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Innovation and Technology Contributing to the Development of Fostera™ PCV MH

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Most progressive pork producers have adopted health programs that include vaccination against porcine circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (M. *hyo*) since these pathogens can impose significant financial harm to unprotected herds. Though separate vaccinations for each pathogen have been historically required, the recent advent of combination vaccines containing both antigens offers to help reduce the labor and animal stress involved in vaccination protocols.

Development of a PCV2/M. *hyo* combination vaccine involves much more than simply mixing two existing products into one bottle. Rather, multiple technical factors can impact safety, efficacy, and ease-of-use (e.g., field mixing, multiple doses, etc.). To represent an advance in vaccination technology, a PCV2/M. *hyo* combination vaccine would ideally help provide efficacy and safety similar to that of monovalent vaccines, be cost-effective, and be easy to use (single dose, one bottle, little or no mixing, good flow through a syringe, etc.). Therefore, Zoetis has devoted significant research and development to deliver a highly effective and user-friendly single-dose PCV2/M. *hyo* combination vaccine, employing scientific expertise and innovation to create a novel state-of-the-art product.

Development of a combination vaccine involves much more than simply mixing existing products.

Fostera™ PCV MH

Fostera™ PCV MH, from Zoetis, is the first and only combination, **one-bottle** vaccine that helps to protect swine from ***both*** porcine circovirus-associated disease (PCVAD) and enzootic mycoplasma pneumonia after administration of a ***single dose*** (other combination vaccines require mixing in the field or multiple doses). Fostera PCV MH was uniquely developed to ensure proper antigen/adjuvant balance, helping deliver safe and effective protection against both PCVAD and enzootic mycoplasma pneumonia.

A single 2-mL intramuscular (IM) dose of Fostera PCV MH is licensed for the vaccination of healthy pigs at 3 weeks of age or older as an aid in preventing viremia, lymphoid depletion, and colonization of lymphoid tissue caused by PCV2; and as an aid in reducing PCV2 viral shedding and enzootic pneumonia caused by M. *hyo*. Thus, Fostera PCV MH helps provide disease protection afforded by other monovalent Zoetis products: protection from M. *hyo* similar to RespiSure-ONE,® and protection against PCVAD similar to Fostera PCV, all in a convenient one-bottle, one-dose formulation.

Zoetis researchers let science, not expedience, lead the way in their efforts to develop Fostera

PCV MH. The extensive research required to create a combination product that offered safety and efficacy similar to current monovalent vaccines like RespiSure-ONE and Fostera PCV forced scientists to evaluate and optimize each step of the development and production process. Multiple obstacles were encountered, and each required research effort to identify, test, and implement an innovative solution. Thus, the dual-antigen, single-dose Fostera PCV MH vaccine is founded on strong scientific principles and outcomes that have resulted in a labor-saving advance for vaccination protocols.

Better Compatibility

The primary problem that had to be overcome in developing Fostera PCV MH concerned the lack of compatibility of the M. *hyo* antigens grown with porcine serum and the PCV antigens when formulated together in a vial. Specifically, binding of antibodies from the porcine serum to the PCV antigens created technical issues with respect to antigen potency.

