GR

ORIGINAL SUBMISSION

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April 21, 2008

Via Overnight Mail

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
4300 River Road
College Park, Maryland 20740-3835

Re: GRAS Notification for ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate Intended for Use as an Ingredient in Food

Dear Dr. Martin:

Pursuant to proposed 21 C.F.R. § 170.36 and on behalf of Proliant Health Ingredients, Inc., we are submitting the enclosed notification to the Food and Drug Administration (FDA) indicating that ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it has been determined, on the basis of scientific procedures, to be generally recognized as safe (GRAS) when used as an ingredient in various foods at use levels of 0.2% to 5% in the finished food. On February 14, 2008, on behalf of Proliant Health Ingredients, we withdrew without prejudice an earlier notice, dated September 6, 2007 (GRAS Notice No. GRN 234), in order to avoid having FDA reject the submission due to lack of sufficient information. Specifically, there are twelve areas where FDA indicated more information is needed.

In order to assist FDA in its review of this new notification, this letter quotes each of the twelve comments contained in a December 31, 2007 e-mail message from Harold Woodall and identifies the responsive changes that are incorporated into this revised notice.

<u>FDA's Comments and Corresponding Changes Made in Response</u>

1. On pg 14, you report a protein digestibility of 94.4% for Bovine Globulin but your notice does not include information about how your calculation was derived. Additionally, the reported 94.4% digestibility value appears to conflict with the statement in Attachment 19 that the IgG in bovine globulin is resistant to digestion. Please explain how you calculated the 94.4% digestibility value and discuss how that value correlates to the high concentration of IgG in your product.

   - Table 3, now on page 16, was modified to show that the correct figure for protein digestibility is 93.4% rather than 94.4%.

- The PDCAAS worksheet in Attachment 12, where the protein digestibility value was incorrectly stated, was updated by substituting 93.4% for 94.4%.

- An explanation of how the protein digestibility figure is derived was added to Table 3 on page 16.

- Beginning at the top of page 14 and ending on page 16, the section "2. ImmunoLin® is a High Quality Protein" was revised to explain and reconcile the high digestibility value of IgG with the statement that IgG is partially resistant to digestion.

2. Under the Identity Section on pg 5, we noticed a typographical error in which Bovine Blobulin rather than Bovine Globulin is incorrectly listed as the chemical name. Please confirm if this is an error.

   - The word "globulin" was substituted for the incorrect word, "blobulin," on page 5.

3. Under the manufacturing section on pg 7 you mention that plasma is pumped into an insulated silo and "is maintained at 45°F or below for microbial stability". Please define the term "microbial stability" in the context of your notice.

   - A sentence was added immediately after use of the term "microbial stability" on page 7 to make it clear that microbial stability in this context refers to the state of being resistant to change in regard to the level of microorganisms present in plasma during the holding period.

4. Under the manufacturing section on pg 7 (bullet #11), there is reference made to "a commonly used GRAS food ingredient." The identity and use of a substance are inherent in GRAS determinations. Without knowing either the identity of the ingredient or whether the ingredient is GRAS for this use, we cannot evaluate the safety of the manufacturing process. Please either (1) identify the commonly used GRAS food ingredient that you refer to or (2) provide a statement confirming that the substances are GRAS for use as described in your method of manufacture. Also in this section (bullet #11), there is a reference made to "diagram, Figure 1". We could not find a Figure 1 but assume you are referring to Attachment 6 or 7. Please confirm.

   - Statement No. 11 on page 8 was revised to clarify that while the substance is a commonly used GRAS food ingredient, it is not used here as a food ingredient, but as a processing aid.

- Statement No. 6 on page 9 was revised for clarification on this point as well.

- Statement No. 11 on page 8 was revised also by changing the reference to "diagram, Figure 1" to "Attachment 7, Delipidation."

5. Under the Estimated Dietary Intake section on pg 11, there appears to be a miscalculation for the maximum use level of Bovine Globulin. You calculated 0.02 g ImmunoLin®/g food x 1500 g food to be 35 grams. Please re-calculate and correct or explain how you derived 35 grams as the maximum use level. Similarly, on pg 11, there appears to be a miscalculation under footnote #3. You calculated the total calories to be 1185. Please re-calculate and correct or explain how you derived the total calories.

   - The number 35 was deleted and the number 30 was added in its place on page 11 at the second paragraph under "VII. Estimated Dietary Intake."

   - The number 1185 was deleted and the number 1985 was added in its place on page 11 in footnote No. 3.

6. Under the GRAS determination section on pg 12, please explain how you converted the ADI for man from 2000 ppm to get the value of 100 mg/kg bw/day.

   - Footnote No. 6 was added on page 12 to explain the ADI conversion.

7. On pg 14, you state that "Isoleucine is the limiting protein." Isoleucine is not a protein, but an amino acid. Please provide a corrected statement.

   - The word "protein" was deleted and the word "amino acid" was added in its place in the sentence "Isoleucine is the limiting amino acid," which is now at the top of page 16.

8. In Table 3 of pg 14 you provide FAO Values. We noticed that there is no score reported for tryptophan. If this is intentional, please explain why a score has not been reported.

   - The uncorrected amino acid score of 1.82, which had been inadvertently omitted, was added to Table 3, now on page 16.

   - The PDCAAS worksheet in Attachment 12, where the uncorrected amino acid score for tryptophan had been inadvertently omitted, was also updated by the addition of the value 1.82.

9. In Attachment 1, please provide the units for the nutritional profile.

   - Attachment 1 was revised to provide units for the nutritional profile.

10. In Attachments 1 - 4, you include the compositional analysis of Bovine Globulin. Is this data from one lot or an average from multiple lots? A minimum of three lots is typically needed for statistical considerations. Please confirm the number of lots used in your analyses.

    - The second paragraph on page 5, under "B. Chemical Composition," was revised to state the number of lots used in the analysis.

11. Please provide a narrative explaining the data in the table in Attachment 5 that supports your statement on page 6 of your submission that your product is stable from between 2-4 years.

    - A narrative section was added to the first paragraph at the top of page 6 to explain the stability testing data presented in Attachment 5.

    - The table in Attachment 5 was also revised to make the data more clear.

12. In Attachment 6, you provide a schematic diagram on the isolation procedure for Bovine Globulin. The diagram is un-readable. Please provide a clearer copy.

    - A larger and clearer copy of the schematic diagram in Attachment 6 is included with this notification.

The only other corrections to the previous notification pertain to spelling or formatting mistakes. The changes noted in the bullet points above represent the only substantive differences between this notification and GRAS notification No. 234, which FDA reviewed previously. Enclosed please find three copies and one electronic copy on disk of this new notification and accompanying documents.

* * *

We trust that all of FDA's questions and concerns from the earlier notice have been addressed in this resubmission. If you have any questions regarding this notification, please contact me at the above number or email address.

Cordially yours,

John B. Dubeck

Enclosures

cc: Mel Vanden Berg

RECEIVED
APR 22 2008
BY:----------

Before the
FOOD AND DRUG ADMINISTRATION
Department of Health and Human Services
Washington, D.C.

GRAS NOTIFICATION

Name of Notifier:	Proliant Health Ingredients, Inc.
Post Office Address:	All communications on this matter are to be sent to Counsel for the Notifier: John B. Dubeck Keller and Heckman LLP 1001 G Street, N.W., Suite 500 West, Washington, D.C. 20001. Telephone: (202) 434-4125
Name of Substance:	ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate
Intended Use:	As a protein supplement in selected foods
Date:	April 18, 2008
Submitted by:	John B. Dubeck Keller and Heckman LLP Counsel for Proliant Health Ingredients, Inc.

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I. Administrative Information

A. Name and Address of the Notifier

Proliant Health Ingredients Inc.
2425 Oak Tree Court
Ankeny, Iowa 50021

All Communications on this matter are to be sent to Counsel for the Notifier:

John B. Dubeck
Keller and Heckman LLP
1001 G Street, N.W., Suite 500 West
Washington, D.C. 20001
Telephone: (202) 434-4125
Facsimile: (202) 434-4646
Email: Dubeck@khlaw.com

B. Name of the Notified Substance

The common and usual name of this product is bovine globulin concentrate. The brand name for Proliant Inc's product is ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate.

C. Intended Conditions of Use

Bovine globulin concentrate is intended to be added as a protein supplement to dairy foods, juices, snack foods, beverages and meal replacements in amounts that typically vary from 0.2 – 2% (2,000 to 20,000 parts per million). The maximum use level would be 5% of the finished food product (50,000 ppm). The amount of protein added daily to the diet for users of the product is estimated to be 5-50% of the RDI or 2.5-25 grams, depending on the level of consumption and the variety of food types consumed. No special target population is envisioned. The population consuming dairy, juice, snack foods, beverages and meal supplements is very diverse and widespread.

D. Basis for GRAS Determination

The described use of bovine globulin concentrate is generally recognized as safe (GRAS) based on scientific procedures, in accordance with 21 C.F.R. § 170.30, as discussed more fully in the accompanying summary of the basis for the GRAS determination.

E. Statement of Availability of Data

The data and information that are the basis for the Notifier's GRAS determination are provided in the Attachments to this Notification or will provided to the Agency upon request by the U.S. Food and Drug Administration (FDA).

* * * *

The foregoing and attached information considered, it is respectfully submitted that the use of ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate in various foods as an ingredient at levels of 0.2% to 5% in the finished food is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it is generally recognized as safe.

Respectfully Submitted,

Proliant Health Ingredients, Inc.

By

John B. Dubeck
Keller and Heckman LLP
COUNSEL FOR THE NOTIFIER

II. Identity of the Notified Substance

A. General Description

The notified substance is ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate (hereafter "ImmunoLin®" or "bovine globulin"). Bovine globulin is a spray-dried powder obtained from bovine blood. The finished product is ≥ 90% protein, ≤ 1.5 % fat, and ≤ 8% water. At least 45% of the protein component is immunoglobulin G (IgG).

Common Name: Bovine globulin
Chemical Name: Bovine globulin
Brand Name: ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate
CAS Reg. No: There is no CAS Registration number for bovine globulin.

B. Chemical Composition of Bovine Globulin

Bovine globulin is a spray-dried powder product obtained by removing the fibrin and albumin proteins from liquid bovine plasma by chemical and mechanical processes. The major component (> 90%) of ImmunoLin® consists of glycoproteins. The majority of these glycoproteins are folded into classic immunoglobulin (Ig) structures consisting of two light chains (~20,000 Daltons) and two heavy chains (~50,000-70,000 Daltons) linked together with disulfide bonds. Butler (1974), Lacelles (1970). Among the Ig's known to be present are IgG_1, IgG_2 and IgG fragments, IgA, secretory IgA, IgM, and IgE.

The chemical composition of ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate is set forth in Attachments 1, 2, 3, and 4. The data in these attachments represent the average of a minimum of four lots. Attachment 1 provides the composition of a typical batch of ImmunoLin®, including protein, carbohydrate, fat, ash, minerals and vitamins. Attachment 2 identifies and quantifies the different proteins present in the product. Attachment 3 shows the overall amino acid profile of the total protein in the product. Attachment 4 provides the detailed composition of the fatty acids in the product including the relative concentrations of saturated, monounsaturated and polyunsaturated acids.

No recombinant Bovine Somatotropin (rBST) hormones are present in the bovine globulin. Federal law prohibits the use of rBST in beef cattle, and ImmunoLin® is produced using only blood sourced from beef cattle.

C. Physical Properties of Bovine Globulin

Bovine globulin is a cream colored powder with a neutral flavor and odor. Bovine globulin is soluble in water and its major component is protein in the form of glycoproteins. Bovine globulin is a spray-dried protein, and it has the density and hydroscopicity typical of such proteins.

D. Stability of Bovine Globulin

Proliant's bovine globulin is stable for a minimum of two years when it is stored in the original container in a cool, dry environment. Stability data is presented in Attachment 5. ImmunoLin® samples from the lot numbers listed in the table were tested at the time of manufacture, which ranged from April 2003 – November 2006. The retained samples were then stored under ambient warehouse conditions and retested in May 2007, at which time the oldest sample was four years old. The lack of progressive decline in percent IgG (BIgG) over the four-year test period indicates that ImmunoLin® is stable from between 2 – 4 years. Proliant's bovine globulin contains no preservatives.

E. Manufacturing Process

The manufacturing process for bovine globulin can be divided into two parts. Part A concerns plasma collection and Part B addresses globulin production. Illustrated descriptions of Parts A and B of the manufacturing process are provided at Attachments 6 and 7, respectively. The manufacturing process is summarized below.

Bovine Plasma Collection (Part A)

1. United States Domestic cattle, grown without the use of feed containing specified-risk-materials (SRMs), are inspected and approved for slaughter by USDA/FSIS inspectors, in USDA/FSIS inspected facilities, using USDA/FSIS-approved slaughter and inspection criteria. Only healthy cattle are approved for slaughter, and only cattle approved for slaughter are sources of bovine blood for bovine globulin.

2. After being stunned, each bovine is hung in the air by the hind feet and the technician makes a longitudinal cut in the animal's dewlap. A cleaned and sanitized stainless steel bucket with a specially-designed oval opening containing the anticoagulant sodium citrate is placed inside the animal's open dewlap and hung by two hooks. The technician then cuts the jugular vein, which allows the blood to drain into the stainless steel bucket. After all of the animal's blood has drained into the bucket, the bucket is removed from the carcass and hung on a moving, indexed chain. The chain and attached bucket travel in sequence with the animal carcass until the carcass passes post-mortem inspection by the USDA/FSIS inspector.

3. After the slaughtered animal passes post-mortem inspection by the USDA/FSIS inspector (to ensure the slaughtered animal was healthy and not diseased), the collected blood is combined with other animals' blood in a storage tank. If a slaughtered animal fails the post-mortem inspection, the blood from this animal, as well as the blood from the animals immediately adjacent to (i.e.,, immediately before and after) the "failed" animal, is discarded into a rendering trough and excluded from the bovine globulin manufacturing process as a measure to prevent any potential cross-contamination. Blood from each animal is

not commingled until the subsequent animal in the processing line passes post-mortem inspection.

4. The blood collection process is designed to ensure the cleanliness and suitability for food use of the blood collected. The bucket's design, combined with its placement inside the dewlap, ensures that the bucket opening is exposed only to the clean inner surfaces of the dewlap, away from any hair, manure, etc. that might be on the carcass's surface. Both the buckets and the knives used in the blood collection process are cleaned with hot water (180°F) between uses as required by USDA. Furthermore, all buckets are thoroughly washed between uses in with cleaning and sanitizing agents that have been approved for use in contact with food. The blood collection step occurs while the animal's carcass is still intact, so no exposure to specific risk materials (SRMs; brain matter, spinal cord, etc.) occurs. Finally, the centrifugation and subsequent steps that process plasma into the globulin concentrate require two filtration steps through 200-mesh sock filters. While this filtration is intended primarily to eliminate any coagulated blood that occurs during the collection process, it also will remove any other extraneous material present in the blood.

5. The storage tank of bovine blood is emptied into a centrifuge, where the red blood cells are separated from the plasma and diverted for other uses. The majority of leukocytes precipitate out with the red blood cells, as both are heavier than plasma. The majority of the dissolved inorganic and organic substances present in the blood are retained with the plasma.

