

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 Replacement 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 réglementaire 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



Jason Szeto¹, Arun Beharry², Tricia Chen³, Eric Zholumbetov⁴, Emilie Daigneault⁵, Marin Ming⁶, Iain Lounsbury⁷, Nelson Eng⁸, Nemika Thangavadi⁹, Robbie Jin¹, Aurelie Denis-Jacquot¹, Bahram Behnam Azad¹, Meili Li¹, Diana Ketzner¹, Marcus Liu¹, Sophia S. Lee¹, Kai He¹, and Beata Gajewska¹

¹Sanofi Analytical Sciences Immunology, Toronto, ON, Canada; ²Medical Sciences, Western University, London, ON, Canada; ³Sanofi QC Immunochromatography, Toronto, ON, Canada; ⁴Sanofi QC Analytical Excellence, Toronto, ON, Canada; ⁵Department of Immunology, University of Toronto, Toronto, ON, Canada; ⁶Sanofi Biostatistical Biostatistics, Toronto, ON, Canada; ⁷Sanofi QC Analytical Excellence Biostatistics, Toronto, ON, Canada; ⁸Sanofi Analytical Sciences, Toronto, ON, Canada.

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. Quadracl 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 Quadracl 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 Quadracl vaccine

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

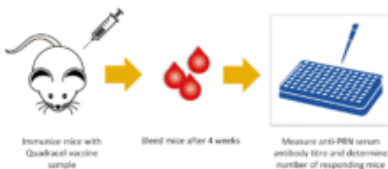


Figure 2. Overview of PRN antigenicity (potency) ELISA



RESULTS

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

Anti-PRN mAb Class	Sequence ID	Amino Acids	Epitope	Monoclonality	Spiking Type	Anti-binding to Pertactin	Relative to Reference	Relative to Reference	Inhibition of PRN-coated bead binding
3-1	SP21	67-107	Non-relevant	Non-relevant	Non-relevant	<2%	100	100	0.7
3-2	SP22	112	100-200	100% Consistent	100%	<2%	100	100	2.1
3-3	SP23	120	100-200	100% Consistent	100%	<2%	100	100	0.7
3-4	SP24	130	224-244	100% Consistent	100%	<2%	100	100	0.1
3-5	SP25	148	26-46 and 76-97	100% Consistent	100%	<2%	100	100	0.1
3-6	SP26	163	67-100/100-130	100% Consistent	100%	<2%	100	100	0.1

¹Data from Zhu et al. *Biomol* 2021, 17, 4030030. ²Data from He et al. *J Pharm Sci* 2020, 109, 1602-1607, showing the percentage of positively stained bacteria as determined by flow cytometry. ³Data from same reference showing relative bead binding percentage capacity.

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

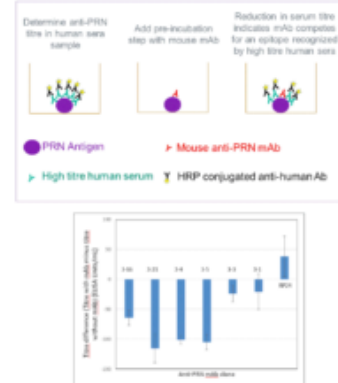


Figure 4. Example 4-PL response curves from PRN antigenicity ELISA

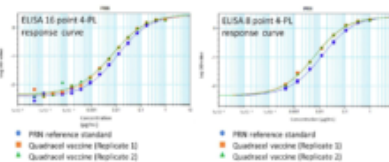


Figure 5. Confirming Specificity of PRN Antigenicity ELISA

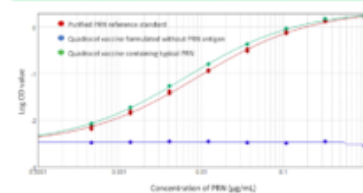


Figure 6: Lot-to-lot Consistency of PRN Antigenicity in Quadracl Vaccine Lots Observed with ELISA

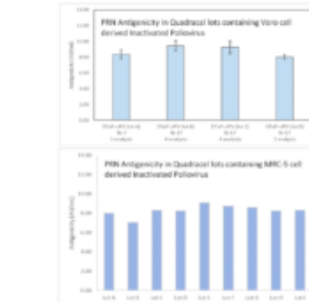


Figure 7. PRN Antigenicity Results are Aligned Between Quadracl and Pentacel

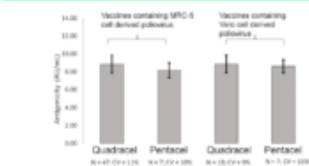


Figure 8. Stability Indication of PRN Antigen using the Antigenicity ELISA

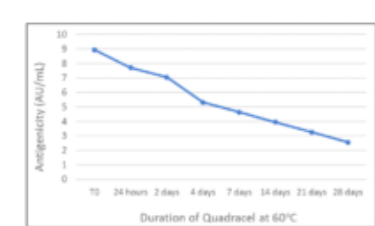


Table 2. Validation of PRN Antigenicity ELISA

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 (92% 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 (R2 > 0.98 from 5 levels)
Specificity	No signal in Quadracl 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 Quadracl samples

Treatment	ELISA fold	# of Mouse Responders Meeting Criteria 1	# of Mouse Responders Meeting Criteria 2	Antigenicity (AU/mL)
Room temp	2.5	Yes	6.1	8.98
14 days, 60 °C	5.5	Yes	3.4	7.77
7 days, 60 °C	5.8	Yes	3.5	7.05
4 days, 60 °C	2.0	Yes	4.3	5.32
7 days, 60 °C	6.9	Yes	4.0	4.64
14 days, 60 °C	4.5	Yes	8.7	3.96
21 days, 60 °C	4.8	Yes	3.7	3.28
28 days, 60 °C	5.1	Yes	2.0	2.38

¹ Quadruple mean average (antibody titer) is shown as a fold increase over the minimum positive QC acceptance criteria. Any value equal to or greater than 1 indicates the QC has met the acceptance criteria.

² Indicates whether the number of responding mice met/exceeded the minimum acceptance criteria.

CONCLUSIONS

- An *in vitro* PRN potency assay has been developed for Quadracl 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 Quadracl
- 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 Quadracl
- CBER has since accepted this proposal and work is currently underway in Sanofi QC Immunochromatography 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.

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Disclosures:

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REFERENCES:

- Szeto et al. *Vaccines* 2023, 11(2), 275.
- Zhu et al. 2021, *Biomol*, 17, e2100308.
- He et al. 2020, *J Pharm Sci*, 109, 1602.

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