Pfizer Animal Health
Technical Bulletin

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Next generation PreZent™-A adjuvant system: Key to enhanced protection conferred by BVDV (Types 1 and 2) components of *CattleMaster®GOLD™*

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Key Points

- The new *CattleMaster® GOLD™* line of multivalent vaccines has label claims for protecting against the birth of persistently infected calves caused by BVDV (Types 1 and 2).
- *PreZent™-A*, a next generation adjuvant system, drives the enhanced immunogenicity of the killed BVDV fractions, thus accounting for the vaccine's unique clinical efficacy.
- Antigen-Quil A-cholesterol nanocomplexes, carried on microdroplets of *Amphigen®*, are thought to attract the body's antigen-presenting cells (APCs), resulting in strong, long-lasting immune responses.
- In efficacy studies, *PreZent-A*-adjuvanted BVDV safely initiated immunologic and fetal protection responses comparable to those generated by modified-live virus antigens.

Historically, killed BVD vaccines have been used to prevent acute disease, its attendant immunosuppression, respiratory and enteric signs, and reproductive loss. Until the July 2004 introduction of *CattleMaster® GOLD™*, however, no killed BVD vaccine had provided sufficient protection to obtain a label claim for aiding in the prevention of persistently infected calves, the primary source of BVD disease spread. Previous studies attempting to demonstrate efficacy of inactivated vaccines against fetal challenge produced either varying degrees of success or used a BVDV challenge model that failed to consistently affect control animals.[1-6] These earlier generation products were incapable of initiating the robust immune responses necessary to protect the developing fetus during the first 125 days of gestation, the period of greatest fetal susceptibility. For this reason, considerable research has been undertaken in recent years to devise new methods of enhancing the immunogenic potential of killed BVD vaccines.

The innovative technologies used in the development and production of *CattleMaster GOLD* have achieved this objective. On the basis of results obtained in prelicensing efficacy trials, *CattleMaster GOLD*

received the first BVD Type 1 and Type 2 fetal protection claim ever granted to a killed BVDV vaccine licensed in the U.S. In these trials, prebreeding vaccination of BVDV-seronegative cows protected 100% of their fetuses against BVDV Type 1 or BVDV Type 2 challenges that produced persistent infection in 100% of fetuses from nonvaccinated dams challenged with BVDV Type 1 and 88% of fetuses from nonvaccinated dams challenged with BVDV Type 2.[7,8] (More detailed information about these studies can be found in the Pfizer Technical Bulletin "*CattleMaster GOLD*: Inactivated BVDV fractions aid in the prevention of persistently infected calves.")

Key to the vaccine's BVDV fetal protection claim is its breakthrough adjuvant system—tradenamed *PreZent-A*. Adjuvant systems like *PreZent-A* have been shown to induce very strong and long-lasting immune responses, which appear similar to those normally associated with a live infection.[9]

This bulletin presents detailed information about the components of the *PreZent-A* adjuvant system and reviews current scientific understanding of the ways in which this type of adjuvant system interacts with the immune system.

Adjuvant Components

The diluent component of *CattleMaster GOLD* contains a mixture of inactivated BVD viruses, viral particles, and adjuvant. Immunologically, the most important components of this preparation are the E1 and E2 glycoproteins, which are located on the envelope of the BVD virus (Figure 1).

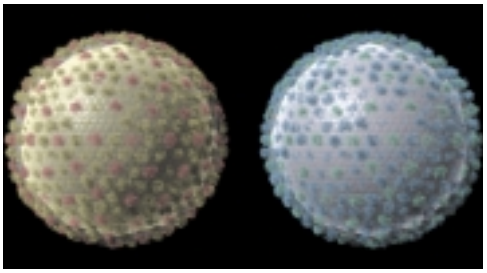


Figure 1 – The immunologically important E1 and E2 glycoproteins are depicted as knob-like projections on the surface of BVD viruses. (Color of each virus envelope is for illustrative purposes only.)

PreZent-A Glossary of Terms

The *PreZent-A* adjuvant system is a product of breakthrough science, and some of the language of its innovative technologies may be new to many readers. Key terms used in describing *PreZent-A* and the way it reacts with the immune system are defined below.