6. The plasma is cooled to below 45°F by running it through a plate chiller.

7. Plasma is pumped into an insulated silo, where it is held for pickup. The silo is maintained at 45°F or below for microbial stability. Under these conditions there is no change in the level of microorganisms during the holding period.

8. Plasma is removed from the silo and loaded into a cleaned, sanitized and dedicated Proliant Health Ingredients, Inc. trailer and brought to the processing facility. The lot code of the plasma shipment is identified by USDA seal on the shipping documents.

Bovine Globulin Production (Part B)

9. The temperature of each load of raw plasma is checked upon arrival at the manufacturing plant and before unloading. Any load that exceeds 45°F is rejected and shipped to a facility that processes inedible plasma.

10. Accepted plasma is off-loaded into the facility and transported via a cleaned and sanitized stainless steel pipe to a stainless steel holding tank, where it is stored at $\leq$ 45°F.

11. A commonly used GRAS food ingredient (under FDA's food additive regulations) is added as a processing aid to precipitate the fat and fibrin fractions, as well as any remaining leukocytes. (*See* Attachment 7, Delipidation).

12. The plasma is centrifuged to separate the lipids (fat), fibrin, and any remaining leukocytes from the blood serum.[1] This also removes from the blood serum the majority of cholesterol and phospholipids, as well as all of the processing aid used in Manufacturing Step #11 (*supra*).

13. 2 Normal hydrochloric acid (2N HCl) is added to the blood serum to adjust the pH to 4.2 or below. Acidification of the blood serum creates an unsuitable environment for viruses and microbes.

14. Common GRAS food ingredients are added to the blood serum as processing aids to help reduce clotting, to stabilize trace metals, and as a sequestrant to aid the separation of the serum into globulin and albumin.

15. The serum is centrifuged to separate the globulin and albumin components.

16. The globulin is collected and a solution of 15% Sodium Hydroxide is added to adjust the pH back to neutral.

17. The globulin undergoes ultrafiltration using a dialysis membrane to substantially remove all processing aids (including those functioning as pH adjusting agents, anticoagulants, trace metal stabilizers, and/or sequestrants). All remaining substances are present at levels that either comply with an approved food additive regulation or are affirmed by FDA regulation as generally recognized as safe.

18. The remaining globulin is concentrated by ultrafiltration to a level of 15% protein on a liquid basis ($\geq$ 85% on a dry basis).

19. The globulin is spray-dried in a dryer with an inlet temperature of 350°F - 495°F and an exit temperature of between 194°F - 206°F. The drying step produces a product with no more than 8% moisture and a water activity level of less than 0.50. After drying, the globulin is stored in a holding silo until being packaged.

20. The globulin powder is passed through a 30 mesh sieve, weighed, and packaged in 25 kg or larger sized poly-lined bags that are compliant with FDA regulations for food-contact materials. The filled bags are labeled, coded, and placed on a pallet.

21. All packaged product is stored at ambient temperature in an approved facility until released for shipment. The preferred storage of the product is in a cool, dry

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[1] *See* Particle Analysis Report at Attachment 8.

environment. Due to the low water activity level and acidification during processing, the product will not spoil in extreme conditions.

III. Regulatory Status of Food Processing Aids Used

The following processing aids are utilized in the ImmunoLin® manufacturing process:

1. Hydrochloric Acid - Generally Recognized as Safe (21 C.F.R. § 182.1057); acidulant, buffer, neutralizing agent.

2. Sodium Hydroxide - Generally Recognized as Safe (21 C.F.R. § 182.1763); alkalant.

3. Sodium Citrate - Generally Recognized as Safe (21 C.F.R. § 182.1751); blood anticoagulant.

4. A phosphate sequestrant, which is commonly used as a food ingredient and which has been affirmed by FDA as Generally Recognized as Safe.

5. A trace metal stabilizer and chelating agent, which is a common food ingredient and the use of which is consistent with an approved food additive regulation.

6. A precipitation aid, which is commonly used as a food ingredient consistent with an approved food additive regulation, and is used here as a processing aid that is later removed by centrifugation.

IV. Product Specifications

Specifications for food grade ImmunoLin® are shown below in Table 1. Compositional limits are established for protein, dry matter, ash content. Laboratory analyses indicate the absence of heavy metals, with arsenic not detected at < 1 ppm, lead not detected at < 0.5 ppm, and mercury not detected at < 0.05 ppm. There is nothing inherent in the raw material or in Proliant's manufacturing equipment or processes that would be a source of heavy metal contamination, and laboratory analyses confirm this. Accordingly, no specifications are included for these substances; this is consistent with Food Chemical Codex's policy of excluding heavy metal specifications when there is no need.

Microbiological specifications have also been established. The pH reduction to a strongly acidic state, combined with the spray drying process that yields a product with a water activity level of not more than 0.50 during the manufacturing process ensures that no microbes or viruses are present in the finished globulin product.

All raw materials are of a purity and quality suitable for the intended use. The product is manufactured in accordance with current good manufacturing practices for food.

Table 1. Product Specifications for ImmunoLin®			
Test	**Specification**	**Test Method**	**Reference**
Protein	≥ 85%	A.O.A.C. 16 Ed	992.15
IgG	≥ 45%	Radial ImmunoDiffusion (Internal method)	Etzel *et al.* 1997
Fat	≤ 1.5%	Acid hydrolysis A.O.A.C. 16 Ed	954.02
Moisture	≤ 8%	A.O.A.C. 16 Ed	934.01
Ash	≤ 3%	A.O.A.C. 16 Ed	942.05
Microbiological			
Total plate count	≤ 10,000 cfu/g	A.O.A.C.16 Ed.	990.12
Salmonella	ND	FDA BAM 8 Ed	Chap 5
Listeria	ND	FDA BAM 8 Ed A.O.A.C. 16 Ed	999.04
Staph. Aureus	ND	A.O.A.C.16 Ed.	975.55
E. Coli	ND	A.O.A.C.16 Ed.	992.30
ND = None Detected in sample			

V. Level of Use and Intended Technical Effect

Proliant's ImmunoLin® is intended to be used as an ingredient in dairy food products, fruit and/or vegetable juices, snack foods, beverages, and meal replacements. Typical use levels will vary from 0.2% to 2.0%, with a maximum use level of 5% of the food (2,000 to 20,000 ppm typical; 50,000 ppm maximum). *See* Table 2, below.

Table 2. Requested Food Types and ImmunoLin® Use Levels		
Foods	**Use Levels (ppm)**	**Intended Uses**
Dairy foods	2,000 – 50,000	Protein supplement
Fruit/Vegetable Juices	2,000 – 50,000	Protein supplement
Snack foods	2,000 – 50,000	Protein supplement
Beverages	2,000 – 50,000	Protein supplement
Meal replacements	2,000 – 50,000	Protein supplement

Bovine globulin is considered a high value protein, and its use is intended to provide only a portion – rather than be the sole source – of protein in food. This purpose can be achieved via the addition of small amounts – generally 2% or less – to food. Due to its nature, bovine

globulin has a self-limiting level of use in food. Indeed, use levels of bovine globulin greater than 5% in food lead to adverse, undesirable flavor profiles. Furthermore, due to the higher financial cost of globulin proteins as compared to other proteins that are widely available for supplementation, food processors are likely to use ImmunoLin® in a blend with less costly proteins.

VI. Methodology for Analysis of IgG in Bovine Globulin

The IgG fraction of the protein in bovine globulin is analyzed by Radial ImmunoDiffusion (RID). RID analysis utilizes the diffusion of antigen from a circular well into a homogeneous gel containing specific antiserum for each particular antigen. A detailed description of the analytical method is provided in Attachment 9.

VII. Estimated Dietary Intake

When estimating consumer exposure to a dietary substance, FDA assumes that approximately 1,500 grams of liquid food and 1,500 grams of solid food is consumed daily by the average adult male.[2] In a balanced diet, the 1,500 grams of solid food is allotted as approximately 300 g of carbohydrate, 50 g of protein, 65 g of fat, 25 g of fiber.[3] This level of protein intake is consistent with FDA's established Recommended Daily Intake (RDI) of 50 grams for protein.

As indicated above, Proliant's bovine globulin product will typically be added to dairy foods, fruit and/or vegetable juice products, snack foods, beverages, and meal replacement products at a level of 0.2 to 2.0%. Using the higher 2% use level, a maximum of 30 grams of solid food could be ImmunoLin® (0.02 g ImmunoLin®/g food x 1500 g food = 30 g ImmunoLin®). This calculation greatly exaggerates the expected amount of ImmunoLin® in the diet because it assumes ImmunoLin® will be added to all foods, rather than only certain targeted foods. Additionally, it assumes that ImmunoLin® is the sole protein used for supplementation which, as noted above, is highly unlikely due to its higher cost as compared to other readily available protein sources. Nonetheless, this exercise establishes that an intake level of 30grams is a conservative estimate of the amount of ImmunoLin® that a very determined consumer of ImmunoLin® might ingest. A more reasonable estimate of the expected dietary intake of ImmunoLin® is 2.5 to 25 grams per adult per day, which is 5% - 50% of the Daily Reference Value (DRV) of 50 g for protein[4], depending on the level of consumption and the variety of food types consumed.

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[2] This calculation assumes for simplicity that the liquid portion of the diet does not contribute any calories.

[3] If the calories in the solid components are summed, the total equals FDA's recommended daily caloric intake. (4 Cal/g × 300 g) + (4 Cal/g × 50 g) + (9 Cal/g × 65 g) = 1200 Cal + 200 Cal + 585 Cal = 1985 Calories (approximately equal to 2000 Calories).

[4] *Accord* 21 C.F.R. § 101.9(c)(9).

No special target population is envisaged; the population consuming dairy foods, fruit and vegetable juice products, snack foods, beverages and meal supplement products is very diverse and widespread.

VIII. Basis for the GRAS Determination

There is a vast amount of scientific literature attesting that proteins and protein supplements have been safely used for many years in human food. This GRAS Notification for bovine globulin relies primarily on the generally recognized safety of dietary proteins, the specific amino acid composition of the notified protein (bovine globulin), and Proliant's purification process that consistently produces bovine globulin that meets rigorous and defined purity and protein quality specifications. In addition, we present data substantiating that ImmunoLin® contains no chemical impurities, that Bovine Spongiform Encephalopathy (BSE)-related food safety measures are in place and are effective, and that humans and animals have safely consumed bovine globulin as a component of their diet without harm.

A. Bovine Plasma and Bovine Globulin are Recognized, Safe Protein Sources

Bovine plasma has been used for its protein content in both human food and animal feed for many, many years. There is a vast amount of literature attesting to the safe and wide human food use of bovine plasma in products such as surimi, sausage, and processed meats. FDA has considered the safety of bovine plasma, and in 1992 the Agency issued a letter stating that it would not contest a self-determined GRAS position for this ingredient.[5] The bovine globulin that is the subject of this GRAS Notification is a high quality, purified protein extract derived from bovine plasma which is of equivalent or superior protein quality.

Conventional scientific support for a GRAS substance usually rests on animal safety studies demonstrating a valid No Observed Effect Level (NOEL), to which is applied a 100-fold safety factor to determine an Acceptable Daily Intake (ADI). In the present situation, however, the required degree of exaggeration in the diet of animals needed to achieve a 100-fold safety factor is not possible because the subject protein is both substantial in the diet and non-toxic (it has a high NOEL). To wit, application of a 100-fold safety factor to a NOEL of 20% or 200,000 ppm in the diet (which is a reasonable maximum dietary level for protein that could be incorporated into the diet of a laboratory animal), would yield an ADI for man of 2,000 ppm or 100 mg/kg bw/day in the diet.[6] This corresponds to a daily adult intake of only 7 grams of protein per person per day (g/p/d) for an adult weighing 70 kilograms (kg), whereas the nominal adult DRV for protein intake is 50 g/p/d.

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[5] Letter from Alan M. Rulis, Ph.D., Director, Division of Food and Color Additives, Center for Food Safety and Applied Nutrition, FDA, to John Dubeck, Keller and Heckman, April 23, 1992; copy provided at Attachment 10.

[6] The conversion factor for relating ppm in the diet to mg/kg bw/day for a chronic feeding study in rats is 0.05. *See* Association of Food and Drug Officials. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. (1959). Alternatively, if we assume consumption rates are comparable: 2000 ppm = 2 g/kg or a total of 6 g protein for a 3 kg human diet.

For macro foods such as protein, FDA recognizes that alternate means of demonstrating safety arenecessary and appropriate, and it has approved the use of food substances at substantial levels with less than 100-fold safety factors (*e.g.*, vitamins, olestra).[7] Both FDA and the World Health Organization/Food and Agriculture Organization (WHO/FAO) have recognized that if a substance is metabolized in the body to common food metabolites, it may be unreasonable to insist on a 100-fold safety factor.[8] Indeed, in its 1997 proposed rule, FDA described the data to be used in satisfying the technical element of scientific procedures and specifically stated:

> "However, depending on the circumstances, other scientific data and scientific information such as relating to chemical identity or characteristic properties of a substance, as well as methods of manufacture, could support, and in some cases be sufficient to satisfy that element."[9]

B. Traditional Safety Considerations for Protein Concentrates are Addressed

Traditional safety concerns for protein concentrates generally have included: (1) the possible formation of unique amino acids in processing (*e.g.*, exposure to high temperatures can cause lysoalanine production from potato protein), (2) inadequate nutritional quality of the protein due to insufficient amounts of essential amino acids, (3) the presence and potential concentration of toxic impurities when evaporations or extractions are involved in the processing, and (4) the potential for allergenicity. As explained below, the manufacturing procedure for ImmunoLin® satisfactorily addresses each of these potential issues so they are not problematic for ImmunoLin®.

1. ImmunoLin® Manufacturing Process Yields No Unique Amino Acids

Proliant's ImmunoLin® production process calls for the protein to be separated from bovine plasma at room temperature and immediately cooled. Only mechanical processes, centrifugation and filtration are used in the separation phases and only pH changes are involved in separating the albumins from the globulins. No other chemical changes are involved. The only time heat is utilized in the production process is during spray-drying, during which the protein's temperature is moderated by the evaporating water. Furthermore, very little water remains with the protein by the time it exits the dryer, so water-facilitated heat-induced molecular rearrangements of the protein are unlikely.

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7 *See e.g.,* final rule authorizing the use of olestra as a food additive. 61 Fed. Reg. 3118, 3120 (January 30, 1996).

8 *Id.*

9 62 Fed. Reg. 18938, 18942 (April 17, 1997) "Substances Generally Recognized as Safe," Proposed Rule.

2. ImmunoLin® is a High Quality Protein

The essential amino acid composition of ImmunoLin® is shown below in Table 3, along with a comparison to the amino acid composition of protein obtained from eggs and beef.[10] Each of the nine essential amino acids are present in ImmunoLin® in amounts that exceed or are comparable to the standard reference, egg white protein, which is used to determine the Protein Digestibility Corrected Amino Acid Score (PDCAAS).[11] A dietary protein containing all nine of the essential amino acids in the proportions required by the body is considered a high quality protein.

