Elanco Titanium®

TECH REPORT
Elanco research information for beef consultants

Summary of Licensing Approval Studies for Titanium® 5 + PH-M Demonstrating Effectiveness, Noninterference and Safety

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Background information and implications

Four studies were conducted and submitted to the U.S. Department of Agriculture (USDA), Center for Veterinary Biologics (CVB), to obtain licensing approval to market Titanium® 5 + PH-M.

This research demonstrated Titanium 5 + PH-M met requirements for effectiveness against five viruses: bovine viral diarrhea (BVD), types 1 and 2, infectious bovine rhinotracheitis (IBR), bovine parainfluenza$_3$ (PI$_3$), and bovine respiratory syncytial virus (BRSV); and two bacteria: *Mannheimia haemolytica* and *Pasteurella multocida* when administered to healthy cattle 2 months of age and older. It also established noninterference between the viral and bacterial components of the vaccine.

The safety study established the field safety of Titanium 5 + PH-M.

Titanium 5 + PH-M noninterference and efficacy research

Introduction

Studies were conducted to document that the Titanium 5 and PH-M components do not interfere with one another when combined into a single vaccine: Titanium 5 + PH-M. This research also demonstrated the vaccine's effectiveness against five important viruses and two bacterial pathogens associated with bovine respiratory disease (BRD), confirming previous efficacy research submitted to USDA to gain approval for marketing the components as individual vaccines.

Mannheimia haemolytica challenge study[1]

Study design

Research investigators procured 25 healthy crossbred beef calves at approximately 2 months of age. They used nasal swabs to confirm negative isolation of *M. haemolytica* and *P. multocida*, and evaluated antibody titer levels and physical appearance. The calves were held with maternal cows in Missouri from prevaccination through the pre-challenge period, and assigned randomly to one of three groups (Table 1).

Table 1. *M. haemolytica* challenge study design[1]

Randomly allocated on Day -35	Revaccinated on Day -14	Day 0	Day 4
GROUP 1: 10 head **2 mL PH-M*, SQ**	**2 mL PH-M***	*M. haemolytica* challenge (transthoracic)	Surviving animals sacrificed: all necropsied to evaluate lung lesions
GROUP 2: 10 head **2 mL Titanium 5 & PH-M*, SQ**	**2 mL Titanium 5 & PH-M***		
Group 3: 5 head **Control**	No treatment		

Clinical signs and rectal temperatures were observed on Days 0-4

Ten calves in Group 1 received 2 mL of a PH-M* vaccine subcutaneously (SQ) in the neck away from the suprascapular lymph node. The 10 calves in Group 2 received a 2 mL, SQ dose of Titanium 5 + PH-M in the neck. Five control calves were not vaccinated. Calves in Groups 1 and 2 were revaccinated with the same vaccine on the opposite side of the neck 21 days later. All calves were removed from the cows and shipped to a ranch in Texas for the challenge study. On Day 0 (14 days after revaccination), a transthoracic dose of *M. haemolytica* was given to each calf. Investigators monitored rectal temperatures and observed clinical signs at least three times daily for the next four days. They necropsied dead animals and evaluated lung lesions each day, with surviving calves sacrificed and necropsied on Day 4. Blood samples were taken on Days -51, -35, -14 and 0.

Statistical analysis

Statisticians used three methods to evaluate efficacy results of this challenge study:

1. Mann-Whitney U test
2. Prevented fraction (decreased chance of a vaccinate becoming a case)
3. Mitigated fraction (increased chance that a vaccinate's case will be less severe)

Study results

This study looked at survival rate (Figure 1), total temperature score, total clinical score, estimated total lung-lesion score (ETLLS), bacterial recovery and serological (blood antibody) testing.

Analysis showed no statistically significant ($P = 0.01$) difference between the two groups of vaccinates on any measured parameter. When comparing the vaccinated groups (Groups 1 and 2) to the unvaccinated controls (Group 3), the researchers found the results for vaccinates were significantly ($P = 0.01$) different from the control calves (Table 2). This established noninterference between the viral and bacterial components of Titanium 5 + PH-M in the presence of an *M. haemolytica* challenge.

M. haemolytica was isolated from all lung-lesion samples except for those from one calf, confirming *M. haemolytica* was the causative agent of the calf mortality and lung lesions.