Amphiphilic	Denoting a molecule, such as detergents or wetting agents, that contains groups with characteristically different properties, e.g., both hydrophilic and hydrophobic properties
Glycoprotein	Any of a class of conjugated proteins in which the nonprotein group is a carbohydrate; sometimes restricted to proteins containing small amounts of carbohydrate; most viral glycoproteins occur as membrane-anchored spikes extending outward from the envelope of enveloped viruses; elicits an immune response, part of which is directed specifically against the carbohydrate group
Microdroplet	In reference to *Amphigen*, an oil droplet following high shear force processing; the vehicle to which multiple nano-complexes (see below) of Quil A-cholesterol-antigen are attached for presentation to the immune system
Nano-complex	A structure, measured in units of one-thousand-millionth (10^{-9}) of a meter; *PreZent-A* nano-complexes consist of Quil A-cholesterol-antigen (intact virus or fragmented piece of viral membrane with attached glycoprotein) after high shear force processing; highly immunostimulatory
Polymer, polymeric	A compound, usually of high molecular weight, formed by combination of simpler molecules (monomers)

Because antibodies produced against E2 (formerly known as gp53) have been associated with virus neutralization,[10-13] a primary objective of killed vaccine development is enhancing the presentation of high quantities of envelope glycoprotein to the immune system.

The *PreZent-A* adjuvant system is composed of Quil A, cholesterol, antigen, and *Amphigen.* The Quil A, cholesterol, and *Amphigen* components act in a complementary fashion to bind with, and optimally present, high levels of E2 antigen to the body's antigen-presenting cells (APCs). Immunogenicity is further enhanced by high shear force processing, which maximizes the surface area for antigen presentation. What follows is a description of the individual components along with a theoretical discussion of the roles each plays in helping initiate robust immune responses in vaccinated cattle.

Quil A

The first adjuvant component is Quil A, a highly refined form of the plant-derived biochemical saponin. Chemically, Quil A has three distinct areas with respect to polarity: 1) a lipophilic/hydrophobic terminal anchor—depicted in greyish white in the computer graphic (Figure 2), 2) a triterpenoid, hydrophobic core—shown in green, and 3) multiple hydrophilic, immunostimulatory sugar units—shown in red.[14] The hydrophilic components are carbohydrates and the triterpenoid component is quillaic acid.[14] The high capacity of Quil A to strongly induce both cell-mediated and humoral immune responses is widely recognized.[9,15,16]

Cholesterol

The second component of the *PreZent-A* adjuvant system is cholesterol. Cholesterol is a fat-like bipolar (hydrophilic and hydrophobic) substance of animal origin. When added in the appropriate proportion to Quil A, cholesterol eliminates some of the less desirable effects of Quil A without compromising its immunostimulatory capacity and helps create a truly unique adjuvant delivery system.[9] Quil A has a strong affinity for cholesterol, perhaps caused by the similarity of the hydrophobic core in combination with the presence of

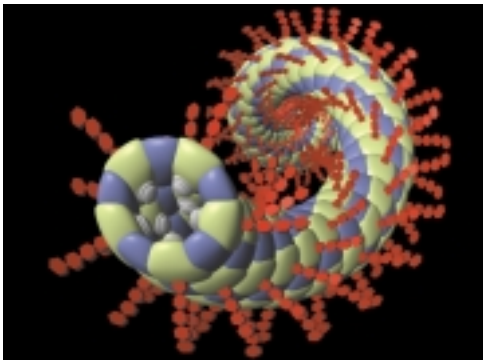


Figure 2 – Cholesterol binds with Quil A, eliminating the molecule's undesirable properties while leaving the highly immunostimulating sugar units (red) exposed.