Protein Digestibility – As described in footnote 11, the PDCAAS expresses the quality of a protein based on the amino acid requirements of humans, as adjusted by correcting for digestibility. Digestibility is determined by the rat fecal protein digestibility assay of the subject protein. The validity of this aspect of the PDCAAS has been questioned by researchers who argue that fecal digestibility overestimates the nutritional value of a protein because amino acids that move beyond the terminal ileum are unlikely to be absorbed by the body for protein synthesis.[12] As noted in the text accompanying Table 3, the protein digestibility value used to establish the PDCAAS for ImmunoLin® is based upon ileal measurements in pigs of spray-dried plasma. While spray-dried plasma has less IgG than ImmunoLin®, additional data from human studies suggest that a 93.4% protein digestibility value for ImmunoLin® is conservative notwithstanding evidence that oral IgG possesses immunological activity when ingested and a study (Roos *et al.*, 1995)[13] which shows a significant amount of IgG survives the stomach and small intestines to reach the colon intact.

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[10] The amino acid content values for ImmunoLin® set forth in Table 3 are an average of the values obtained by laboratory analysis of two composite samples, each of which was sourced from five individual lots. Certificate of Analysis CDR-31312008-0 (dated 8/7/02).

[11] The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a method for evaluating protein quality based on the needs of human children that was adopted by FDA and the FAO/WHO in 1993. A protein with a PDCAAS of 1.0 is a complete protein and has the same score as milk protein and egg white. To determine a protein's PDCAAS , calculate the amount (in mg/g) of each amino acid in the food and divide that amount by the relevant FAO value, which is the amount of that amino acid (in mg/g) required in the diet of a 2 – 5 year old child. This yields an "Uncorrected Amino Acid Score" for each amino acid in the food. All uncorrected amino acid scores are compared, and the lowest uncorrected amino acid score is the limiting amino acid in the food. Multiply the value of this limiting amino acid by the percent digestibility of ImmunoLin® to yield the PDCAAS for the product. A worksheet showing all calculations to determine ImmunoLin®'s PDCAAS is set forth in Attachment 12.

[12] *See e.g.*, Schaafsma G (2000) The Protein Digestibility–Corrected Amino Acid Score. *Journal of Nutrition*, 130:1865S-1867S.

[13] Roos N, Mahe S *et al.* (1995) 15N –labeled immunoglobulin from bovine colostrum are partially resistant to digestion in human intestine *Journal of Nutrition*, 125: 1238-1244.

Kelly *et al.* (1997)[14] measured the survival of orally administered bovine immunoglobulin (IgG) concentrate with an IgG level comparable to ImmunoLin® in the human gastrointestinal tract of six healthy volunteers under varying conditions, *i.e.*, with food, with antacid, during antacid secretory therapy, and in enteric capsules. When fed with food, the survivability of the oral IgG was only 1.6% of the applied dose, fully consistent with traditionally measured plasma digestibility values. Only with the use of enteric coated capsules to shelter the IgG was there a highly significant degree of survival through the intestinal tract (32.7%). In companion *in vitro* experiments with IgG added to gastric fluid at pH 1-2, there was a rapid and near complete degradation of the IgG binding capacity. Both the *in vivo* and *in vitro* studies demonstrate that IgG is almost entirely digested in the GI tract, but that a small proportion does escape digestion, and can still be detected in the colon, where it can have measurable antitoxin, neutralizing activity.

Bogstedt and Robinison (1997)[15] studied the survival of IgG in the GI tract of four healthy volunteers by using different protocols aimed at prolonging IgG survival. These authors also found virtually all detectable IgG was digested, and only minute amounts of active antibody (< 0.01%) were found in the stools.

Both of the papers above cite the paper by Roos *et al.* (1995) that appears to conflict with their findings. Roos *et al.* found that 19% of the ingested IgG was still immunologically active in the ileum. In the Roos *et al.* study, however, the subjects were fasted, which significantly reduced the transit time. This was apparently considered sufficient by Kelly *et al.* to explain the discrepant results. Bogstedt *et al.* also suggested the possibility that there could still be some digestibility occurring in the ilea, which could account for both the significant levels of active IgG in the ileum and the much lower levels in the feces. The high digestibility of bovine plasma protein containing IgG is supported by the studies in pigs by Pierce *et al.* (2005)[16] and in studies in malnourished children by Lembcke, Peerson and Brown, (1997).[17]

So while there is research that suggests IgG has an immunological effect and that there is low IgG activity in the colon under some circumstances, it is clear that oral immunoglobulin still serves as a highly digestible protein.

Amino Acid Composition – As demonstrated in Table 3, the PDCAAS for ImmunoLin® is 0.92, meaning that it is a complete protein with adequate levels of all the essential amino acids.

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[14] Kelly CP *et al.* (1997) Survival of anti-clostridium difficile bovine immunoglobin concentrate in the human gastrointestinal tract. *Antimicrobial Agents and Chemotherapy, 41(2):* 236-241.

[15] Borstedt A K, Hammarstrom, Robertson A-K (1997) Letter to the Editor. Survival of Immunoglobulins from different species through the gastrointestinal tract in health human adult volunteers: Implications for human therapy. *Antimicrobial Agents and Chemotherapy, 41(10):* 2320.

[16] Pierce JL, Cromwell GL, Lindemann , *et al.* (2005) Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. *Journal of Animal Science*, 83: 2878-2885.

[17] Lembcke JL, Peerson M, and Brown HH (1997) Acceptability, Safety, and Digestibility of Spray-dried bovine serum added to diets of recovering malnourished children. *Journal of Pediatric Gastroenterology and Nutrition*, 25:381-384.

Isoleucine is the limiting amino acid, with an uncorrected amino acid score of 0.98; all the other amino acids exceed the levels of the reference protein.[18] The analytical data upon which these values are based appears in Attachment 11. While it is extremely unlikely that an individual would derive all his dietary protein from ImmunoLin® for an extended period, the extremely high quality of ImmunoLin®'s protein makes such an intake completely safe and acceptable.

Table 3. Essential Amino Acid Composition of Immunolin®: Comparison with Other Proteins

Amino Acid	Protein content (mg/g) protein in:			FAO Value	Uncorrected AA Score
	Egg	Beef	ImmunoLin®		
Histidine	22	34	24	19	1.28
Isoleucine	54	48	27	28	0.98
Leucine	86	81	79	66	1.20
Lysine	70	89	61	58	1.05
Methionine + Cystine	57	40	11 + 21 = 32	25	1.28
Phenylanine + Tyrosine	93	80	42 + 50 = 92	63	1.46
Threonine	47	46	66	34	1.93
Tryptophan	17	12	20	11	1.82
Valine	66	50	82	35	2.35

ImmunoLin® has 93.4 % digestibility* **PDCAAS = 0.98 x .934 = 0.915 ~ 0.92**

* Digestibility is calculated from the data in Table 5 of Gottlob *et al.*, (2006). This number represents an average of the standardized ileal digestibility in pigs of all the amino acids analyzed from an oral feeding study of spray-dried plasma protein.

3. <u>ImmunoLin® Production Method Does Not Concentrate Impurities</u>

Proliant's production process ensures that any potential impurities in the bovine blood are not concentrated in the ImmunoLin® bovine globulin product. The production process contains two factors that could affect impurities in the globulin – one that might increase the amount (concentration) and one that definitely will decrease the amount (dilution). As explained below and as substantiated by analytical results presented in Attachments 13 - 18, any concentration that may hypothetically occur will be entirely offset by the known dilution effect so no concentration can occur.

The first factor is dilution. The bovine blood collected and used to make the product is the composite of the blood from as many as 1,800 animals, obtained from no less than 35 to 50 cattle producers or feed lots. This greatly dilutes the amount of any impurities that may have

[18] If desired, caseinate complements can be added to beef plasma to make up for any deficiencies in isoleucine and methionine content. Wismer-Pederson. (1979). Utilization of animal blood in meat products. *Journal of Food Technology*; 33(8): 76-80.

existed in the blood of a few of the animals. For example, if a few animals had out-of-tolerance lead residues, these residues would effectively disappear after their blood was combined with that of the other animals in the bulk blood tank.

The second factor is concentration. The separation of the protein fraction from the composite raw plasma in the ImmunoLin® manufacturing process causes the immunoglobulin fraction to be concentrated. Thus, any impurities that might preferentially combine with the protein fraction could potentially be concentrated in the final product. Since the non-immunoglobulin protein fraction (mainly albumin) accounts for approximately 7% of the raw plasma and the immunoglobulin protein fraction constitutes approximately 1.7% of the raw plasma, any impurities that are primarily associated with the combined protein fraction could result in a roughly 10-fold concentration of impurities in the protein fraction over that present in the raw plasma. In light of this potential for concentration, a determination of the potential presence of impurities must be evaluated using the immunoglobulin fraction itself (*i.e.* the ImmunoLin®). Summarized below are the analytical results of ImmunoLin® evaluated for pesticide residues, heavy metal contaminations, and antibiotic residues.

Pesticides – Four samples of ImmunoLin® were evaluated using an organochlorine pesticide screen, which tests for the 61 common chlorinated pesticides approved by EPA. The four samples of ImmunoLin® were sourced from different lots, produced on different days, and are representative of Proliant's blood supply source.[19] Only chlorobenzilate (< 0.4 ppm) and polychlorinated biphenyls (< 0.250 ppm) exceeded the < 0.100 ppm level. All samples were below the 2.0 ppm tolerance that USDA uses for muscle meat. The analytical results are provided at pages 3-5 of Attachments 13, 14, 15, and 16 and pages 1-2 of Attachment 17.

Heavy metals – The same four samples of ImmunoLin® that were tested for pesticide content also were tested for arsenic, lead and mercury content. The results indicated that arsenic was present at less than 0.99 ppm, lead was present at less than 0.5 ppm and mercury was present at less than 0.05 ppm. These results are provided on page 3 of Attachments 13, 14, 15, and 16.

Antibiotics – A composite sample obtained from 10 separate lots was analyzed for antibiotic residues, notably sulfa drugs and the tetracyclines. Sulfadimethoxine, sulfathiosol, sulfamethazine, and sulfaquinoxaline were all present at < 0.01 ppm. Tetracycline,

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[19] The analytical results for the four samples appear on Certificate of Analysis (COA) No. CDR-31648961-1 (dated 3/19/07; copy provided as Attachment 13) (this COA supercedes COA No. CDR-31648961-0, which was incomplete); COA No. CDR-31658783-0 (dated 3/19/07; Attachment 14); COA No. CDR-31658784-0 (dated 3/19/07; Attachment 15); and COA No. CDR-31658785-0 (dated 3/19/07; Attachment 16). Each of the samples was obtained from different production batches of ImmunoLin®, produced on different days. Proliant produces ImmunoLin® from blood sourced from two slaughterhouses; both are owned by the same company and both slaughter only "fat cattle" (2 to 3 years of age; no cows) obtained from similar sources. No differences are expected between plasma produced from blood obtained from the two facilities.

chlortetracycline and oxytetracycline were present at < 0.1 ppm. The analytical results are provided at page 3 of Attachment 17.[20]

4. Blood Products from U.S. Cattle Pose No BSE Risk

There currently is minimal official concern regarding the safe consumption of bovine blood, bovine plasma, or protein products derived from bovine blood. This confidence is based on several considerations, including the essentially zero natural prevalence of bovine spongiform encephalopathy (BSE) in cattle in the United States, which demonstrates the effectiveness of the BSE-prevention and sanitation practices FDA and USDA mandate. Additionally, numerous, well-controlled and properly executed animal studies have demonstrated the complete absence of any recorded infectivity of blood. This absence of infectivity is why food and feed officials have excluded bovine blood from the category of Specific Risk Materials (SRMs), whose use in animal feed or human food is prohibited. Finally, any potential concentration of BSE-infective prions in immunoglobulins during the ImmunoLin® production process is more than offset by the dilution effect resulting from the pooling of blood from many cattle. A complete and thorough discussion of the scientific nature and causes of BSE, the effectiveness of existing food safety measures, and the reasons why ImmunoLin® is safe to consume are set forth in Attachment 18.

5. No Potential for Allergenicity

It is highly unlikely that the proposed use of ImmunoLin® as a supplemental protein ingredient in several food categories will result in allergic reactions. The proteins in bovine plasma have never been associated with allergic responses despite wide used in the animal feed and food industries. Food products containing blood-derived proteins have been consumed in food for centuries without allergic responses. The Food Allergen Labeling and Consumer Protection Act (FALCPA), which specifically addresses allergenic material, excludes bovine protein from its list of allergens.[21] Furthermore, there are no reports of allergic responses due to environmental exposure to bovine protein in the hundreds of manufacturing and food processing facilities that use bovine protein-derived products, even in facilities where some inhalation exposures of bovine plasma dust could reasonably be expected to occur.

6. Animal Studies Show Bovine Plasma Consumption is Safe

Spray-dried blood and plasma proteins have long been recognized as high quality feed ingredients for farm animals, including swine, cattle and poultry. Blood proteins have an excellent amino acid profile and high digestibility. In addition to use as a source of protein, spray-dried animal plasma also has been recommended for inclusion in animal diets as a source

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[20] COA No. CDR-30338990-0 (dated 9/22/04). The limits of detection for arsenic, lead, and mercury levels indicated in Attachments 13-16 are lower than those set forth in Attachment 17. This is due to increased sample sizes in the more recent analyses, which concentrates any potential impurities in the sample and allows a more accurate determination of the amount of impurities in the sample.

[21] Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282); enacted August 2, 2004.

of immunological support. It has been used in the diets of commercially reared, early weaned pigs for about 20 years. Today, the vast majority of all pig starter feeds (*i.e.*, fed to pigs immediately after weaning) used in the U.S. contain spray-dried animal plasma (SDAP) at levels of 2 to 4% of the formulation.

Most of the animal studies on bovine plasma have shown improved feed intake, animal growth, feed efficiency, and animal health when animals' diets were supplemented with SDAP. Furthermore, no adverse affects have been reported. Coffey and Cromwell reviewed over 60 studies that have been conducted on the safety and nutritional performance of spray dried plasma fed at doses from 2% to 14% to weaning pigs.[22] These studies comprise literally millions of animals and represent a safety and nutritional dossier rarely found in support of a food ingredient.[23] A general summary of SDAP-fed animal studies, with references to the copious amount of published scientific literature, is presented in Attachment 19. Since the spray dried animal plasma used in these studies contained from 10% to 25% immunoglobulin, the studies are useful surrogates for the safe ingestion of pure immunoglobulin.

7. Human Studies Show Bovine Plasma Consumption is Safe

Several human studies on plasma immunoglobulin have been conducted. Two studies in human infants and young children showed that oral administration of human serum immunoglobulin results in a faster recovery from acute rotaviral gastroenteritis.[24,25] Both of these studies also showed that the ingestion of immunoglobulin by human infants and young children is well tolerated. A third clinical study on human subjects also found that bovine blood plasma added to the diets of severely malnourished Peruvian infants and very young children (9 to 25 months of age) was well tolerated.[26]

As noted above, a common observation when supplementing animal diets with immunoglobulin is increased weight gain. Experimental trials in newly weaned farm animals have demonstrated that incorporation of spray-dried bovine or porcine blood plasma into their feeds increases their dietary intakes and produces greater weight gains compared with control diets.[27] This weight gain in animals inspired several researchers to conduct a study to see

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22 Coffey Rd and Cromell GK. (2001). Use of spray-dried animal plasma in diets for weanling pigs. *Pig News and Information*; 22(2): 38N-48N.

23 *Id.* The studies focused mainly on nutritional performance (*e.g.,* overall health, behavior, food efficiency, weight gain and other measures of development). No evidence of organ pathology was suggested and no pathology or histology was performed as would be in a conventional safety study.