Figure 1. Survival rate of calves after *M. Haemolytica* challenge[1]

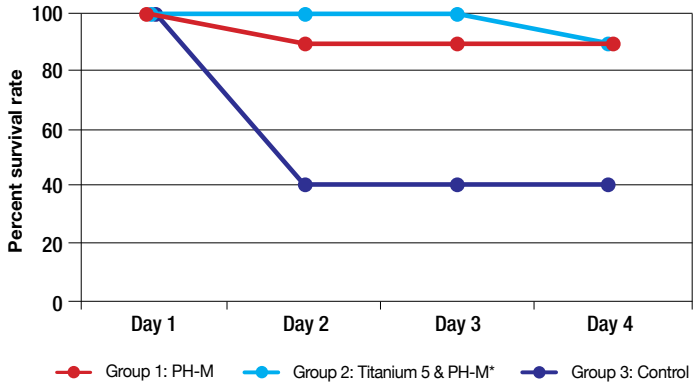


Table 2. *M. haemolytica* challenge study results[1]

	PH-M (Group 1)	Titanium 5 & PH-M* (Group 2)	Control (Group 3)
Animals, No.	10	10	5
Average total temperature score$^{\Delta}$	3.5[a]	3.2[a]	8.6[b]
Average total clinical score$^{\Delta\Delta}$	1.2[a]	1.2[a]	7.2[b]
Preventable fraction, mortality, %	83‡	83‡	
Mitigated fraction, lung lesions, %	82‡	82‡	
Average estimated total lung-lesion score, cm^3†	540.6[a]	476.1[a]	1718.9[b]
Leukotoxin (LKT) antibody titer			
Prevaccination	2.2	1.6	1.8
Pre-challenge	4.1[a]	4.2[a]	1.8[b]
M. haemolytica antibody titer			
Prevaccination	1.0	1.1	1.0
Pre-challenge	4.9[a]	4.9[a]	1.2[b]

[a,b]Significance at $P < 0.01$.
‡Significance at $P = 0.05$.
$^{\Delta}$During each observation, calves were assigned a temperature score (0: < 104°F, 1: 104°F – 104.9°F, 2: 105°F – 105.9°F, 3: ≥ 106°F). Scores from all observations were added to create a total temperature score for each calf, with animals that died before Day 4 arbitrarily assigned a "9" — one point higher than the highest recorded total score.
$^{\Delta\Delta}$Clinical signs observed included nasal discharge, coughing, anorexia, depression and respiratory effort.
†Assumes 1 g of lung tissue equals 1 cm^3. The ETLLS was calculated as the sum of the estimated lung lesions on all caudal lobes.

MLV-component noninterference study[2]

Study design

This research trial involved 80 healthy Holstein steers in Iowa that were approximately 9 months of age and weighed from 400 to 800 pounds. Trial investigators confirmed each was seronegative (serum neutralization titer ≤ 2) for BVD, types 1 and 2, IBR and PI_3, and had not been vaccinated previously for any of these viruses. The steers were assigned randomly to one of three groups (Table 3) and commingled in outdoor pens with a barn, with vaccinates housed separately from unvaccinated controls.

On Day 0, 35 steers in Group 1 received a 2 mL SQ dose of Titanium 5 + PH-M and the 35 steers in Group 2 received a 2 mL, SQ dose of Titanium 5, with all vaccinations administered in the neck. Twenty-one days later, Group 1 steers were revaccinated with a 2 mL, SQ PH-M and modified-live virus (MLV) BRSV vaccine, while Group 2 received a 2 mL, SQ MLV BRSV vaccine. The 10 control steers in Group 3 were not vaccinated.

Blood samples were taken on Days 0, 28, 35 and 42, then analyzed for antibody titers for BVD, types 1 and 2, IBR and PI_3 (Table 4).

Statistical analysis

For each vaccine fraction, log-transformed antibody titers were estimated for each treatment group, then compared using 90% confidence intervals (CI) to evaluate noninterference. While a sample size of 30 steers per treatment group was expected to provide adequate power to demonstrate noninterference, researchers included 35 vaccinates per group.

Study results

Serum neutralization assays were used to measure the level of antibody titers against BVD, types 1 and 2, IBR and PI_3 delivered by Titanium 5 + PH-M, and to compare them to antibody titers of Titanium 5 vaccinates and control steers.

Data showed the titers of the two groups of vaccinated steers were equivalent for all four viral strains, with all titers significantly different from those of unvaccinated controls. This demonstrated there was no interference between the viral and bacterial components of Titanium 5 + PH-M in delivering an immune response against BVD, types 1 and 2, IBR and PI_3.