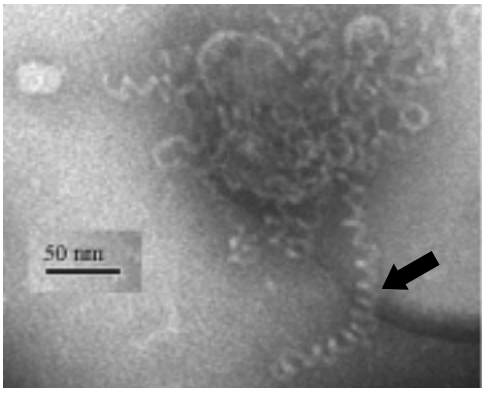


Figure 3 – Transmission electron micrograph of a coiled Quil A-cholesterol helix (arrow) prior to high shear force processing. Magnification: X 120,000

bulky carbohydrate groups enabling effective shielding of the hydrophobic cholesterol molecules. At the molecular level (Figures 2 and 3), multiple helix-like structures with an open core form as the cholesterol (shown in blue) interacts with Quil A in an aqueous environment. The hydrophilic immunostimulating sugar units are exposed on the outer surface, and the lipophilic/hydrophobic terminal anchor lines the interior of the helix.

Antigen

When the Quil A-cholesterol complexes are added to killed BVD virus preparations, the components of the helixes preferentially attach to the outer viral envelope and become intimately bound with critical glycoprotein antigens (E1 and E2) located on the BVDV envelope. This is thought to occur by hydrophobic interactions, but electrostatic interactions and hydrogen bonds are also possible because of the presence of

carbohydrates that are able to form both.[9] At this intermediate stage of vaccine production, the helices of Quil A and cholesterol can be large polymeric structures closely associated with the key viral antigens (Figure 4). Ultimately, the addition of amphiphilic antigen results in an adjuvant with an antigen insertion, so that when antigen-presenting cells engulf the Quil A-cholesterol adjuvant, they also consume the antigen. This method of antigen delivery and presentation distinguishes the *PreZent-A* adjuvant system from any other system currently used in veterinary medicine for enhancing the immunogenicity of killed BVD viruses.

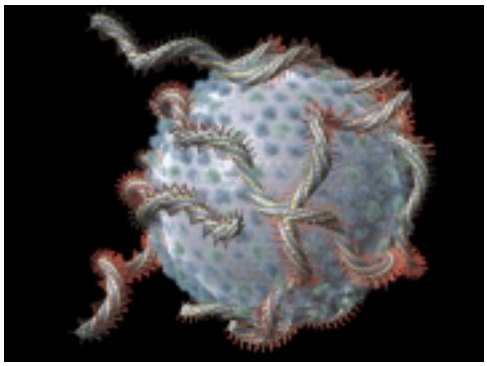


Figure 4 – Quil A-cholesterol-BVDV antigen complex before high shear force processing. Multiple Quil A-cholesterol helixes attach to the viral envelope in close association with key glycoprotein antigens. These helical structures surround the virus envelope, with the immunostimulatory sugar units (red) of Quil A highly exposed.

Amphigen

Immunogenicity of the Quil A-cholesterol-antigen complexes is further potentiated by the addition of *Amphigen.* Composed of a lecithin-derived phospholipid and a glycolipid surfactant in a light, highly refined oil, *Amphigen* offers the advantages of an oil-based adjuvant (in particular, a prolonged immune response) while minimizing adverse local reactions, such as lumps, abscesses, and granulomas at the injection site. Key to understanding *Amphigen*'s immune-enhancing and safety effects is the lecithin surfactant to which vaccine antigen attaches. Researchers have discovered that lecithin contains stereochemical configurations (the spatial placement of atoms within a molecule) that mimic those of a cow's body cells. This "naturalizes" the oil and makes it more accessible to the cells of a cow's immune system. Aluminum hydroxide, a commonly used adjuvant in cattle vaccines, and conventional oil adjuvants lack this stereochemical compatibility.

Because of its low viscosity, *Amphigen* makes *CattleMaster GOLD* highly syringeable and minimizes reactions upon injection. In comparison, thicker water-in-oil-based vaccines often are extremely difficult to draw up into a syringe and historically have been associated with long-lasting reactions at the site where the products are administered.