24 Turner RB, Kelsey DK. (1993). Passive Immunization for the Prevention of Rotovirus Infection in Young Infants. *Pediatric Infectious Diseases Journal*; 12(9): 718-722.

25 Guarino A. Cahani RB, *et al.* (1994). Oral Immunoglobulins for Treatment of Acute Rotoviral Gastroenteritis. *Pediatrics*; 93: 12-16.

26 Lembcke, Peerson, Brown, (1997).

27 Pierce JL *et al.*,(2005).

whether this same benefit could be observed among malnourished Guatamalan infants.[28] The study examined malnourished infants starting at aged 6-7 months and followed their development for 8 months. Two of the study groups consumed 3.1 grams of spray-dried bovine serum concentrate per day for 60 days. The concentrate contained 22% IgG, which resulted in a daily consumption of 0.68g of IgG per infant. A total of 63 infants that consumed the concentrate completed the 8 month observation period, and no effects on morbidity indices due to the supplement were observed. Additionally, no increases in frequency of respiratory or diarrheal infections related to bovine serum concentrate supplementation were observed.

IX. Conclusion

The studies, analytical data, and information presented above and in the Attacments to this Notification demonstrate the basis of the Notifier's position that ImmunoLin® Brand Spray-Dried Bovine Globulin Concentrate may properly be deemed to be generally recognized as safe, based on scientific procedures, for use in selected foods as a protein supplement at use levels of 0.2% to 5% in the finished food. As such, ImmunoLin® is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

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[28] Begin F, Santizo M-C, Peerson JM, Torun B , Brown KH. (2004). Effects of Bovine Serum Concentrate, With or Without Supplemental Micronutrients, on the Growth , Morbidity and Micronutrient Status of Young Children in Low Income, Peri-Urban Guatemalan Communities. Copy accessible at: www.ncbi.nlm.nih.gov/sites/entrez?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17299460.

1

ATTACHMENT 1

Nutritional Profile

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The Science of Protein.

ImmunoLin®
Spray-Dried Bovine Serum Globulin
Typical Nutritional Profile

MACRONUTRIENTS	
Protein	89.9%
Moisture	6.6%
Ash	2.0%
*Carbohydrates	1.3%
Sucrose	<0.1%
Dextrose	<0.1%
Glucose	<0.1%
Fructose	<0.1%
Lactose	<0.1%
Maltose	<0.1%
Fat	0.2%
Cholesterol	14 mg/100g
*Total Calories	367 K Cal/100g
MINERALS	
Calcium	147 mg/100g
Chloride	110 mg/100g
Magnesium	26 mg/100g
Phosphorus	543 mg/100g
Potassium	40 mg/100g
Sodium	461 mg/100g
VITAMINS	
Vitamin C	<1.0 mg/100g
Vitamin B_1	0.1 mg/100g
Vitamin B_{12}	<0.1 mg/100g
Vitamin B_2	<0.1 mg/100g
Vitamin A	835 IU/100g

Rev 01/11/2008

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ATTACHMENT 2

Proteins in ImmunLin

Proteins in ImmunoLin®

Theoretical Values

Data is represented as % of total, dry basis (%db)

Major Classes of Proteins	% of Total Protein	Major Protein(s)
Gamma globulin	60 - 70%	Bovine IgG, IgM, IgA
Beta globulins	15 - 18%	Transferrin
Alpha proteins	10 - 15%	Alpha-1 AcidGlycoProtein
Albumin	5 - 10%	Albumin

Actual Data by Analysis

Total Protein (db) ≥90%

The proteins break down further as follows:

Gamma globulins (RID) *			59%
	IgG total	54%	
	IgG-1	41%	
	IgG-2	13%	
	IgA	0.3%	
	IgM	5.0%	
Beta proteins			18%
Alpha proteins			10%
Albumin (AGE) **			5%

* Radialimmunodiffusion

** Agarose gel electrophoresis

3

ATTACHMENT 3

Typical Amino Acid Profile

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The Science of Protein.

ImmunoLin®
Spray-Dried Bovine Serum Globulin
Typical Amino Acid Profile

	*Typical (g)
Alanine	4.1
Arginine	5.3
Aspartic Acid	9.2
Cystine	2.1
Glutamic Acid	12.9
Glycine	4.1
Histidine	2.4
Isoleucine	2.7
Leucine	7.9
Lysine	6.1
Methionine	1.1
Phenylalanine	4.2
Proline	6.7
Serine	9.3
Threonine	6.6
Tryptophan	2.0
Tyrosine	5.0
Valine	8.2

Amino acids as % of total amino acids

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ATTACHMENT 4

Fatty Acid Profile

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The Science of Protein.

ImmunoLin®
Spray-Dried Bovine Serum Globulin
Fatty Acid Profile

SATURATED	FORMULA	%
CAPROIC	6:0	0.4
CAPRYLIC	8:0	0.8
CAPRIC	10:0	0.3
UNDECANOIC	11:0	<0.1
LAURIC	12:0	0.4
TRIDECANOIC	13:0	<0.1
MYRISTIC	14:0	1.2
PENTADECANOIC	15:0	0.3
PALMITIC	16:0	16.9
MARGARIC	17:0	0.3
STEARIC	18:0	16.9
EICOSANOIC	20:0	<0.1
BEHENIC	22:00	0.6
TRICOSANOIC	23:00	0.2
LIGNOCERIC	24:0	<0.1

MONOUNSATURATED	FORMULA	%
MYRISTOLEIC	14:1	0.6
PENTADECENOIC	15:1	0.5
PALMITOLEIC	16:1	1.5
MARGAROLEIC	17:1	0.9
ELAIDIC	16:1	<0.1
OLEIC	18:1	23.5
GADOLEIC	20:1	0.3
HENEICOSANOIC	21:0	0.8
ERUCIC	22:1	0.3
NERVONIC	24:1	<0.1

POLYUNSATURATED	FORMULA	%
LINOLEIC	18:2	29.2
LINOLELAIDIC	18:2	<0.1
LINOLENIC	18:3	1.6
GAMMA LINOLENIC	18:3	<0.1
EICOSADIENOIC	20:2	1.0
EICOSATRIENOIC (2 forms)	20:3	0.5
ARACHIDONIC	20:4	0.8
EICOSAPENTAENOIC	20:5	0.2
DOCOSADIENOIC	22:2	<0.1
DOCOSAHEXAENOIC	22:6	<0.1

SATURATED FATTY ACID	-	**TOTAL**	38.3
MONOUNSATURATED FATTY ACID	-	**TOTAL**	28.4
POLYUNSATURATED FATTY ACID	-	**TOTAL**	33.3

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ATTACHMENT 5

Stability Table

Immunolin Stability Testing for GRAS submission
5/2/2007

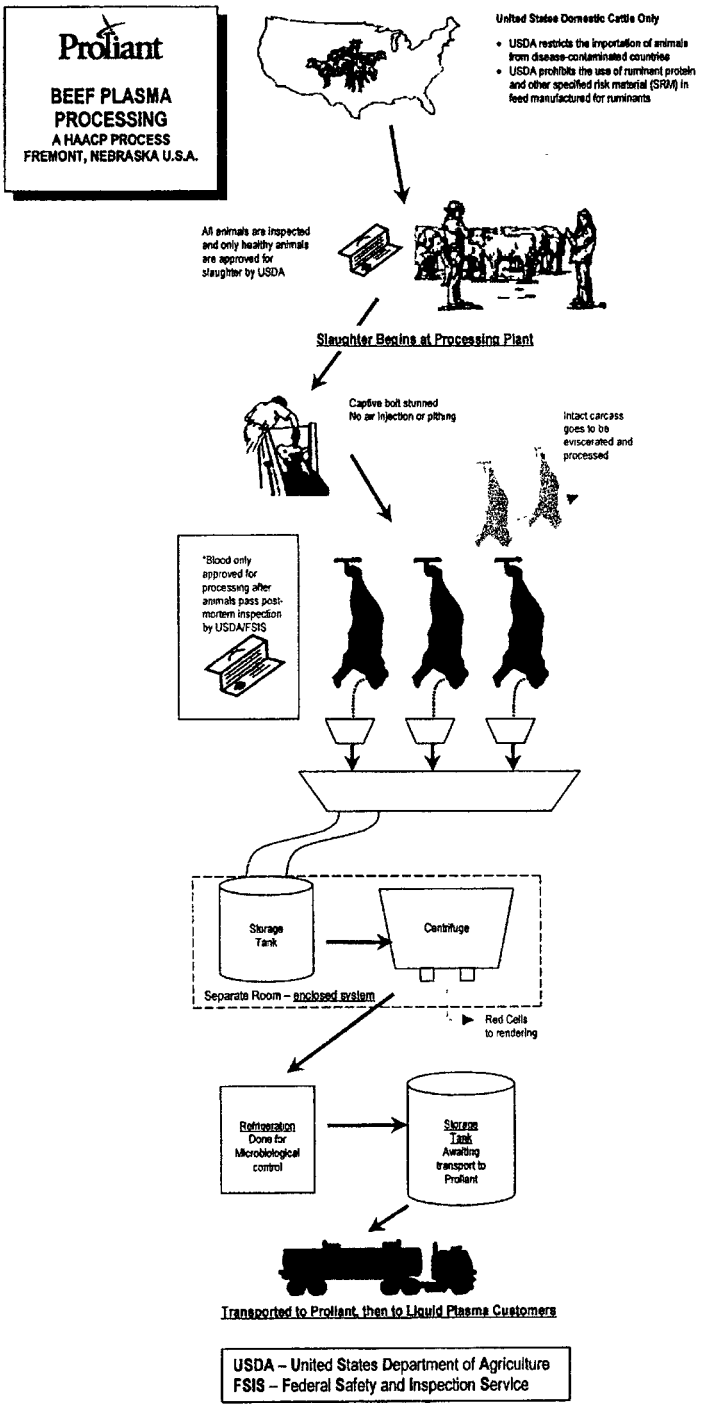
Lot #	Month/Year of ORIGINAL * Testing	BIgG - COBAS ORIGINAL *	Protein ORIGINAL *	Moisture ORIGINAL *	Protein (db) ORIGINAL *	Month/Year of RETEST ++	Age of Sample RETEST ++ (months)	BIgG -COBAS RETEST ++	Protein RETEST ++	Moisture RETEST ++	Protein (db) RETEST ++
BB63260101	Nov-06	58.0	92.50	5.01	97.51	May-07	6	56.0	92.38	5.00	97.38
BB62610101	Sep-06	60.0	94.38	4.72	99.10	May-07	8	58.0	92.69	4.71	97.40
BB61940101	Jul-06	56.0	90.31	4.62	94.93	May-07	10	54.0	92.88	4.63	97.51
BB52640101	Sep-05	56.0	92.31	5.26	97.57	May-07	20	56.0	91.31	5.35	96.66
BB51680101	Jun-05	54.0	91.50	5.82	97.32	May-07	23	52.0	90.69	5.99	96.68
BB50880101	Mar-05	58.0	91.56	5.36	96.92	May-07	26	56.0	90.56	6.22	96.78
BB43130101	Nov-04	50.0	90.56	6.76	97.32	May-07	30	50.0	89.69	7.25	96.94
BB41070101	Apr-04	54.0	89.72	6.51	96.23	May-07	37	52.0	87.81	8.23	96.04
BB40610101	Mar-04	54.0	90.49	5.86	96.35	May-07	38	54.0	89.00	7.51	96.51
BB33090101	Nov-03	50.0	92.32	4.85	97.17	May-07	42	56.0	89.69	7.51	97.20
BB31650101	Jun-03	56.0	90.75	5.28	96.03	May-07	47	50.0	89.38	7.94	97.32
BB30920101	Apr-03	52.0	90.95	5.36	96.31	May-07	49 (4 years)	54.0	88.31	7.64	95.95

* Testing done at time of manufacture

++ Retests were performed on the retain samples collected at time of manufacture

6

ATTACHMENT 6



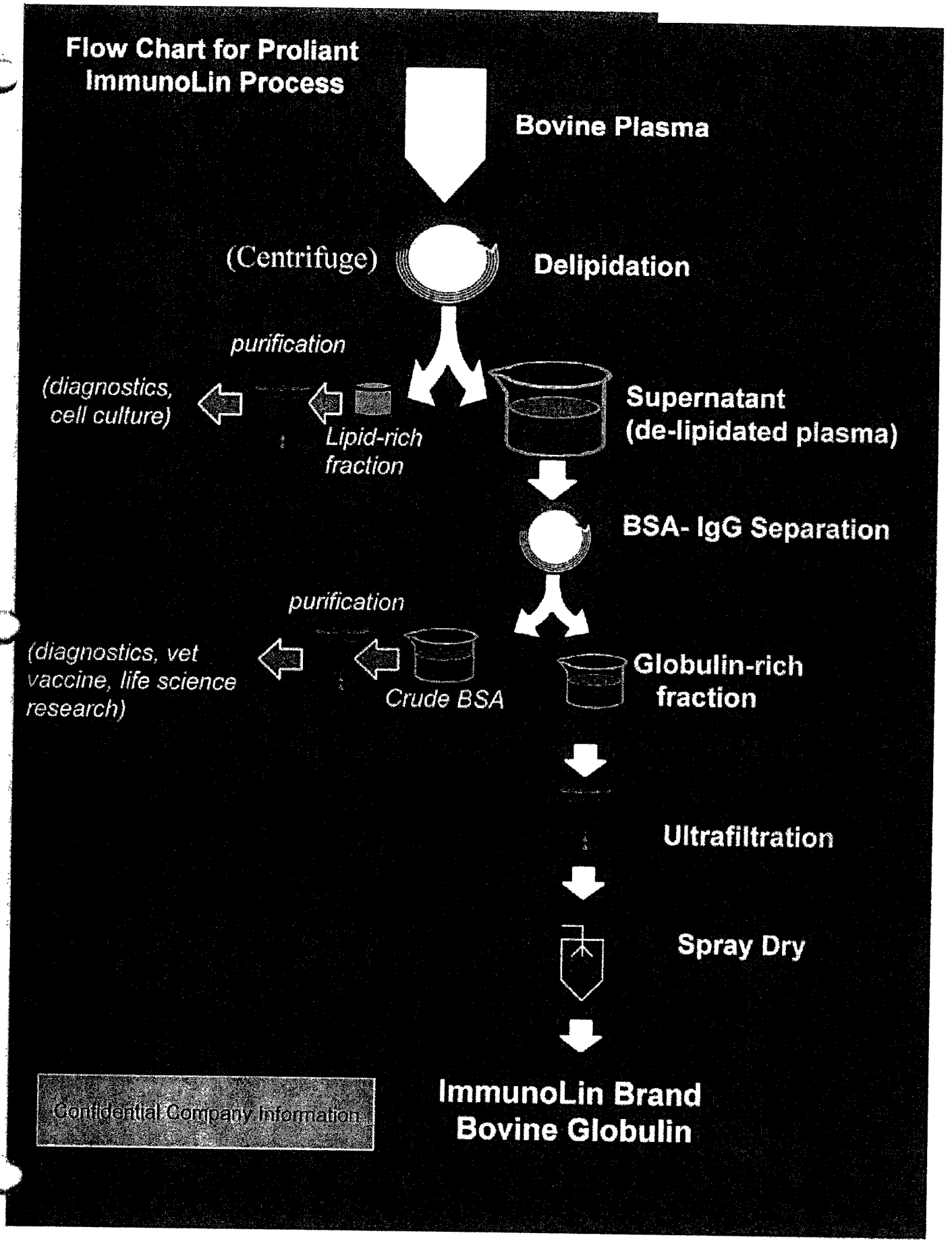
000044

000044

7

ATTACHMENT 7

Process Flow Chart


Flow Chart for Proliant ImmunoLin Process
Bovine Plasma
(Centrifuge)
Delipidation
purification
(diagnostics, cell culture)
Lipid-rich fraction
Supernatant (de-lipidated plasma)
BSA- IgG Separation
purification
(diagnostics, vet vaccine, life science research)
Crude BSA
Globulin-rich fraction
Ultrafiltration
Spray Dry
ImmunoLin Brand Bovine Globulin
Confidential Company Information

8

ATTACHMENT 8

Particle Analysis Report

Particle Characterization Laboratory
Beckman Coulter, Inc
MS 22-B02, P O Box 169015
Miami, FL 33116-9015

Particle Characterization Laboratory

Particle Analysis Report

BECKMAN COULTER™
Particle Characterization
MS 11800 SW 147 Ave.
Miami, FL 33196-9015

Particle Size Distribution Analysis Report

Mark Kuennen

Proliant Inc.