Table 3. MLV-component noninterference study design[2]

Randomly allocated on Day 0	Revaccinated on Day 21	Day 28	Day 35	Day 42
GROUP 1: 35 head **2 mL Titanium 5 + PH-M, SQ** ●	**2 mL PH-M*, BRSV**			
GROUP 2: 35 head **2 mL Titanium 5, SQ** ●	**2 mL BRSV**	●	●	●
Group 3: 10 head **Control** ●	No treatment			

● **Blood samples collected to assess & compare immunological response**

Table 4. Average serum neutralization antibody titers (log 2) in MLV-component noninterference study[2]

	Titanium 5 + PH-M (Group 1)	Titanium 5 (Group 2)	Control (Group 3)
BVD, type 1	795[a]	670[a]	≤ 2[b]
BVD, type 2	34[a]	27[a]	≤ 2[b]
IBR	32[a]	35[a]	≤ 2[b]
PI_3	30[a]	30[a]	≤ 2[b]

[a,b]Significance using a 90% CI.

BRSV challenge study[3]

Study design

This research included 37 healthy, colostrum-deprived Holstein calves — 36 intact males and one female — from two South Dakota dairies, ranging in age from 27 to 57 days at the start of the study. Trial investigators confirmed each was seronegative (serum neutralization titer ≤ 2) for BRSV and negative for BVD persistent infection as determined by ear-notch immunohistochemistry. The calves were assigned randomly to one of two groups (Table 5) and housed outdoors in natural conditions.

Table 5. BRSV challenge noninterference study design[3]

Randomly allocated on Day -2, vaccinated on Day 0	Revaccinated on Day 21	Days 41-42	Day 49
GROUP 1: 21 head **Titanium 5 & PH-M*, SQ, 2 2-mL doses**	**Titanium BRSV & PH-M*, 2 2-mL doses**	BRSV intranasal challenge	Surviving animals sacrificed; all necropsied for evaluation
GROUP 2: 16 head **Control (Titanium 4 & PH-M*, SQ, 2 mL dose)**	**PH-M*, 2 mL dose**		

On Day 0, the 21 calves in Group 1 received the components of Titanium 5 + PH-M via two 2 mL, SQ doses. Researchers administered the components of Titanium 4 (BVD, types 1 and 2, IBR, PI_3) and PH-M in 2 mL, SQ doses to the 16 calves serving as controls in Group 2. All injections were made in the neck.

Twenty-one days later, Group 1 calves were revaccinated with a 2 mL dose of Titanium BRSV and a 2 mL dose of PH-M, and Group 2 calves received a 2 mL dose of PH-M. The vaccines were given SQ in the opposite side of the neck.

On Day 41, calves were moved to an indoor pen in a climate-controlled challenge facility, where they were challenged with BRSV intranasally after arrival and again on Day 42. Investigators monitored rectal temperatures, observed clinical signs and collected nasal secretions using swabs from Day 40 through Day 49. Blood samples were taken from all calves on Days 0 and 41, with Control calves also sampled on Day 21. Researchers euthanized and necropsied calves on Day 49, and evaluated lung lesions and lung-tissue samples.

Statistical analysis

All analysis variables were analyzed using prevented fractions. The relative risk and corresponding exact 95% confidence interval were computed, with the entire 95% CI greater than 0% when the prevented fraction is significantly different ($P < 0.05$) from 0%.

Four calves were removed after the study began, leaving 18 calves in Group 1 and 15 calves in Group 2. This sample size provided adequate power to demonstrate effectiveness against BRSV and compatibility of the vaccine's components.

Study results

This study looked at total percent lung-lesion score (TPLLS), nasal-swab and lung-tissue BRSV isolation, rectal temperature, as well as clinical signs, including nasal discharge, ocular lesions or discharge, respiratory effort, respiratory rate, coughing, depression and appetite.

Investigators designated a calf to be a positive case if its TPLLS was > 10%, or if its TPLLS was > 0% and < 10%, and its score was positive (equal to 1, indicating a clinically significant presence of the virus) for at least one of the following: nasal shedding, lung-tissue virus isolation (VI), lung-tissue fluorescent antibody (FA). A calf was determined to be negative if its TPLLS was zero, or if its TPLLS was > 0% and < 10%, and its score was negative for nasal shedding, lung VI and lung FA, indicating clinically significant presence of virus was not detected.