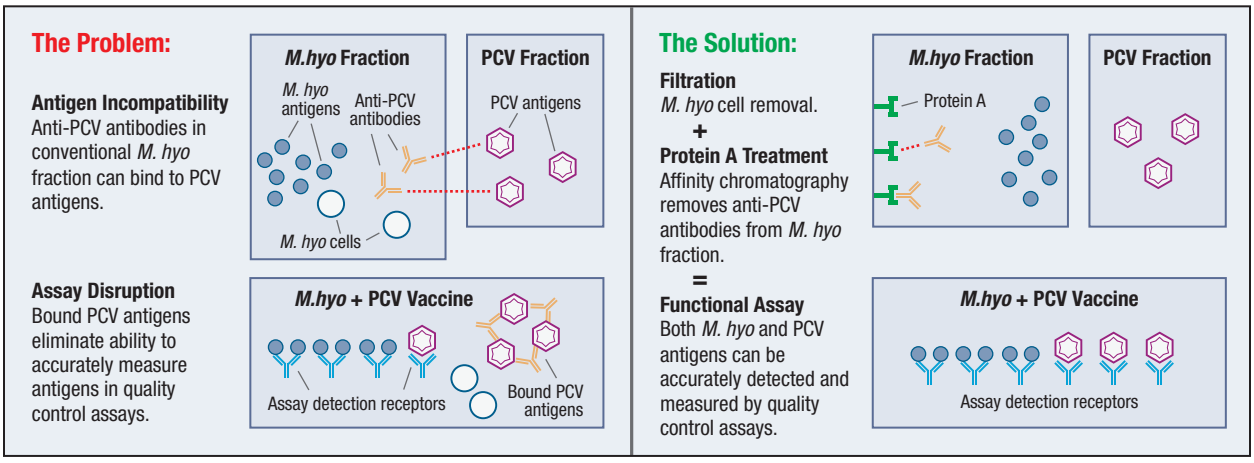
Porcine serum, a critical component of M. *hyo* growth medium, contains a wide variety of antibodies with specificity to many antigens (including PCV2). Unfortunately, when combined with PCV antigens in a combination vaccine, anti-PCV antibodies in the M. *hyo* fraction can bind to the vaccinal PCV antigens, thereby eliminating the ability to detect or measure the antigens in quality control assays (Figure 1). To modify this significant problem, Zoetis investigators developed and tested an improved downstream production process for the M. *hyo* fraction used in Fostera PCV MH.

A Bacteria-Free Vaccine

During cell growth of mycoplasmas, immunogenic components are released into the culture broth. This has been demonstrated in previous research where protection against M. *hyo* was achieved when using the cell-free supernatant of an M. *hyo* culture as compared to using sedimented, whole M. *hyo* cells.[1]

A similar strategy is applied to the production process of the M. *hyo* component of Fostera PCV MH, with M. *hyo* cells filtered away from the fluid portion which is rich in M. *hyo* antigens. As a result, the M. *hyo* antigens in Fostera PCV MH represents a bacterial cell-free product, meaning that only soluble M. *hyo* proteins are used to induce an immune response in vaccinated pigs instead of the whole inactivated microbe. This approach offers several advantages, such as helping improve safety by removing unnecessary components from the vaccine, and allowing for a more targeted immune response.

Figure 1. Incompatibility of conventional *M. hyo* antigens with PCV antigens, and use of Protein A chromatography to remove troublesome anti-PCV antibodies.



Protein A technology helps remove anti-PCV antibodies from the M. hyo antigen fluid, thus improving antigen compatibility.

Protein A: Applying Innovation to Build Compatibility

Simply removing the M. *hyo* cells does not render the M. *hyo* antigens compatible with PCV2 antigens. Zoetis scientists introduced a step that removes the troublesome anti-PCV antibodies from the M. *hyo* containing fluid: 'Protein A' chromatography. Protein A is a cell wall component of *Staphylococcus aureus* bacteria and specifically binds to antibodies (Figure 2). Notably, Protein A is commonly used in human medicines for its ability to purify IgG immunoglobulins (Table 1 shows some examples of Protein A use in monoclonal antibody therapy, including treatments for human cancer and autoimmune disorders). Thus, Protein A acts like a biomolecular 'sponge' that selectively binds and removes antibodies.

For production of Fostera PCV MH, cell-free M. *hyo* culture fluid (containing critical M. *hyo* antigens) is passed through a Protein A chromatography column to remove the antibodies. The resultant fluid is a purified and substantially IgG antibody-reduced M. *hyo* fraction (Figure 1).