2425 SE Oak Tree Court
Ankeny, IA 50021

Reference: CE 18046 Customer PO:Visa

18-May-07

Dear Customer:

The particle size distribution analysis of your sample(s) has been completed using Beckman Coulter MS3 Particle Counter/Size Analyzer utilizing the electrical sensing zone technology, the Coulter Principle.

Enclosed please find a description for the sample preparation and a summary of the analysis result. The analysis printout for your sample(s) including particle size distribution and size statistics is attached.

If there are any questions in regard to this analysis, please do not hesitate to contact me at (800) 327-6531, ext. 304314.

Thank you for utilizing the Beckman Coulter Particle Characterization's Science and Technology Laboratory for your needs.

Sincerely,

BEST ORIGINAL COPY

Edgar Martinez

Beckman Coulter Particle Characterization limits the liability for any claims to the cost of the sample analyses. Beckman Coulter Particle Characterization shall not be liable for any incidental or consequential damages (including damages for loss of business, profits or like) arising out of the use of the results from the present analyses. Some states do not allow the limitation or exclusion of liability for incidental or consequential damages so the above limitation may not apply to you. Beckman Coulter Particle Characterization Laboratory is not an FDA registered contract laboratory.

000051

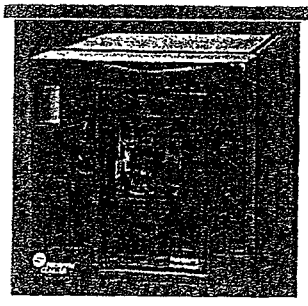
Sample Preparation Procedure

The samples were mixed by hand inversion and blended with 60:40 Isoton II / Glycerol in a round bottom beaker with stirrer. The analyses were performed with a 2000-µm aperture using Time/Volumetric Control Mode

Summary of Particle Size Analysis

Sample	Counts/ml	Number Mean Size (µm)
Lot # BB71280101 Incoming Bovine Plasma	268	57.36
Lot # BB71280101 Bovine Globulin Concentrate	16	51.70

BEST ORIGINAL COPY

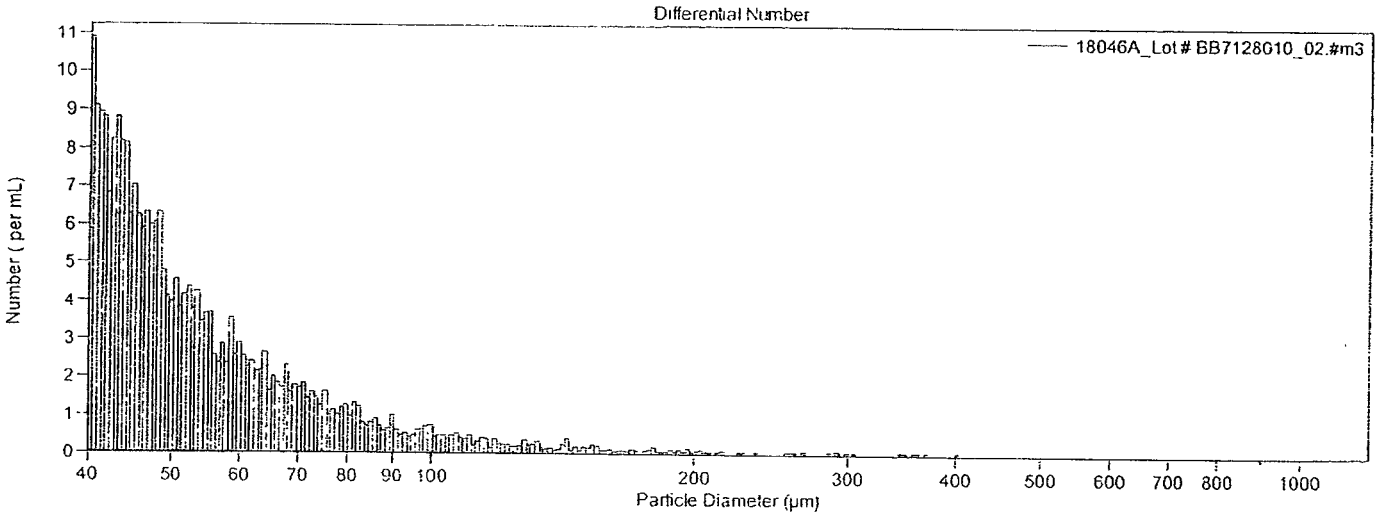


The Multisizer 3 Particle Size Analyzer and Particle Counter employs the well-known Coulter Principle to count and size particles in electrolytic suspension from 0.4 – 1200 µm. Its unparalleled high resolution enables the measurement of both narrow and mixed populations of particles.

000052

Beckman Coulter Particle Characterization Laboratory is not an FDA registered contract laboratory.

File name: C:\MS\Samples Proliant\18046\18046A_Lot # BB7128010_02.#m3
Group ID: 18046A
Sample ID: Lot # BB7128010
Size bins: 300 from 40 µm to 1200 µm
Sigma: 7.782 (Coincidence corrected)
Acquired: 15:40 17 May 2007



Number Statistics (Arithmetic) 18046A_Lot # BB7128010_02.#m3

Calculations from 40.00 µm to 1,200 µm

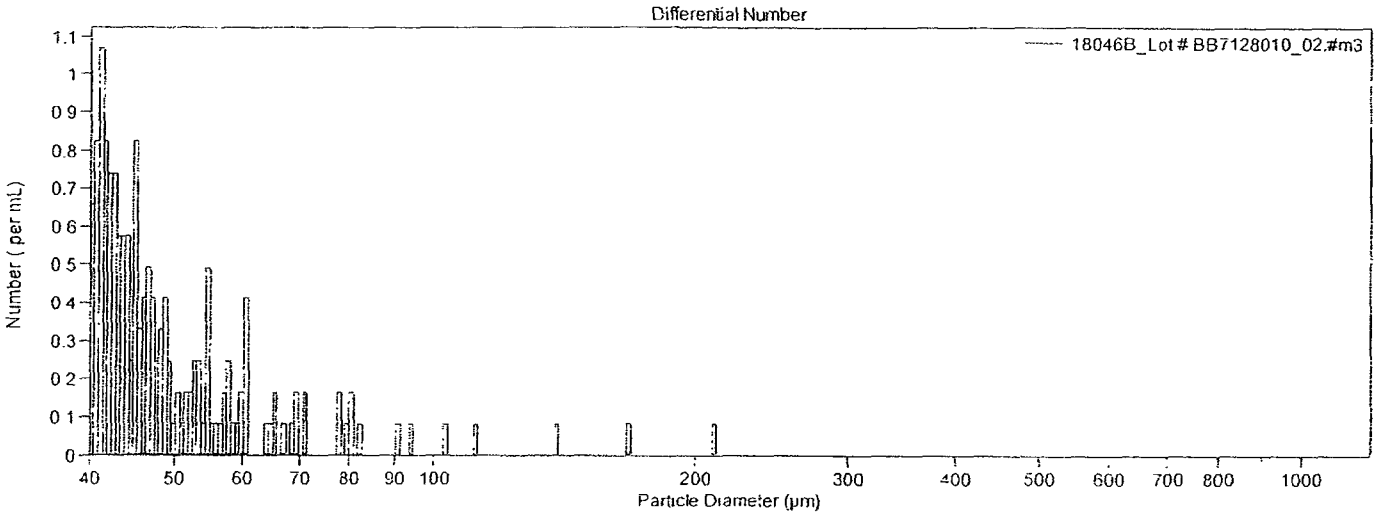
Number:	268.2 per mL		
Mean:	57.36 µm	S.D.:	24.59 µm
Median:	49.22 µm	C.V.:	42.9%
Mode:	40.23 µm	Skewness:	4.440 Right skewed
		Kurtosis:	33.92 Leptokurtic

d_{10}: 41.27 µm d_{50}: 49.22 µm d_{90}: 81.11 µm

18046A_Lot # BB7128010_02.#m3

Particle Diameter µm	Number % >	Number per mL >
50	47.9015	128.488
60	27.1772	72.8983
70	16.5387	44.3623
80	10.4876	28.1311
100	4.94674	13.2688
150	1.18329	3.17398
200	0.373184	1.00101
250	0.205605	0.551514
350	0.0601578	0.161377
400	0.0115967	0.0311279
450	0	0

File name: C:\MS\Samples\Proliant\18046\18046B_Lot # BB7128010_02 #m3
Group ID: 18046B
Sample ID: Lot # BB71280101
Size bins: 300 from 40 um to 1200 µm
Sigma: 189 (Coincidence corrected)
Acquired: 17:07 17 May 2007



Number Statistics (Arithmetic) 18046B_Lot # BB7128010_02.#m3

Calculations from 40.00 µm to 1,200 um

Number:	15.54 per mL		
Mean:	51.70 µm	S.D.:	19.66 µm
Median:	45.51 µm	C.V.:	38.0%
Mode:	41.15 µm	Skewness:	4.745 Right skewed
		Kurtosis:	29.11 Leptokurtic

d_{10}: 40.95 um d_{50}: 45.51 um d_{90}: 66.70 µm

18046B_Lot # BB7128010_02.#m3

Particle Diameter µm	Number % >	Number per mL >
50	31.9149	4.95979
60	15.0659	2.34134
70	7.93651	1.23339
80	5.14518	0.799596
100	2.6455	0.411129
150	1.0582	0.164452
200	0.529099	0.0822258
250	0	0
350	0	0
400	0	0
450	0	0

000054

9

ATTACHMENT 9

Radial Immunodiffusion

Pages 000057-000062 removed under the Privacy Act of 1974.

10

ATTACHMENT 10

Letter from FDA

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington DC 20204

APR 23 1992

Mr. John Dubeck
Keller and Heckman
1001 G Street, N.W.
Washington, D.C. 20001

Dear Mr. Dubeck:

This letter is in response to your letter of January 14, 1992 requesting clarification of the CFSAN's position on the use of beef blood plasma in food.

Further review of current and past uses of beef blood plasma in human food has triggered a reassessment of CFSAN's position on this issue. You have shown that there may be some basis for manufacturers making an independent GRAS determination for this ingredient. Although we will not consider affirmation of that determination without a petition, we also do not wish to contest that claim at this time. Because the USDA's Food Safety and Inspection Service (FSIS) is responsible for inspecting all beef and beef products produced or sold in this country, collection of beef plasma must be under USDA inspection. Further, the ingredient must be declared by its common or usual name on the ingredient label. We presume that this name would be "beef plasma" or "beef blood plasma."

Please feel free to contact us again should you have questions about any of the above.

Sincerely yours,

Alan M. Rulis, Ph.D.
Director
Division of Food and Color Additives
Center for Food Safety
and Applied Nutrition

LAW OFFICES
KELLER AND HECKMAN

1001 G STREET, N.W.
SUITE 500 WEST
WASHINGTON, D.C. 20001

(202) 434-4100
TELEX 49 95551 "KELMAN"
TELECOPIER (202) 434-4646

JOSEPH E. KELLER
JEROME H. HECKMAN
WILLIAM H. BORGHESANI, JR.
MALCOLM D. MACARTHUR
WAYNE V. BLACK
TERRENCE D. JONES
MARTIN W. BERCOVICI
JOHN S. ELDRED
WILLIAM L. KOVACS
CAROLE C. HARRIS
MICHAEL F. MORRONE
MARK FOX EVENS
JOHN B. RICHARDS
JOHN B. DUBECK
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MELVIN S. DROZEN
SHIRLEY S. FUJIMOTO
LAWRENCE P. HALPRIN
RALPH A. SIMMONS
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GAREN E. DODGE
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S. CRAIG TAUTFEST
MARK A. SIEVERS
MICHAEL R. BENNET
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DAVID G. SARVADI*
GLEN FRANKLIN KOONTZ
CATHERINE R. NIELSEN
KRIS ANNE MONTEITH
ELLIOT BELILOS
MARK L. ITZKOFF
MARC BEREJKA
JUSTIN P. McCARTHY
KENNETH A. OLSEN
JEFFREY S. LANG*
ARCHIE L. HARRIS, JR.*
BRIAN T. ASHBY
T. PHILLIPS BECK*
ARTHUR S. GARRETT III
RICK D. RHODES*
LESLIE E. SILVERMAN*
FRANK C. TORRES III*
BRYANT ROBINSON III

*NOT ADMITTED IN D.C

SCIENTIFIC STAFF
DANIEL S. DIXLER, PH. D
CHARLES V. BREDER, PH. D.
ROBERT A. MATHEWS, PH. D
JOHN P. MODDERMAN, PH. D
HOLLY HUTMIRE FOLEY
JUSTIN C. POWELL, PH. D.
JANETTE HOUK, PH. D.
LESTER BORODINSKY, PH. D.

TELECOMMUNICATIONS ENGINEER
CHARLES F. TURNER

January 14, 1992

WRITER'S DIRECT DIAL NUMBER
(202) 434-4125

HAND DELIVERED

Mr. John E. Thomas
Food and Drug Administration
Mail Code HFF-314
200 C Street, S.W. (FB-8)
Washington, D.C. 20204

Re: Beef Plasma

Dear John:

As we discussed on the telephone today, it is urgent that FDA promptly resolve the misinformation that the Food and Drug Administration (FDA) has been responsible for spreading regarding the regulatory status of beef plasma. You have agreed in our previous conversations that this is an issue that should be resolved promptly and we have provided you with a proposed draft of the type of letter that would eliminate confusion. Please note that we are not requesting a GRAS Affirmation. We are simply requesting that FDA acknowledge that it is not aware of any known safety concerns with the product and that FDA has not conducted the in-depth review necessary for it to draw a conclusion one way or another with regard to its GRAS status. Since that would appear to be the truth, we can see no reason why FDA should not write such letter.

Indeed it is the misleading letter from Dr. Manjeet Singh dated July 18, 1991, that has given rise to this confusion. The statement in that letter that anything which is not approved as a food additive or affirmed as generally recognized as safe is illegal is grossly misleading and in flat contradiction to FDA's own regulations.

We have been patient in awaiting your follow through on a promise to resolve this situation promptly. While we have exercised patience, however, our competitors are actively exploiting the situation. As evidence of this I am enclosing a copy of a one page article that appeared in the December 1991 issue of Food Processing Magazine. The product disparagement contained in that article is directly attributable to the misleading statements that have been made by FDA. Since you expressed an interest in the dehydrated raw potato product, I am also enclosing a copy of the patent referred to in the article.