Analysis showed vaccinated calves had fewer positive cases of BRSV (Figure 2), with a calculated preventable fraction of 43% (Table 6). Additional study results appear in Table 6. Regarding clinical signs observed, there was no statistically significant difference between vaccinated calves and controls.

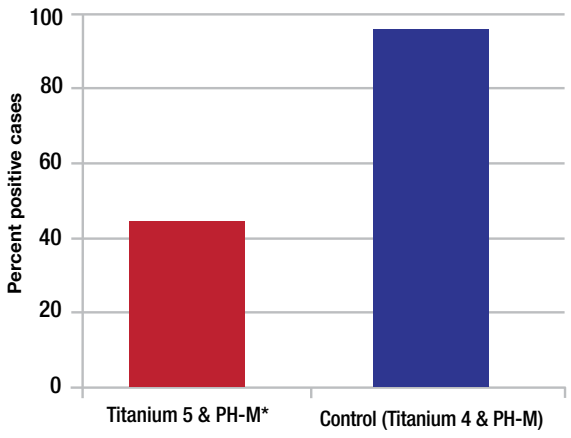
This study established noninterference between the viral and bacterial components of Titanium 5 + PH-M in the presence of a BRSV challenge.

Table 6. BRSV challenge study results[3]

	Titanium 5 & PH-M* (Group 1)	Control (Group 2)
Animals, No.	18	15
Preventable fraction for positive cases of BRD, %	43[Δ]	
Positive cases, %	44	93
Nasal BRSV VI positive cases, %	39	93
Lung-tissue BRSV VI positive cases, %	0	27
Lung-tissue BRSV FA positive cases, %	11	73
Average total lung-lesion score, %	1.04	8.55

[Δ]Lower exact 95% confidence limit (CL) of 13.2%; upper CL of 62.4%.

Figure 2. Positive cases after BRSV challenge[3]



Titanium 5 + PH-M safety research

Introduction

A study was conducted to demonstrate target-animal safety for Titanium 5 + PH-M. This research showed the vaccine was safe for use in non-pregnant cattle 60 days of age and older under field conditions when administered subcutaneously.

Field-safety study[4]

Study design

This field-safety evaluation included 458 healthy calves at four locations in Nebraska, Oklahoma and Texas. The calves represented a variety of cattle — crossbred and purebred cattle, including both beef and dairy breeds; steers, heifers and a bull; and calves from 30 days to 8 months of age at the start of the study (Figure 3).

On Day 0, all calves received a 2 mL dose of Titanium 5 + PH-M administered SQ in the right side of the neck. Twenty-one days later, the calves were revaccinated with a 2 mL, SQ dose in the left side of the neck. (Table 7) After vaccination, research investigators observed calves for signs of immediate hypersensitivity, anaphylaxis or toxicity for two to three hours, and again from Hour 18 to Hour 24.

Investigators monitored calves individually for adverse reactions** daily for at least 14 days after vaccination, and palpated injection sites four to six days after vaccination, and again on Days 21 and 42. Injection-site swellings were measured in cubic centimeters, with the cube root calculated to comply with Veterinary Dictionary for Drug Regulatory Activities (VEDDRA) definitions.

Calves were returned to owners at the study's conclusion.

Study results

Veterinary investigators did not observe any immediate hypersensitivity, anaphylaxis or toxicity during the observation periods within the first 24 hours after vaccination or revaccination.

The only adverse events observed relating to the vaccine during the 42-day trial were minimal, and consisted of swelling at the injection site (Figure 4). These swellings decreased in size and most were not apparent 21 days after vaccination, with 80% of calves exhibiting no swelling on Day 21 and 83% of calves having no swelling on Day 42.

This research established the field safety of Titanium 5 + PH-M as well as fallout vaccines made from the components of Titanium 5 + PH-M.