Shear Force Processing

The final step in production of the *PreZent-A* adjuvant system is high shear force processing of the Quil A-cholesterol-antigen-*Amphigen* preparation. Shear force processing has two primary effects: 1) It ensures a highly uniform and stable mixture of submicron-sized (0.1 to 0.2 μm) microdroplets of *Amphigen* (see box, page 5) and 2) It affects the length of the helical coils of Quil A-cholesterol. The coils become fragmented, forming short-chain helices that bind to the BVD viral envelope and align with the E1 and E2 glycoproteins in somewhat of a pattern. The resulting nano-complexes of Quil A-cholesterol-antigen become orientated on the surface of microdroplets of *Amphigen* with the immunostimulatory sugar units protruding outwards. Figure 5 depicts this nano-complex as an intact virus surrounded by regularly arranged Quil A-cholesterol helices. It is likely that the disruptive nature of shear force processing results in the formation of many smaller nano-complexes composed of Quil A-cholesterol helices

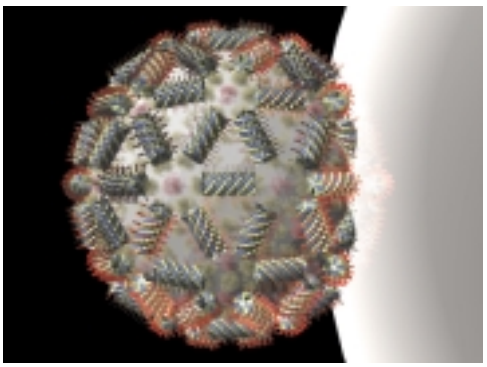
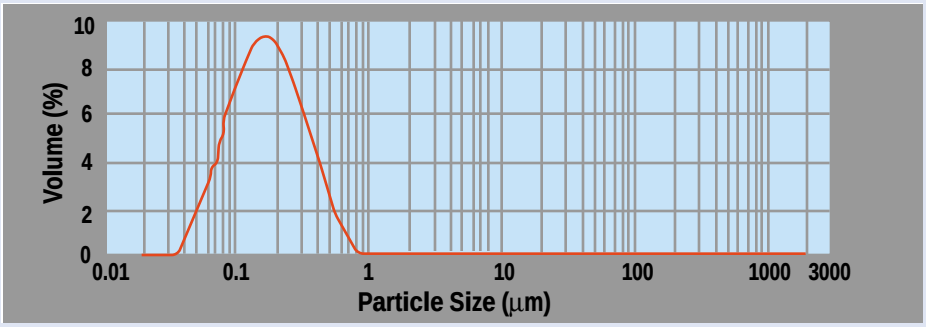


Figure 5 – *Amphigen* microdroplet with nano-complexes. Multiple nano-complexes are attached to the outer surface of each microdroplet.

Product Consistency and Extended Shelf Life: Additional Benefits of Antigen-Adjuvant Blending

The proprietary shear force processing used in manufacturing *CattleMaster GOLD* results in diluent that has unparalleled uniformity and consistency for a veterinary vaccine. Vaccines prepared with this technology are much less likely to separate or cream and remain stable for extended shelf life. Most importantly, the resulting microdroplets offer a high surface area for antigen presentation, and they are optimally sized for active and direct uptake by antigen presenting cells of the immune system.



Particle size distribution of the BVDV-adjuvant blend is highly uniform due to high shear force processing of the Quil A-cholesterol-BVD antigen-*Amphigen* complexes.

attached to the glycoproteins of fragmented pieces of the viral envelope. The majority of the nano-complexes are probably embedded in the surface of the *Amphigen* microdroplets, but a proportion will also be free in the vaccine diluent (Figure 6).

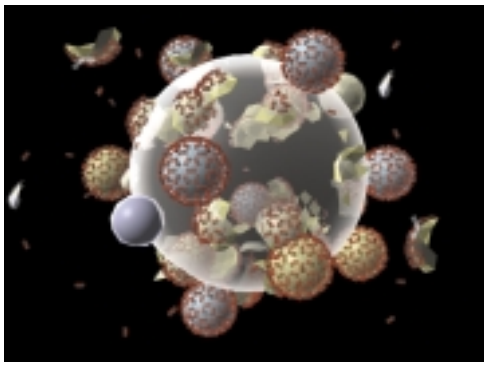


Figure 6 – Microdroplet with smaller nano-complexes composed of Quil A-cholesterol helices intimately bound with glycoproteins on fragmented pieces of virus envelope.