If an appropriate clarification is not issued promptly, the upcoming "season" will be lost to competitive products. Thank you for your attention to this matter.

Very truly yours,

John B. Dubeck

Enclosures

cc: L. R. Lake
Janice Oliver
Alan M. Rulis
Richard J. Ronk

JBD\dle
H:\H&S\OTR_FDA\AM6910\JBD2106L.DLE
File: American Meat Protein

11

ATTACHMENT 11

Silliker COA

SILLIKER, Inc.
Iowa Laboratory
405 Eighth Avenue SE
Cedar Rapids, IA 52401-2153
319/ 366 3570 Fax 319/ 366 4018

CERTIFICATE OF ANALYSIS

COA No:	CDR-31312008-0
Supersedes:	None
COA Date	8/7/06
Page 1 of 2	

TO:
Mr. Chad Peterson
Product Release Coordinator
Prollant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	7/29/06

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #–	Laboratory ID:	307282684
Desc. 2:	SKU #56001	Condition Rec'd:	NORMAL
Desc. 3:	ImmunoLin	Temp Rec'd (°C):	25
Desc. 4:	Composite		
Desc. 5:	Lot #BB61560101, BB61580101, BB61650101,		
Desc. 6:	BB61630101, BB61670101		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Amino Acids Complete			USDA MSS2 (1993)	8/4/06	CHG
Aspartic Acid	8.67	%			
Threonine	6.23	%			
Serine	8.68	%			
Glutamic Acid	12.05	%			
Glycine	3.83	%			
Alanine	3.81	%			
Valine	7.69	%			
Methionine	1.05	%			
Isoleucine	2.57	%			
Leucine	7.48	%			
Tyrosine	4.58	%			
Phenylalanine	3.89	%			
Lysine	5.70	%			
Histidine	2.27	%			
Arginine	4.97	%			
Proline	6.41	%			
Hydroxyproline	<0.01	%			
Cystine	1.80	%			
Tryptophan	1.85	%			

The results of these tests relate only to the samples tested. This report shall not be reproduced except in full, without the written approval of the laboratory.

SILLIKER, Inc.
Iowa Laboratory
405 Eighth Avenue SE
Cedar Rapids, IA 52401-2153
319/ 366 3570 Fax 319/ 366 4018

CERTIFICATE OF ANALYSIS

COA No:	CDR-31312008-0
Supersedes:	None
COA Date	8/7/06
Page 2 of 2	

TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	7/29/06

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #--	**Laboratory ID:**	307282876
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	25
Desc. 4:	Composite		
Desc. 5:	Lot #BB61730101, BB61710101, BB61800101,		
Desc. 6:	BB61820101, BB61870101		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Amino Acids Complete			USDA MSS2 (1993)	8/7/06	CHG
Aspartic Acid	8.59	%			
Threonine	6.10	%			
Serine	8.81	%			
Glutamic Acid	12.16	%			
Glycine	3.82	%			
Alanine	3.83	%			
Valine	7.74	%			
Methionine	1.06	%			
Isoleucine	2.58	%			
Leucine	7.44	%			
Tyrosine	4.77	%			
Phenylalanine	4.04	%			
Lysine	5.77	%			
Histidine	2.29	%			
Arginine	5.06	%			
Proline	6.25	%			
Hydroxyproline	<0.01	%			
Cystine	2.16	%			
Tryptophan	1.99	%			

Jodene Jurgens, Ph.D. Laboratory Director

Noted Test Locations: CHG-Silliker, Inc. Illinois Laboratory, 1304 Halsted Street, Chicago Heights, IL 60411

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ATTACHMENT 12

ATTACHMENT 12

PDCAAS Worksheet for ImmunoLin®

Amino Acid	AA As Recvd Sample 1	Adjusted to 100g Protein	AA As Recvd Sample 2	Adjusted to 100g Protein	Adjusted to 100g Protein Average	Mg/g	FAO Value	Uncorrected AA Score	PDCAAS 93.4% Digestibility
Aspartic Acid	8.67	9.27	8.59	9.09	9.18	92			
Threonine	6.23	6.66	6.1	6.46	6.56	66	34	1.93	
Serine	8.68	9.28	8.81	9.33	9.30	93			
Glutamic Acid	12.05	12.88	12.16	12.87	12.88	129			
Glycine	3.83	4.09	3.82	4.04	4.07	41			
Alanine	3.81	4.07	3.83	4.05	4.06	41			
Valine	7.69	8.22	7.74	8.19	8.21	82	35	2.35	
Methionine	1.05	1.12	1.06	1.12	1.12	11	25	1.28	
Isoleucine	2.57	2.75	2.58	2.73	2.74	27	28	0.98	0.92
Leucine	7.48	8.00	7.44	7.88	7.94	79	66	1.20	
Tyrosine	4.58	4.90	4.77	5.05	4.97	50	63	1.46	
Phenylalanine	3.89	4.16	4.04	4.28	4.22	42			
Lysine	5.7	6.09	5.77	6.11	6.10	61	58	1.05	
Histidine	2.27	2.43	2.29	2.42	2.43	24	19	1.28	
Arginine	4.97	5.31	5.06	5.36	5.34	53			
Proline	6.41	6.85	6.25	6.62	6.73	67			
Hydroxyproline	0	0.00	0	0.00	0.00	0			
Cystine	1.8	1.92	2.16	2.29	2.11	21			
Tryptophan	1.85	1.98	1.99	2.11	2.04	20	11	1.82	
Total	93.53	100.00	94.46	199.00					
	1.069176		1.058649						

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ATTACHMENT 13

Silliker COA

SILLIKER®
Food Safety & Quality Solutions
SILLIKER, Inc.
Iowa Laboratory
405 Eighth Avenue SE, Cedar Rapids, IA 52401-2153
Tel. 319/ 366 3570 Fax. 319/ 366 4018

CERTIFICATE OF ANALYSIS

COA No:	CDR-31648961-1
Supersedes:	CDR-31648961-0
COA Date	3/19/07
Page 1 of 5	

COPY TO:
Mr. Mark D. Kuennen
QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/23/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1: LIMS Lab #177347
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70480101
Desc. 6: Pallet #–

Laboratory ID: 309047842
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Aerobic Plate Count	100	/g	AOAC 966.23	2/25/07	
Coliforms - 3 tube MPN	<3	/g	AOAC 966.24	2/25/07	
E. coli - 3 tube MPN	<3	/g	AOAC 966.24	2/25/07	
Staphylococci - coag. positive	<10	/g	AOAC 975.55	2/25/07	
Yeast and Mold			FDA-BAM, 7th ed.	2/28/07	
Yeast	<10	/g			
Mold	<10	/g			

Desc. 1: LIMS Lab #177347
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70480101
Desc. 6: Pallet #–

Laboratory ID: 309047962
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Salmonella - ELFA	Negative	/100g	Proliant Inc.	2/25/07	

Desc. 1: LIMS Lab #177347
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70480101
Desc. 6: Pallet #–

Laboratory ID: 309047978
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Ash	1.84	%	AOAC 920.153	3/2/07	WIS
Moisture - Forced Air Oven	4.60	%	AOAC 930.15	3/1/07	WIS
Protein - Combustion			AOAC 992.15	3/2/07	WIS
Protein Factor	6.25	-			
Protein	92.25	%			

The results of these tests relate only to the samples tested. This report shall not be reproduced except in full, without the written approval of the laboratory.

SILLIKER®
Food Safety & Quality Solutions
SILLIKER, Inc.
Iowa Laboratory
405 Eighth Avenue SE, Cedar Rapids, IA 52401-2153
Tel. 319/ 366 3570 Fax. 319/ 366 4018

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COA Date	3/19/07
Page 2 of 5	

COPY TO:
Mr. Mark D. Kuennen
QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/23/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177347	**Laboratory ID:**	309047994
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70480101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Scorched Particles	7.5	mg	ADPI (1990) p. 32	2/27/07	CCA

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SILLIKER®
Food Safety & Quality Solutions
SILLIKER, Inc.
Iowa Laboratory
405 Eighth Avenue SE, Cedar Rapids, IA 52401-2153
Tel. 319/ 366 3570 Fax. 319/ 366 4018

CERTIFICATE OF ANALYSIS

COA No:	CDR-31648961-1
Supersedes:	CDR-31648961-0
COA Date	3/19/07
Page 3 of 5	

COPY TO:
Mr. Mark D. Kuennen
QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/23/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177347	**Laboratory ID:**	309048198
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70480101		
Desc. 6:	Pallet #--		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Arsenic	<0.099	mg/100g	AOAC 984.27	3/1/07	CHG
ICP Sample Prep - Microwave	Microwave	-	AOAC 984.27	3/1/07	CHG
Lead	<0.050	mg/100g	AOAC 984.27	3/1/07	CHG
Mercury	<0.05	ppm	EPA 7471	2/28/07	MID
Organochlorine Pesticide Screen			CDFA Method	3/13/07	EMA
a, b, d - BHC	< 0.010	ppm			
Alachlor, (Lasso)	< 0.020	ppm			
Aldrin	< 0.010	ppm			
Benfluralin, (Balan, Benefin)	< 0.020	ppm			
BHC, (Benzahex)	< 0.010	ppm			
Bifenox, (Modown)	< 0.050	ppm			
Bifenthrin	< 0.020	ppm			
Bromacil	< 0.040	ppm			
Captafol	< 0.040	ppm			
Captan	< 0.020	ppm			
Chlordane	< 0.250	ppm			
Chlorobenzilate, (Acaraben)	< 0.400	ppm			
Chlorthalonil, (Bravo)	< 0.010	ppm			
Cyanazine	< 0.100	ppm			
Cyfluthrin	< 0.040	ppm			
Cypermethrin	< 0.040	ppm			
Dacthal, (Chlorthal)	< 0.020	ppm			
DDD	< 0.020	ppm			
DDE	< 0.020	ppm			
DDT	< 0.020	ppm			
Dichlobenil, (Casoron)	< 0.030	ppm			
Dicloran, (Botran)	< 0.020	ppm			
Dicofol, (Kelthane)	< 0.050	ppm			
Dieldrin	< 0.010	ppm			
Endosulfan alpha	< 0.010	ppm			
Endosulfan beta	< 0.010	ppm			
Endosulfan sulfate	< 0.010	ppm			

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CERTIFICATE OF ANALYSIS

COA No:	CDR-31648961-1
Supersedes:	CDR-31648961-0
COA Date	3/19/07
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QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/23/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177347	**Laboratory ID:**	309048198
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70480101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Endrin	< 0.010	ppm			
Esfenvalerate, (Asana)	< 0.030	ppm			
Ethalfiluralin, (Sonalan)	< 0.030	ppm			
Fluvalinate	< 0.040	ppm			
Folpet	< 0.050	ppm			
Heptachlor	< 0.010	ppm			
Heptachlor epoxide	< 0.020	ppm			
Iprodione, (Rovral)	< 0.050	ppm			
Lindane, (gamma-BHC)	< 0.010	ppm			
Linuron	< 0.020	ppm			
Methoxychlor	< 0.050	ppm			
Metribuzin	< 0.020	ppm			
Mirex	< 0.020	ppm			
Myclobutanil, (Rally)	< 0.050	ppm			
Oxadiazon, (Ronstar)	< 0.050	ppm			
Oxyfluorfen, (Goal)	< 0.040	ppm			
Pendamethalin	< 0.050	ppm			
Penthachloronitrobenzene (PCNB)	< 0.020	ppm			
Perthane, (Ethylan)	< 0.100	ppm			
Polychlorinated Biphenyls	< 0.250	ppm			
Profluralin, (Tolban)	< 0.020	ppm			
Pronamide, (Kerb, Propyzamide)	< 0.050	ppm			
Pyrethrins, (Total)	< 0.050	ppm			
Tetradifon, (Tedion)	< 0.020	ppm			
Tridimefon, (Bayleton)	< 0.020	ppm			
Trifluralin, (Treflan)	< 0.020	ppm			
Vegadex, (Diethyldithlocarb. acid)	< 0.050	ppm			
Vinclozolin, (Ronlian)	< 0.020	ppm			
Alert (Chlorfenapyr)	<0.040	ppm			
Boscalid	<0.020	ppm			
Dichlone	<0.050	ppm			
Endosulfans (Total)	<0.010	ppm			

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Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/23/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177347	**Laboratory ID:**	309048198
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70480101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Fenhexamid	<0.030	ppm			
Indoxacarb	<0.030	ppm			
Pentachloroanaline (PCA)	<0.010	ppm			
Procymidone	<0.020	ppm			
Propanil	<0.050	ppm			
Toxaphene (Attac)	<0.250	ppm			
Trifloxystrobin	<0.030	ppm			
Triflumazole	<0.050	ppm			
Deltamethrin	<0.020	ppm			
Fenpropathrin	<0.010	ppm			
Lambda Cyhalothrin	<0.010	ppm			
Permethrin (Pounce)	<0.100	ppm			
Tralomethrin	<0.020	ppm			

Jodene Jürgens, Ph.D. Laboratory Director

Noted Test Locations: WIS-Silliker, Inc. Wisconsin Laboratory, 3688 Kinsman Boulevard, Madison, WI 53704
Noted Test Locations: CCA-Silliker, Inc. Northern California Laboratory, 1548 Cummins Drive, Modesto, CA 95358-6412
Noted Test Locations: CHG-Silliker, Inc. Illinois Laboratory, 1304 Halsted Street, Chicago Heights, IL 60411
Noted Test Locations: MID-Midwest Laboratories, Inc., 13611 B Street, Omaha, NE 68144-3693
Noted Test Locations: EMA-Environmental Micro Analyses, Inc., 40 N. East Street, Suite B, Woodland, CA 95776

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ATTACHMENT 14

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Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1: LIMS Lab #177458
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70500101
Desc. 6: Pallet #–

Laboratory ID: 309077108
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Aerobic Plate Count	700	/g	AOAC 966.23	3/1/07	
Coliforms - 3 tube MPN	<3	/g	AOAC 966.24	3/1/07	
E. coli - 3 tube MPN	<3	/g	AOAC 966.24	3/1/07	
Staphylococci - coag. positive	<10	/g	AOAC 975.55	3/1/07	
Yeast and Mold			FDA-BAM, 7th ed.	3/4/07	
Yeast	<10	/g			
Mold	<10	/g			

Desc. 1: LIMS Lab #177458
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70500101
Desc. 6: Pallet #–

Laboratory ID: 309077128
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Salmonella - ELFA	Negative	/100g	Proliant Inc.	3/1/07	

Desc. 1: LIMS Lab #177458
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70500101
Desc. 6: Pallet #–

Laboratory ID: 309077138
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Ash	1.60	%	AOAC 920.153	3/2/07	WIS
Moisture - Forced Air Oven	4.67	%	AOAC 930.15	3/1/07	WIS
Protein - Combustion			AOAC 992.15	3/2/07	WIS
Protein Factor	6.25	-			
Protein	92.44	%			

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Ankeny, IA 50021-7102

Received From:	Boone, IA
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Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177458	**Laboratory ID:**	309077142
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70500101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Scorched Particles	7.5	mg	ADPI (1990) p. 32	3/2/07	CCA