By using Protein A to help remove PCV antibodies (and other irrelevant IgG antibodies) from the M. *hyo* fraction, the PCV antigens in the combination vaccine remains efficacious. In fact, research evaluating multiple combinations of whole-cell and fluid-only versions of M. *hyo* antigens confirmed that the cell-free, Protein A-treated M. *hyo* antigens were highly efficacious.[5] Protein A-treated M. *hyo* antigens and PCV antigens were also shown to be highly efficacious when in combination and did not interfere with each other.[6,7]

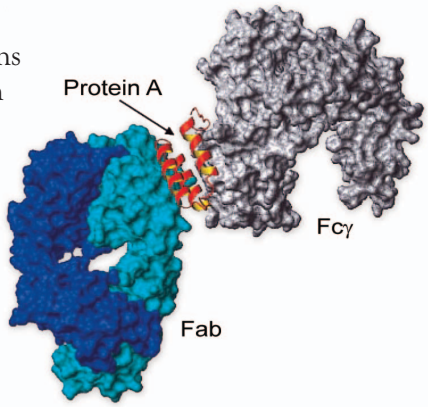


Figure 2. Illustration of *Staphylococcus aureus* Protein A antibody binding activity (Fab = Ig fragment responsible for antigen recognition; Fcγ = constant region of IgG involved in effector functions).[2]

In summary, the improved vaccine production process enables the 'combinability' of the 2 antigens (by reducing anti-PCV2 and other IgG antibodies) and likely imparts a better safety profile by removing unnecessary protein immunological agents from the vaccine. Furthermore, after vaccine administration, the immune cells of the pig can be directed to relevant M. *hyo* antigens.

Table 1 – Examples of human monoclonal antibody therapies that utilize Protein A technology.[3,4]

Antibody	Brand name	Company	Indication/targeted disease
Bevacizumab	Avastin®	Genentech/Roche	Colorectal cancer, macular degeneration
Belimumab	Benlysta®	GlaxoSmithKline	Systemic lupus erythematosus
Certolizumab pegol	Cimzia®	UCB	Crohn's disease
Cetuximab	Erbitux®	Bristol-Myers Squibb/Lilly/Merck	Colorectal cancer, Head and neck cancer
Trastuzumab	Herceptin®	Genentech	Breast cancer
Adalimumab	Humira®	Abbot	Several auto-immune disorders
Canakinumab	Ilaris®	Novartis	Cryopyrin-associated periodic syndrome

Identifying the Ideal Adjuvant

The final piece of the puzzle for investigators was adjuvant research — finding the right adjuvant to simultaneously optimize the efficacy of both antigens in Fostera PCV MH. Researchers conducted multiple experiments[8-10] to determine the safest and most efficacious adjuvant for the combination vaccine, comparing 3 adjuvants:

- 5% Amphigen® (lecithin/mineral oil blend);
- 5% Amphigen + 5% SLCD (sulfolipo-cyclodextrin);
- 10% MetaStim® (also called SP Oil, an oil-in-water emulsion composed of squalane, poloxamer 121, and polysorbate 80).

While each of these adjuvants can be very efficacious when used in a monovalent vaccine, the dynamics of their efficacy and safety had to be investigated when intended for use in a *combination* vaccine like Fostera PCV MH.

Of the 3 adjuvants evaluated, MetaStim was chosen, generating an efficacious and safe profile for use in a combination vaccine. MetaStim has been successfully used worldwide for decades as an adjuvant in legacy Fort Dodge vaccines (many that are now Zoetis products) for swine, bovine, and equine applications, as well as similar formulations in products of other companies. Past experience has consistently demonstrated a good safety profile, including studies indicating that MetaStim is safe and efficacious under field conditions for another prominent Zoetis *M. hyo* vaccine used internationally, Suvaxyn® MH-One. In addition, MetaStim possesses a variety of characteristics relevant to use as a vaccine adjuvant:

- has been shown to help fortify induction of cell-mediated immunity and humoral immunity in horses;[11]
- helps induce potent immune responses to a variety of antigens, including inactivated bacteria, inactivated viruses, and modified-live viruses;
- non-viricidal activity poses no harm to viral antigens;
- non-viscous for easy syringeability.

Conclusions

The development of Fostera PCV MH involved intensive research and well-supported scientific methodology that has resulted in an innovative, one-bottle/one-dose combination vaccine. The ability of Zoetis scientists to produce a safe and highly efficacious PCV2/*M. hyo* combination vaccine represents a significant advancement, simplifying vaccination protocols used by pork producers to help protect the profit potential of their herds.

Fostera™ PCV MH

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