must be greater than or equal to 0.95 for linear regression analyses	The R^2 values generated from the linear regressions were **0.98.** The slope estimate value of **1.0** was obtained for linear regressions.
Range	The range of the assay will be determined with acceptable accuracy, precision and linearity for each impacted product.	The range of the assay is validated for samples containing 2 µg/mL to 10 µg/mL of PRN, corresponding to observed PRN antigenicity of 2.56 AU/mL to 13.67 AU/mL.

sanofi

Specification of PRN Antigenicity for Quadracel and Pentacel[1]

- **Consistency Approach** was taken to set product specification
- **43** production lots (22 Quadracel and 21 Pentacel lots) were tested to set product specification
- **95% confidence, 99% coverage tolerance limits** were calculated as product acceptance criteria (7.1 - 11.2 AU/mL)

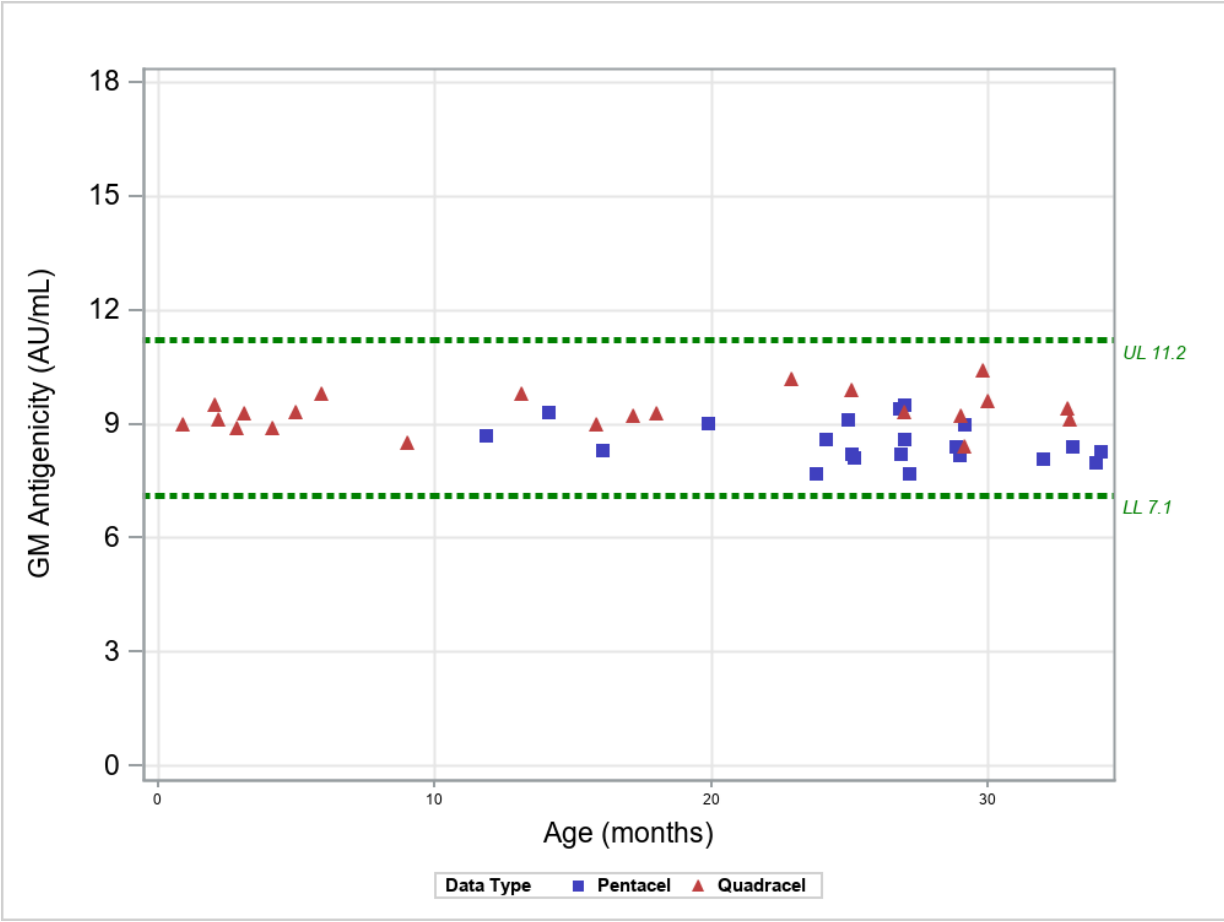


[1]Pentacel contains Quadracel (DTaP-IPV component, liquid formulation) and ActHIB component (lyophilized)

sanofi

Specification of PRN Antigenicity for Stability Monitoring

- **No apparent age effects** were observed from the production lots used for setting release acceptance criteria
- Release specification limits were applied to stability monitoring



sanofi

Conclusion

- An ***in vitro*** **PRN Antigenicity ELISA** has been developed for Quadracel and Pentacel using mAbs that detect relevant PRN epitopes and **is superior to the animal-based potency test** for detecting changes to PRN antigen in heat-stressed Quadracel.
- The PRN Antigenicity ELISA is **stability indicating** and was fully **validated** showing acceptable accuracy, precision, specificity, linearity, and robustness.
- PRN Antigenicity ELISA was successfully **implemented in Quality Control** to replace the PRN mouse immunogenicity assay for release and stability monitoring for Quadracel and Pentacel products
- The *in vitro* PRN Antigenicity ELISA **eliminates the use of animal tests** for PRN potency determination, hence, significantly **reduces false OOS** related to animal test variability
- **Test cycle time for PRN potency was significantly reduced** from two months to one week.
- The implementation of PRN Antigenicity ELISA in Sanofi **demonstrates the company's commitment to 3Rs** (replacement, reduction, and refinement of animal tests).

sanofi

Acknowledgements

Sanofi Toronto Quality Control

Greg Glasier
Meili Li
Sophia Lee
Kristy Yang
Diana Keizner
Eric Zholumbetov
Na Guan
Kimberley Williams
Kai He

Sanofi Toronto Analytical Sciences

Beata Gajewska
Jason Szeto
Nelson Eng
Tricia Chen
Marin Ming

This work was funded by Sanofi.
The author is a Sanofi employee and may hold shares and/or stock options in the company.

sanofi

THANK YOU

sanofi