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Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177458	**Laboratory ID:**	309077171
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70500101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Arsenic	<0.096	mg/100g	AOAC 984.27	3/6/07	CHG
ICP Sample Prep - Microwave	Microwave	-	AOAC 984.27	3/5/07	CHG
Lead	<0.048	mg/100g	AOAC 984.27	3/6/07	CHG
Mercury	<0.05	ppm	EPA 7471	3/6/07	MID
Organochlorine Pesticide Screen			CDFA Method	3/13/07	EMA
a, b, d - BHC	< 0.010	ppm			
Alachlor, (Lasso)	< 0.020	ppm			
Aldrin	< 0.010	ppm			
Benfluralin, (Balan, Benefin)	< 0.020	ppm			
BHC, (Benzahex)	< 0.010	ppm			
Bifenox, (Modown)	< 0.050	ppm			
Bifenthrin	< 0.020	ppm			
Bromacil	< 0.040	ppm			
Captafol	< 0.040	ppm			
Captan	< 0.020	ppm			
Chlordane	< 0.250	ppm			
Chlorobenzilate, (Acaraben)	< 0.400	ppm			
Chlorthalonil, (Bravo)	< 0.010	ppm			
Cyanazine	< 0.100	ppm			
Cyfluthrin	< 0.040	ppm			
Cypermethrin	< 0.040	ppm			
Dacthal, (Chlorthal)	< 0.020	ppm			
DDD	< 0.020	ppm			
DDE	< 0.020	ppm			
DDT	< 0.020	ppm			
Dichlobenil, (Casoron)	< 0.030	ppm			
Dicloran, (Botran)	< 0.020	ppm			
Dicofol, (Kelthane)	< 0.050	ppm			
Dieldrin	< 0.010	ppm			
Endosulfan alpha	< 0.010	ppm			
Endosulfan beta	< 0.010	ppm			
Endosulfan sulfate	< 0.010	ppm			

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Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1: LIMS Lab #177458
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70500101
Desc. 6: Pallet #–

Laboratory ID: 309077171
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Endrin	< 0.010	ppm			
Esfenvalerate, (Asana)	< 0.030	ppm			
Ethalfiluralin, (Sonalan)	< 0.030	ppm			
Fluvalinate	< 0.040	ppm			
Folpet	< 0.050	ppm			
Heptachlor	< 0.010	ppm			
Heptachlor epoxide	< 0.020	ppm			
Iprodione, (Rovral)	< 0.050	ppm			
Lindane, (gamma-BHC)	< 0.010	ppm			
Linuron	< 0.020	ppm			
Methoxychlor	< 0.050	ppm			
Metribuzin	< 0.020	ppm			
Mirex	< 0.020	ppm			
Myclobutanil, (Rally)	< 0.050	ppm			
Oxadiazon, (Ronstar)	< 0.050	ppm			
Oxyfluorfen, (Goal)	< 0.040	ppm			
Pendamethalin	< 0.050	ppm			
Penthachloronitrobenzene (PCNB)	< 0.020	ppm			
Perthane, (Ethylan)	< 0.100	ppm			
Polychlorinated Biphenyls	< 0.250	ppm			
Profluralin, (Tolban)	< 0.020	ppm			
Pronamide, (Kerb, Propyzamide)	< 0.050	ppm			
Pyrethrins, (Total)	< 0.050	ppm			
Tetradifon, (Tedion)	< 0.020	ppm			
Tridimefon, (Bayleton)	< 0.020	ppm			
Trifluralin, (Treflan)	< 0.020	ppm			
Vegadex, (Diethyldithlocarb. acid)	< 0.050	ppm			
Vinclozolin, (Ronlian)	< 0.020	ppm			
Alert (Chlorfenapyr)	<0.040	ppm			
Boscalid	<0.020	ppm			
Dichlone	<0.050	ppm			
Endosulfans (Total)	<0.010	ppm			

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2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177458	**Laboratory ID:**	309077171
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70500101		
Desc. 6:	Pallet #--		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Fenhexamid	<0.030	ppm			
Indoxacarb	<0.030	ppm			
Pentachlororanaline (PCA)	<0.010	ppm			
Procymidone	<0.020	ppm			
Propanil	<0.050	ppm			
Toxaphene (Attac)	<0.250	ppm			
Trifloxystrobin	<0.030	ppm			
Triflumazole	<0.050	ppm			
Deltamethrin	<0.020	ppm			
Fenpropathrin	<0.010	ppm			
Lambda Cyhalothrin	<0.010	ppm			
Permethrin (Pounce)	<0.100	ppm			
Tralomethrin	<0.020	ppm			

Jodene Jürgens, Ph.D. Laboratory Director

Noted Test Locations: WIS-Silliker, Inc. Wisconsin Laboratory, 3688 Kinsman Boulevard, Madison, WI 53704
Noted Test Locations: CCA-Silliker, Inc. Northern California Laboratory, 1548 Cummins Drive, Modesto, CA 95358-6412
Noted Test Locations: CHG-Silliker, Inc. Illinois Laboratory, 1304 Halsted Street, Chicago Heights, IL 60411
Noted Test Locations: MID-Midwest Laboratories, Inc., 13611 B Street, Omaha, NE 68144-3693
Noted Test Locations: EMA-Environmental Micro Analyses, Inc., 40 N. East Street, Suite B, Woodland, CA 95776

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ATTACHMENT 15

Silliker COA

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Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1: LIMS Lab #177511
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70520101
Desc. 6: Pallet #--

Laboratory ID: 309077462
Condition Rec'd: NORMAL
Temp Rec'd (°C): 17

Analyte	Result	Units	Method Reference	Test Date	Loc.
Aerobic Plate Count	1300	/g	AOAC 966.23	3/1/07	
Coliforms - 3 tube MPN	<3	/g	AOAC 966.24	3/1/07	
E. coli - 3 tube MPN	<3	/g	AOAC 966.24	3/1/07	
Staphylococci - coag. positive	<10	/g	AOAC 975.55	3/1/07	
Yeast and Mold			FDA-BAM, 7th ed.	3/4/07	
Yeast	<10	/g			
Mold	<10	/g			

Desc. 1: LIMS Lab #177511
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70520101
Desc. 6: Pallet #--

Laboratory ID: 309077475
Condition Rec'd: NORMAL
Temp Rec'd (°C): 17

Analyte	Result	Units	Method Reference	Test Date	Loc.
Salmonella - ELFA	Negative	/100g	Proliant Inc.	3/1/07	

Desc. 1: LIMS Lab #177511
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70520101
Desc. 6: Pallet #--

Laboratory ID: 309077483
Condition Rec'd: NORMAL
Temp Rec'd (°C): 17

Analyte	Result	Units	Method Reference	Test Date	Loc.
Ash	1.55	%	AOAC 920.153	3/2/07	WIS
Moisture - Forced Air Oven	4.69	%	AOAC 930.15	3/1/07	WIS
Protein - Combustion			AOAC 992.15	3/2/07	WIS
Protein Factor	6.25	-			
Protein	91.88	%			

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Analytical Results

Desc. 1:	LIMS Lab #177511	**Laboratory ID:**	309077489
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	17
Desc. 5:	Lot #BB70520101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Scorched Particles	7.5	mg	ADPI (1990) p. 32	3/2/07	CCA

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Ankeny, IA 50021-7102

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Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177511	**Laboratory ID:**	309077497
Desc. 2:	SKU #	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	17
Desc. 5:	Lot #BB70520101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Arsenic	<0.099	mg/100g	AOAC 984.27	3/6/07	CHG
ICP Sample Prep - Microwave	Microwave	-	AOAC 984.27	3/5/07	CHG
Lead	<0.049	mg/100g	AOAC 984.27	3/6/07	CHG
Mercury	<0.05	ppm	EPA 7471	3/6/07	MID
Organochlorine Pesticide Screen			CDFA Method	3/14/07	EMA
a, b, d - BHC	< 0.010	ppm			
Alachlor, (Lasso)	< 0.020	ppm			
Aldrin	< 0.010	ppm			
Benfluralin, (Balan, Benefin)	< 0.020	ppm			
BHC, (Benzahex)	< 0.010	ppm			
Bifenox, (Modown)	< 0.050	ppm			
Bifenthrin	< 0.020	ppm			
Bromacil	< 0.040	ppm			
Captafol	< 0.040	ppm			
Captan	< 0.020	ppm			
Chlordane	< 0.250	ppm			
Chlorobenzilate, (Acaraben)	< 0.400	ppm			
Chlorthalonil, (Bravo)	< 0.010	ppm			
Cyanazine	< 0.100	ppm			
Cyfluthrin	< 0.040	ppm			
Cypermethrin	< 0.040	ppm			
Dacthal, (Chlorthal)	< 0.020	ppm			
DDD	< 0.020	ppm			
DDE	< 0.020	ppm			
DDT	< 0.020	ppm			
Dichlobenil, (Casoron)	< 0.030	ppm			
Dicloran, (Botran)	< 0.020	ppm			
Dicofol, (Kelthane)	< 0.050	ppm			
Dieldrin	< 0.010	ppm			
Endosulfan alpha	< 0.010	ppm			
Endosulfan beta	< 0.010	ppm			
Endosulfan sulfate	< 0.010	ppm			

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CERTIFICATE OF ANALYSIS

COA No:	CDR-31658784-0
Supersedes:	None
COA Date	3/19/07
Page 4 of 5	

COPY TO:
Mr. Mark D. Kuennen
QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177511	**Laboratory ID:**	309077497
Desc. 2:	SKU #	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	17
Desc. 5:	Lot #BB70520101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Endrin	< 0.010	ppm			
Esfenvalerate, (Asana)	< 0.030	ppm			
Ethalfiluralin, (Sonalan)	< 0.030	ppm			
Fluvalinate	< 0.040	ppm			
Folpet	< 0.050	ppm			
Heptachlor	< 0.010	ppm			
Heptachlor epoxide	< 0.020	ppm			
Iprodione, (Rovral)	< 0.050	ppm			
Lindane, (gamma-BHC)	< 0.010	ppm			
Linuron	< 0.020	ppm			
Methoxychlor	< 0.050	ppm			
Metribuzin	< 0.020	ppm			
Mirex	< 0.020	ppm			
Myclobutanil, (Rally)	< 0.050	ppm			
Oxadiazon, (Ronstar)	< 0.050	ppm			
Oxyfluorfen, (Goal)	< 0.040	ppm			
Pendamethalin	< 0.050	ppm			
Penthachloronitrobenzene (PCNB)	< 0.020	ppm			
Perthane, (Ethylan)	< 0.100	ppm			
Polychlorinated Biphenyls	< 0.250	ppm			
Profluralin, (Tolban)	< 0.020	ppm			
Pronamide, (Kerb, Propyzamide)	< 0.050	ppm			
Pyrethrins, (Total)	< 0.050	ppm			
Tetradifon, (Tedion)	< 0.020	ppm			
Tridimefon, (Bayleton)	< 0.020	ppm			
Trifluralin, (Treflan)	< 0.020	ppm			
Vegadex, (Diethyldithlocarb. acid)	< 0.050	ppm			
Vinclozolin, (Ronlian)	< 0.020	ppm			
Alert (Chlorfenapyr)	<0.040	ppm			
Boscalid	<0.020	ppm			
Dichlone	<0.050	ppm			
Endosulfans (Total)	<0.010	ppm			

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COA No:	CDR-31658784-0
Supersedes:	None
COA Date	3/19/07
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Mr. Mark D. Kuennen
QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	2/27/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177511	**Laboratory ID:**	309077497
Desc. 2:	SKU #	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	17
Desc. 5:	Lot #BB70520101		
Desc. 6:	Pallet #--		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Fenhexamid	<0.030	ppm			
Indoxacarb	<0.030	ppm			
Pentachlororanaline (PCA)	<0.010	ppm			
Procymidone	<0.020	ppm			
Propanil	<0.050	ppm			
Toxaphene (Attac)	<0.250	ppm			
Trifloxystrobin	<0.030	ppm			
Triflumazole	<0.050	ppm			
Deltamethrin	<0.020	ppm			
Fenpropathrin	<0.010	ppm			
Lambda Cyhalothrin	<0.010	ppm			
Permethrin (Pounce)	<0.100	ppm			
Tralomethrin	<0.020	ppm			

Jodene Jürgens, Ph.D. Laboratory Director

Noted Test Locations: WIS-Silliker, Inc. Wisconsin Laboratory, 3688 Kinsman Boulevard, Madison, WI 53704
Noted Test Locations: CCA-Silliker, Inc. Northern California Laboratory, 1548 Cummins Drive, Modesto, CA 95358-6412
Noted Test Locations: CHG-Silliker, Inc. Illinois Laboratory, 1304 Halsted Street, Chicago Heights, IL 60411
Noted Test Locations: MID-Midwest Laboratories, Inc., 13611 B Street, Omaha, NE 68144-3693
Noted Test Locations: EMA-Environmental Micro Analyses, Inc., 40 N. East Street, Suite B, Woodland, CA 95776

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16

ATTACHMENT 16

Silliker COA

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ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	3/1/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1: LIMS Lab #177712
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70550101
Desc. 6: Pallet #--

Laboratory ID: 309096834
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Aerobic Plate Count	100	/g	AOAC 966.23	3/3/07	
Coliforms - 3 tube MPN	<3	/g	AOAC 966.24	3/3/07	
E. coli - 3 tube MPN	<3	/g	AOAC 966.24	3/3/07	
Staphylococci - coag. positive	<10	/g	AOAC 975.55	3/3/07	
Yeast and Mold			FDA-BAM, 7th ed.	3/6/07	
Yeast	<10	/g			
Mold	<10	/g			

Desc. 1: LIMS Lab #177712
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70550101
Desc. 6: Pallet #--

Laboratory ID: 309096867
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Salmonella - ELFA	Negative	/100g	Proliant Inc.	3/3/07	

Desc. 1: LIMS Lab #177712
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70550101
Desc. 6: Pallet #--

Laboratory ID: 309096884
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Ash	1.57	%	AOAC 920.153	3/6/07	WIS
Moisture - Forced Air Oven	4.57	%	AOAC 930.15	3/5/07	WIS
Protein - Combustion			AOAC 992.15	3/7/07	WIS
Protein Factor	6.25	-			
Protein	93.06	%			

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Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	3/1/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177712	**Laboratory ID:**	309096942
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70550101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Scorched Particles	7.5	mg	ADPI (1990) p. 32	3/7/07	CCA

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Product Release Coordinator
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2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	3/1/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1: LIMS Lab #177712
Desc. 2: SKU #56001
Desc. 3: ImmunoLin
Desc. 5: Lot #BB70550101
Desc. 6: Pallet #–