PreZent-A and the Immune System

Clinical and immunological studies have confirmed that the BVD components of *CattleMaster GOLD* stimulate a very strong and long-lasting immune response. The following scenario, which is based on an understanding of antigen presentation using the *PreZent-A* adjuvant system and current knowledge of the immune system, presents a theoretical explanation for how the *PreZent-A* adjuvant system can drive robust, broad-based immunological protection against BVD infection. Key to the proposed mechanism of action is the supposition that the immune system likely reacts to the adjuvanted, killed BVD antigens as if they were live.

Theoretical Basis of Protection

Following vaccine administration, the immunostimulatory sugar molecules on the adjuvant-antigen nano-complexes are recognized by a special class of antigen-presenting cell (APCs) known as dendritic cells (Figure 7). Located throughout the body,

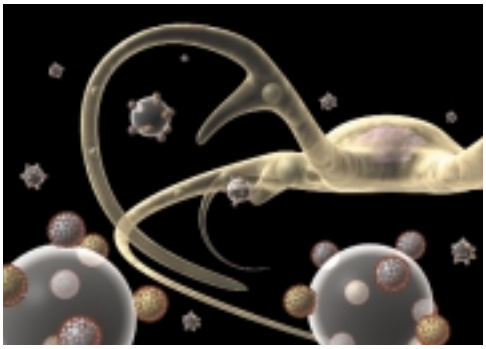


Figure 7 – Following vaccination, tentacled dendritic cells are attracted to sugar units bound in the adjuvant's nano-complexes, pick up antigen, and migrate to lymph nodes.

dendritic cells are particularly abundant in the skin and readily migrate to subcutaneous tissue after *CattleMaster GOLD* is administered. The *PreZent-A* nano-complexes and the microdroplets on which they are carried are all sufficiently small to be directly consumed and processed by the activated dendritic cells. These cells subsequently migrate to lymph nodes, where they process and present antigen to specific receptors on T-lymphocytes (T-cells). The distinctive properties of the adjuvant allow antigen to be displayed on the surface of the dendritic cells complexed with two special molecules called major histocompatibility complex I and major histocompatibility complex II, or MHC I and MHC II (Figure 8). Because antigen processed by the dendritic cells is displayed by both MHC I and MHC II on the cells' outer surface (Figure 9), both of the major types of T-cells (helper and killer T-cells) are likely activated, initiating cell-mediated immunity (CMI) responses (killer T-cells) and providing immunological help (helper T-cells) to both CMI responses and the formation of protective antibodies.

Antigen processed and presented by the MHC I pathway stimulates killer T-cells that are primarily associated with cell-mediated immunity (Figure 10). Killer T-cells destroy host cells infected with BVDV.

Antigen processed and presented by the MHC II pathway stimulates helper T-cells, which respond in two important ways (Figure 11). First, they interact with B-cells, instructing them to become more efficient at producing antibodies to BVDV glycoprotein. Second, they release messenger proteins called cytokines, which activate more killer T-cells and B-cells, further enhancing cell-mediated and humoral antibody responses. Simultaneously stimulated are B-cell and T-cell memory mechanisms, which remain primed to respond quickly and intensely to the same BVDV antigen in a future disease challenge situation.

Concurrent with activation of T-cells by dendritic cells, some free BVDV antigen is

Figure 8 – Major histocompatibility complex I (left) and major histocompatibility complex II molecules located on the surface of dendritic cells likely present BVDV antigen to the humoral and cell-mediated immune systems.

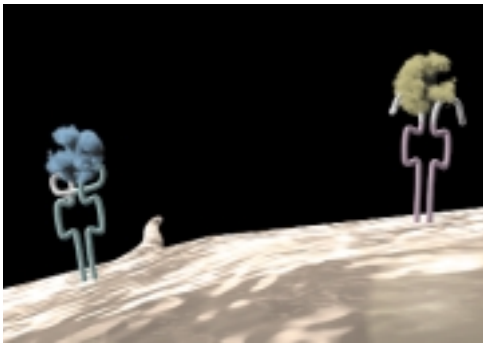


Figure 9 – Hypothetical representation of MHC I (left) and MHC II (right) molecules respectively presenting BVDV antigen to killer T-cells and helper T-cells, thereby activating both CMI and humoral immune responses.