Figure 3. Age of calves enrolled in safety study

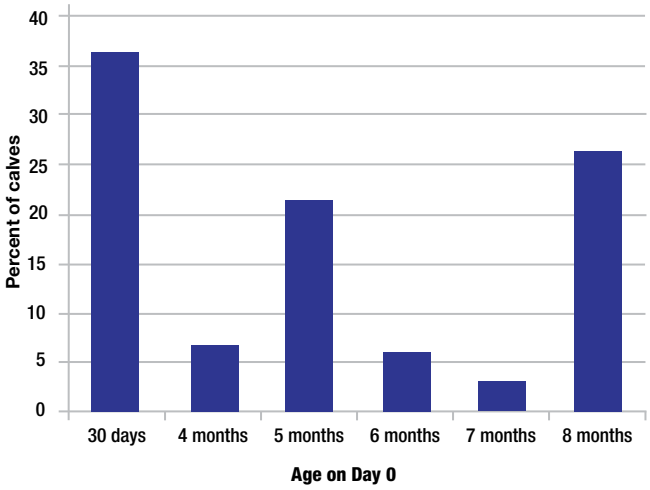
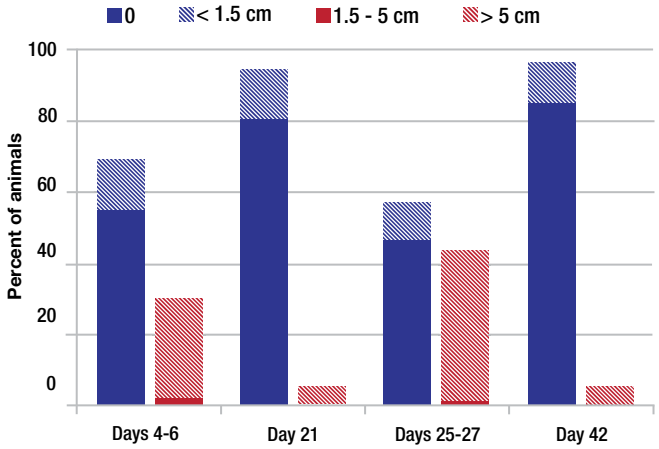


Table 7. Safety study design[4]

Vaccinated on Day 0	Days 4-6	Revaccinated on Day 21	Days 25-27	Day 42
458 calves **2 mL Titanium 5 + PH-M, SQ**	●	**2 mL Titanium 5 + PH-M** ●	●	●

● **Palpated neck**

Figure 4. Post-vaccination injection-site swelling measurements



Day 0 = vaccination; Day 21 = revaccination

Titanium 5 + PH-M indications

Based on research submitted to USDA APHIS's CVB, Titanium 5 + PH-M label indications are for the vaccination of healthy:

- Cattle 60 days of age or older
- Cows and heifers no less than 30 days before breeding
- Pregnant cattle, provided they were vaccinated according to label instructions with Titanium 3, Titanium 4 L5, Titanium 5, Titanium 5 L5 HB or Titanium 5 + PH-M 30 to 60 days before breeding
- Calves nursing pregnant cows, provided their dams were vaccinated as described above

Titanium 5 + PH-M is indicated as an aid in the prevention of disease caused by:

- IBR
- BVD, type 1
- BVD, type 2
- PI_3
- BRSV
- *M. haemolytica*
- *P. multocida*

* *Mannheimia haemolytica-Pasteurella multocida* Bacterin-Toxoid Code 7935.04/G935.01

** Adverse events are categorized according to standardized VEDDRA terms. General categories include, but are not limited to, behavioral disorders, blood and lymphatic system disorders, eye disorders, immune system disorders like anaphylaxis, injection-site reactions, respiratory tract disorders, and systemic disorders like not drinking or eating, or fever.

[1] Milliken, G. A. 2013. *Mannheimia haemolytica* efficacy studies demonstrating the absence of excessive interference of Titanium products with the *Mannheimia haemolytica-Pasteurella multocida* bacterin-toxoid.

[2] Demonstration of the compatibility of components between APHIS product codes 1181.20 (Establishment 213) and G935.04 (Establishment 315) APHIS product code 45B9.20. Study No. 2010-01 Rev. 1.

[3] 2013. Efficacy study for BRSV fraction to demonstrate compatibility of the BRSV component in APHIS product code: 45B9.20 (unlicensed). Study No. 2011-05 Rev. 1.

[4] Porter, M. 2011. Field safety evaluation of bovine rhinotracheitis-virus diarrhea-parainfluenza$_3$-respiratory syncytial virus vaccine, modified live virus, APHIS product code 1181.20 (establishment 213) in combination with *Mannheimia haemolytica-Pasteurella multocida* bacterin-toxoid, APHIS product code: G935.04 (establishment 315). Protocol No. 2011-01, Rev. 02.

The label contains complete use information, including cautions and warnings. Always read, understand and follow all label and use directions.

Do not vaccinate within 21 days of slaughter.

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