Contrôle des vaccins et méthodes alternatives : Sanofi récompensé

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En février dernier, Emmanuelle Coppens, coordinatrice du programme 3R chez Sanofi, a partagé avec nous les efforts et les défis auxquels Sanofi faisait face dans le développement de méthodes alternatives pour le contrôle des vaccins.

Ces efforts ont porté leurs fruits puisque Sanofi s'est vu décerné le prix ATLA Remplacement in Practice Poster lors du 12th World Congress on Alternatives and Animal Use in the Life Sciences. Le poster primé mettait en lumière le remplacement du test d'activité pour un des antigènes d'une combinaison vaccinale pédiatrique alors que les autorités exigent traditionnellement qu'ils soient réalisés sur des animaux (en l'occurrence des souris). Il s'agit d'une première mondiale que d'avoir développé, validé et obtenu l'approbation règlementaire pour une méthode alternative, l'essai d'antigénicité, pour un antigène coquelucheux. Le prix récompense un cas concret avec mise en pratique d'une alternative in vitro.

Removing the Mouse From the House: An ELISA Method to Replace Mouse-based Potency Testing for Pertactin Antigen

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INTRODUCTION

- The '3Rs' principle encourages the Replacement, Reduction, and Refinement of animal testing.
- Many established vaccines, including Sanofi products, continue to rely on animal based tests for release.
- For Sanofi Acel DTaP-IPV vaccines (e.g. Quadracel and Pentacel), the current licensed assay used for assessing *Bordetella pertussis* pertactin (PRN) antigen potency for release and routine stability testing is a Mouse Immunogenicity assay
- Animal tests are resource-intensive and can be challenging due to high variability, high cost, and ethical concerns.
- This mouse-based PRN potency assay was an ideal candidate for the development of an alternative *in vitro* animal-free, ELISA potency (antigenicity) assay using well characterized and relevant anti-PRN monoclonal antibodies.

Data from this poster has been recently published: Szeto *et al.* Vaccines 2023, 11(2), 275.

METHODS

- Several anti-PRN monoclonal antibodies (mAbs) were characterized for affinity, functionality, relevance to human response, ability to detect degraded PRN, and epitope identification
- Two mAbs were selected for development of a sandwich ELISA potency assay (mAb clone 3-5 for capture and mAb clone 3-4 for detection)
- ELISA was assessed for ability to detect degraded PRN, and to monitor lot-to-lot consistency of Quadracel and Pentacel
- ELISA was transferred to QC test lab and fully validated
- Comparison of ELISA versus mouse-based potency test was performed using heat-stressed Quadracel vaccine

Figure 1. Overview of traditional mouse-based Pertactin antigen potency testing

Figure 2. Overview of PRN antigenicity (potency) ELISA

RESULTS

Table 1. Summary of characterization data for anti-PRN monoclonal antibodies

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* Isolates[2]	Relevance to Human Response in SIA	Relevance to Human Response (mAb)	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	520-536, 575-584, 677-685, and 830-837	Conformational	<25%	YES	YES	0.7
3-5	IgG1	0.91	234-244	Likely conformational (formed by linear segments)	<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	30-78 and 809-156	Conformational	<25%	YES	YES	0.1

[1]Data from Zhu *et al.* Biotech. J. 2021; 17, e2100058. [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.

Figure 3. Assessing relevance of anti-PRN mAbs to human response using Serum Inhibition Assay (SIA)

Figure 4. Example 4-PL response curves from PRN antigenicity ELISA

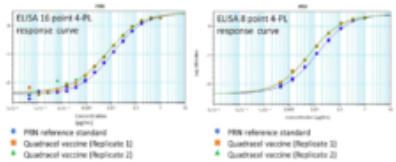

Figure 5. Confirming Specificity of PRN Antigenicity ELISA

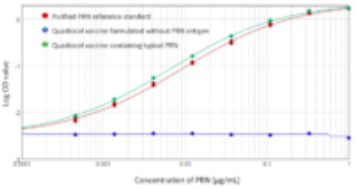

Figure 6: Lot-to-lot Consistency of PRN Antigenicity in Quadracel Vaccine Lots Observed with ELISA

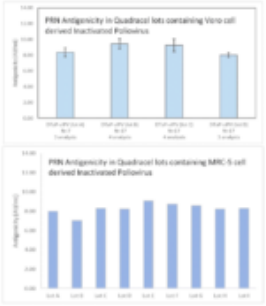

Figure 7. PRN Antigenicity Results are Aligned Between Quadracel and Pentacel

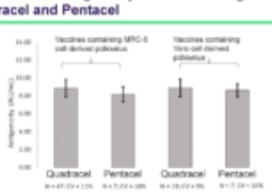

Figure 8. Stability Indication of PRN Antigen using the Antigenicity ELISA

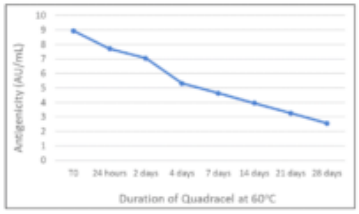

Table 2. Validation of PRN Antigenicity ELISA

Validation of the PRN antigenicity ELISA was performed by assessing the parameters of accuracy, precision (repeatability and intermediate precision), specificity, linearity, and range.

ELISA validation parameter	Validation Criteria	Validation criteria met
Accuracy	Recovery of 80-120% of expected value	Yes (80% recovery at 100% sample level, and 80-90 % recovery at all other sample levels tested)
Intermediate Precision	%CV ≤ 15%	Yes (%CV less than 15% at all sample levels tested)
Repeatability	%CV ≤ 15%	Yes (%CV less than 15% at all sample levels tested)
Linearity	R2 ≥ 0.98	Yes (>0.98 R2 from 5 levels)
Specificity	No signal in Quadracel sample lacking PRN antigen	Yes (no detection of other vaccine components)

Table 3: PRN Mouse Immunogenicity Assay versus PRN Antigenicity ELISA - Assessment of 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	5.95
14 h, 60 °C	5.5	Yes	5.4	Yes	7.77
2 days, 60 °C	5.4	Yes	3.5	Yes	7.85
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	3.36
28 days, 60 °C	5.1	Yes	2.6	Yes	2.56

[1] Geometric mean antibody titer (reciprocal dilution) 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 meets/exceeded the minimum acceptance criteria.

CONCLUSIONS

- An *in vitro* PRN potency assay has been developed for Quadracel and Pentacel using mAbs that detect ***relevant*** PRN epitopes
- The ELISA is stability indicating and was fully validated showing acceptable accuracy, repeatability, intermediate precision, linearity, and specificity
- The PRN antigenicity ELISA is superior to the animal-based potency test for detecting changes to PRN antigen in heat-stressed Quadracel
- This ELISA has since been presented to the Center for Biologics Evaluation and Research (CBER; FDA) as an *in vitro* replacement for the mouse immunogenicity release test for PRN in Quadracel
- CBER has since accepted this proposal and work is currently underway in Sanofi QC Immunochemistry Toronto for implementation
- **Impact:** The PRN Antigenicity ELISA helps move our 3Rs commitments forward, and will contribute to future savings on animal testing. This assay provides a gateway for introduction of other *in vitro* assays for routine release/stability testing.

ACKNOWLEDGMENTS:

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Disclosures

Authors who are/were Sanofi employees may hold shares and/or stock options in the company.

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REFERENCES:

1. **Szeto et al. Vaccines 2023, 11(2), 275.**
2. **Zhu *et al.* 2021, Biotech. J., 17, e2100358.**
3. **He *et al.* 2020, J. Pharm. Sci., 109, 1002.**

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