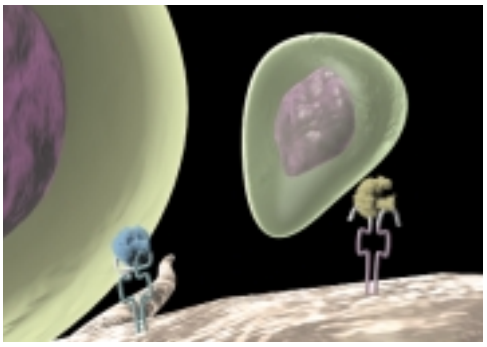


Figure 10 – The MHC I pathway. Activated killer T-cells associated with cell-mediated immunity destroy infected host cells.

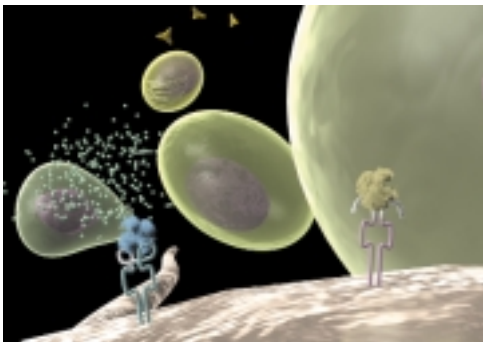


Figure 11 – The MHC II pathway. Helper T-cells: 1) instruct B-cells to produce Y-shaped neutralizing antibodies (background left center) and 2) release cytokines to activate more killer T-cells (left).

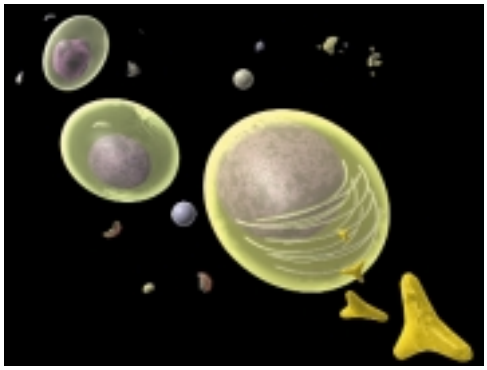


Figure 12 – Y-shaped neutralizing antibodies to BVDV envelope glycoproteins are produced in the lymph nodes following B-cell stimulation of plasma cells (activated in illustration).

likely transported to the lymph nodes, where it is recognized by B-cells. The B-cells are stimulated by both the antigen and helper T-cells to divide and become plasma cells, producing neutralizing antibodies specific to BVDV glycoproteins, and memory cells, providing long-term immunity (Figure 12).

Conclusion

The *PreZent-A* adjuvant system stimulates improved immunologic and protective responses to the killed BVD components in *CattleMaster GOLD*. This is evidenced by the BVDV fetal and respiratory protection label claims and the supporting efficacy studies conducted for licensing. Prior to the introduction of *CattleMaster GOLD*, such levels of protection against BVDV Type 1 and Type 2 fetal infection were associated exclusively with prebreeding administration of only a few modified-live BVDV vaccines.[17,18] Now, dairy and beef producers have the option of using a safe and convenient killed BVDV vaccine to aid in the prevention of persistently infected calves caused by fetal infection with BVDV Types 1 and 2. The ability to initiate fetal protection against BVDV with the pathogen's strong tropism for tissues of the bovine reproductive tract[6,19-22] is likely attributable to the *PreZent-A* adjuvant system. *PreZent-A* enhances immunogenicity of the killed BVDV fractions in *CattleMaster GOLD* to levels beyond those associated with earlier generation killed BVDV vaccines. Of the adjuvants currently used to potentiate killed BVDV antigen, only *PreZent-A* has been shown to stimulate the magnitude and type of immune response that results in BVDV fetal protection.

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