Laboratory ID: 309097144
Condition Rec'd: NORMAL
Temp Rec'd (°C): 18

Analyte	Result	Units	Method Reference	Test Date	Loc.
Arsenic	<0.090	mg/100g	AOAC 984.27	3/7/07	CHG
ICP Sample Prep - Microwave	Microwave	-	AOAC 984.27	3/7/07	CHG
Lead	<0.045	mg/100g	AOAC 984.27	3/7/07	CHG
Mercury	<0.05	ppm	EPA 7471	3/8/07	MID
Organochlorine Pesticide Screen			CDFA Method	3/14/07	EMA
a, b, d - BHC	< 0.010	ppm			
Alachlor, (Lasso)	< 0.020	ppm			
Aldrin	< 0.010	ppm			
Benfluralin, (Balan, Benefin)	< 0.020	ppm			
BHC, (Benzahex)	< 0.010	ppm			
Bifenox, (Modown)	< 0.050	ppm			
Bifenthrin	< 0.020	ppm			
Bromacil	< 0.040	ppm			
Captafol	< 0.040	ppm			
Captan	< 0.020	ppm			
Chlordane	< 0.250	ppm			
Chlorobenzilate, (Acaraben)	< 0.400	ppm			
Chlorthalonil, (Bravo)	< 0.010	ppm			
Cyanazine	< 0.100	ppm			
Cyfluthrin	< 0.040	ppm			
Cypermethrin	< 0.040	ppm			
Dacthal, (Chlorthal)	< 0.020	ppm			
DDD	< 0.020	ppm			
DDE	< 0.020	ppm			
DDT	< 0.020	ppm			
Dichlobenil, (Casoron)	< 0.030	ppm			
Dicloran, (Botran)	< 0.020	ppm			
Dicofol, (Kelthane)	< 0.050	ppm			
Dieldrin	< 0.010	ppm			
Endosulfan alpha	< 0.010	ppm			
Endosulfan beta	< 0.010	ppm			
Endosulfan sulfate	< 0.010	ppm			

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COA No:	CDR-31658785-0
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Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	3/1/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177712	**Laboratory ID:**	309097144
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70550101		
Desc. 6:	Pallet #--		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Endrin	< 0.010	ppm			
Esfenvalerate, (Asana)	< 0.030	ppm			
Ethalfiluralin, (Sonalan)	< 0.030	ppm			
Fluvalinate	< 0.040	ppm			
Folpet	< 0.050	ppm			
Heptachlor	< 0.010	ppm			
Heptachlor epoxide	< 0.020	ppm			
Iprodione, (Rovral)	< 0.050	ppm			
Lindane, (gamma-BHC)	< 0.010	ppm			
Linuron	< 0.020	ppm			
Methoxychlor	< 0.050	ppm			
Metribuzin	< 0.020	ppm			
Mirex	< 0.020	ppm			
Myclobutanil, (Rally)	< 0.050	ppm			
Oxadiazon, (Ronstar)	< 0.050	ppm			
Oxyfluorfen, (Goal)	< 0.040	ppm			
Pendamethalin	< 0.050	ppm			
Penthachloronitrobenzene (PCNB)	< 0.020	ppm			
Perthane, (Ethylan)	< 0.100	ppm			
Polychlorinated Biphenyls	< 0.250	ppm			
Profluralin, (Tolban)	< 0.020	ppm			
Pronamide, (Kerb, Propyzamide)	< 0.050	ppm			
Pyrethrins, (Total)	< 0.050	ppm			
Tetradifon, (Tedlon)	< 0.020	ppm			
Tridimefon, (Bayleton)	< 0.020	ppm			
Trifluralin, (Treflan)	< 0.020	ppm			
Vegadex, (Diethyldithlocarb. acid)	< 0.050	ppm			
Vinclozolin, (Ronlian)	< 0.020	ppm			
Alert (Chlorfenapyr)	<0.040	ppm			
Boscalid	<0.020	ppm			
Dichlone	<0.050	ppm			
Endosulfans (Total)	<0.010	ppm			

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COA No:	CDR-31658785-0
Supersedes:	None
COA Date	3/19/07
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Mr. Mark D. Kuennen
QA Manager
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Ltd.
2425 SE Oak Tree Court
Ankeny, IA 50021-7102

Received From:	Boone, IA
Received Date:	3/1/07

Location of Test: (except where noted)
Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #177712	**Laboratory ID:**	309097144
Desc. 2:	SKU #56001	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin	**Temp Rec'd (°C):**	18
Desc. 5:	Lot #BB70550101		
Desc. 6:	Pallet #–		

Analyte	Result	Units	Method Reference	Test Date	Loc.
Fenhexamid	<0.030	ppm			
Indoxacarb	<0.030	ppm			
Pentachlororanaline (PCA)	<0.010	ppm			
Procymidone	<0.020	ppm			
Propanil	<0.050	ppm			
Toxaphene (Attac)	<0.250	ppm			
Trifloxystrobin	<0.030	ppm			
Triflumazole	<0.050	ppm			
Deltamethrin	<0.020	ppm			
Fenpropathrin	<0.010	ppm			
Lambda Cyhalothrin	<0.010	ppm			
Permethrin (Pounce)	<0.100	ppm			
Tralomethrin	<0.020	ppm			

Jodene Jürgens, PhD. Laboratory Director

Noted Test Locations: WIS-Silliker, Inc. Wisconsin Laboratory, 3688 Kinsman Boulevard, Madison, WI
Noted Test Locations: CCA-Silliker, Inc. Northern California Laboratory, 1548 Cummins Drive, Modesto, CA 95358-6412
Noted Test Locations: CHG-Silliker, Inc. Illinois Laboratory, 1304 Halsted Street, Chicago Heights, IL 60411
Noted Test Locations: MID-Midwest Laboratories, Inc., 13611 B Street, Omaha, NE 68144-3693
Noted Test Locations: EMA-Environmental Micro Analyses, Inc., 40 N. East Street, Suite B, Woodland, CA 95776

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ATTACHMENT 17

Silliker COA

SILLIKER, Inc.
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CERTIFICATE OF ANALYSIS

COA No:	CDR-30338990-0
Supersedes:	None
COA Date	9/22/04
Page 1 of 3	

COPY TO:
Ms. Bonnie J. Humm
Director of Quality Assurance
Proliant Inc. / APC Inc.
2425 SE Oak Tree Court
Ankeny, IA 50021

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Inc.
2425 SE Oak Tree Court
Ankeny, IA 50021

Received From:	Boone, IA
Received Date:	9/1/04
P.O.# / ID:	BB
Location of Test: (except where noted)	Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #--	**Desc. 4:**	BB40090101, BB40200101	**Laboratory ID:**	301789899
Desc. 2:	SKU #56001	**Desc. 5:**	BB40330101, BB40470101	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin Composite	**Desc. 6:**	BB40610101, BB40860101	**Temp Rec'd (°C):**	23

Sample Comment: BB41420101, BB41810101, BB42240101, and BB42260101

Analyte	Result	Units	Method Reference	Test Date	Loc.
Arsenic	<0.22	mg/100g	AOAC 984.27	9/7/04	CHG
ICP Sample Prep - Microwave	Microwave	-	AOAC 984.27	9/7/04	CHG
Lead	<0.11	mg/100g	AOAC 984.27	9/7/04	CHG
Mercury	0.11	ppm	EPA 7471	9/8/04	MID
Organochlorine Pesticide Screen			CDFA Method	9/22/04	EMA
a, b, d - BHC	< 0.010	ppm			
Alachlor, (Lasso)	< 0.020	ppm			
Alert, (Pirate)	< 0.040	ppm			
Aldrin	< 0.010	ppm			
Benfluralin, (Balan, Benefin)	< 0.020	ppm			
BHC, (Benzahex)	< 0.010	ppm			
Bifenox, (Modown)	< 0.050	ppm			
Bifenthrin	< 0.040	ppm			
Bromacil	< 0.040	ppm			
Captafol	< 0.040	ppm			
Captan	< 0.020	ppm			
Chlordane	< 0.250	ppm			
Chlorobenzilate, (Acaraben)	< 0.400	ppm			
Chlorthalonil, (Bravo)	< 0.010	ppm			
Cyanazine	< 0.100	ppm			
Cyfluthrin	< 0.050	ppm			
Cypermethrin	< 0.050	ppm			
Dacthal, (Chlorthal)	< 0.020	ppm			
DDD	< 0.020	ppm			
DDE	< 0.020	ppm			
DDT	< 0.020	ppm			
Dichlobenil, (Casoron)	< 0.030	ppm			
Dichlone, (Phygon)	< 0.050	ppm			
Dicloran, (Botran)	< 0.020	ppm			
Dicofol, (Kelthane)	< 0.050	ppm			
Dieldrin	< 0.010	ppm			
Dyrene, (Anilazine)	< 0.100	ppm			
Endosulfan alpha	< 0.010	ppm			

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Ankeny, IA 50021

ORIGINAL TO:
Mr. Chad Peterson
Product Release Coordinator
Proliant Inc. / APC Inc.
2425 SE Oak Tree Court
Ankeny, IA 50021

Received From:	Boone, IA
Received Date:	9/1/04
P.O.# / ID:	BB
Location of Test: (except where noted)	Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #--	**Desc. 4:**	BB40090101, BB40200101	**Laboratory ID:**	301789899
Desc. 2:	SKU #56001	**Desc. 5:**	BB40330101, BB40470101	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin Composite	**Desc. 6:**	BB40610101, BB40860101	**Temp Rec'd (°C):**	23

Sample Comment: BB41420101, BB41810101, BB42240101, and BB42260101

Analyte	Result	Units	Method Reference	Test Date	Loc.
Endosulfan beta	< 0.010	ppm			
Endosulfan sulfate	< 0.010	ppm			
Endrin	< 0.010	ppm			
Esfenvalerate, (Asana)	< 0.020	ppm			
Ethalfiluralin, (Sonalan)	< 0.030	ppm			
Fluvalinate	< 0.050	ppm			
Folpet	< 0.050	ppm			
Heptachlor	< 0.010	ppm			
Heptachlor epoxide	< 0.020	ppm			
Iprodione, (Rovral)	< 0.050	ppm			
Lindane, (gamma-BHC)	< 0.010	ppm			
Linuron	< 0.020	ppm			
Methoxychlor	< 0.050	ppm			
Metribuzin	< 0.020	ppm			
Mirex	< 0.020	ppm			
Myclobutanil, (Rally)	< 0.050	ppm			
Nitrofen, (TOK)	< 0.050	ppm			
Oxadiazon, (Ronstar)	< 0.050	ppm			
Oxyfluorfen, (Goal)	< 0.040	ppm			
Pendamethalin	< 0.050	ppm			
Penthachloronitrobenzene (PCNB)	< 0.020	ppm			
Permethrin	< 0.100	ppm			
Perthane, (Ethylan)	< 0.100	ppm			
Polychlorinated Biphenyls	< 0.250	ppm			
Profluralin, (Tolban)	< 0.020	ppm			
Pronamide, (Kerb, Propyzamide)	< 0.050	ppm			
Pyrethrins, (Total)	< 0.100	ppm			
Tetradifon, (Tedion)	< 0.020	ppm			
Toxaphene, (Attac, Strobane)	< 0.250	ppm			
Tridimefon, (Bayleton)	< 0.020	ppm			
Trifluralin, (Treflan)	< 0.020	ppm			
Vegadex, (Diethyldithlocarb. acid)	< 0.050	ppm			
Vinclozolin, (Ronlian)	< 0.020	ppm			
Sulfa Drug Screen			AOAC 993.32	9/13/04	BRD

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COA No:	CDR-30338990-0
Supersedes:	None
COA Date	9/22/04
Page 3 of 3	

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Mr. Chad Peterson
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Received From:	Boone, IA
Received Date:	9/1/04
P.O.# / ID:	BB
Location of Test: (except where noted)	Cedar Rapids, IA

Analytical Results

Desc. 1:	LIMS Lab #–	**Desc. 4:**	BB40090101, BB40200101	**Laboratory ID:**	301789899
Desc. 2:	SKU #56001	**Desc. 5:**	BB40330101, BB40470101	**Condition Rec'd:**	NORMAL
Desc. 3:	ImmunoLin Composite	**Desc. 6:**	BB40610101, BB40860101	**Temp Rec'd (°C):**	23

Sample Comment: BB41420101, BB41810101, BB42240101, and BB42260101

Analyte	Result	Units	Method Reference	Test Date	Loc.
Sulfadimethoxine	<0.01	ppm			
Sulfathiazole	<0.01	ppm			
Sulfamethazine	<0.01	ppm			
Sulfaquinoxaline	<0.01	ppm			
Tetracycline Screen			AOAC 967.39	9/13/04	BRD
Tetracycline	<0.10	ppm			
Chlortetracycline	<0.10	ppm			
Oxytetracycline	<0.10	ppm			

Jodene Jurgens, Ph.D. — Laboratory Director

Noted Test Locations: CHG-Silliker, Inc. Illinois Laboratory, 1304 Halsted Street, Chicago Heights, IL 60411
Noted Test Locations: MID-Midwest Laboratories, Inc., 13611 B Street, Omaha, NE 68144-3693
Noted Test Locations: EMA-Environmental Micro Analyses, Inc., 40 N. East Street, Suite B, Woodland, CA 95776
Noted Test Locations: BRD-Barrow-Agee, 1555 Three Pl, Memphis, TN 38116

The results of these tests relate only to the samples tested. This report shall not be reproduced except in full, without the written approval of the laboratory.

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ATTACHMENT 18

BSE Memorandum

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MEMORANDUM

TO: John B. Dubeck

FROM: Robert J. Scheuplein, Ph.D.

DATE: April 13, 2007

RE: Discussion of Potential Safety Concerns Specific to Protein Obtained from Bovine Blood

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There is currently little official concern over the safety of bovine blood, bovine plasma or protein products derived from bovine blood. This confidence is based on several considerations, including the essentially zero natural prevalence of bovine spongiform encephalopathy (BSE) in cattle in the United States, the effectiveness of BSE-prevention and sanitation practices mandated by FDA and USDA, the complete absence of any recorded infectivity of blood in experimental animal studies (which is why blood is excluded from the category of Specific Risk Materials – SRMs – whose use in animal feed or human food is prohibited), and perhaps most importantly, the fact that any possible concentration of BSE-infective prions in immunoglobulins during the ImmunoLin production process would be more than offset by the dilution effect resulting from the pooling of blood from many cattle. A complete and thorough discussion of the scientific nature and causes of BSE, as well as the effectiveness of existing food safety measures and the reasons why consumption of ImmunoLin™ is safe, is set forth below.

1. Scientific Background; Practical Absence of BSE in the United States

Bovine spongiform encephalopathy (BSE) is a brain disease in cattle that is spread by the feeding of the cattle with infected meat and bone meal. The infectious agent is understood to be a prion (PrP or PrP^c). A prion is a small glycosylated protein molecule found mainly in the brain cell membrane. Prions have the ability to undergo a conformational change to a predominantly flattened sheet form and in the process become both infectious and heat and protease resistant. When such an infective prion reaches brain cells, it is thought to be able to act as a template for other prions, "recruiting" them until layers of abnormal protein are formed, which eventually leads to a loss of brain function. The disease has a 4-6 year incubation period in cattle and is invariably fatal. A disease outbreak began in Great Britain in the early 1970s and is thought to have been caused by several factors, including the first-time feeding of meat and bone meal to very young - and hence vulnerable - dairy calves, coupled with changes in the rendering practices of feed stock, which were thought to have increased the infectivity of the feed. At the disease peak in 1987, 37,000 cattle were infected in the UK and ultimately a total of

approximately 180,000 became infected. With the mandated slaughter of clinically diagnosed animals, the banning of ruminant-derived protein in animal feed, and the prohibition of specified bovine offals from the human food or animal feed chain, the epidemic was brought under control in the UK. However it had spread to Europe through the export of British beef and animal feed containing ruminant-protein, and although now diminished in these countries, it is still a matter for regulation and concern.

In the 30 years since BSE was discovered and the ensuing epidemic in the United Kingdom and Western Europe, only three cows have been found in the United States that were infected with BSE. Evidence indicates that two of these cows were of Canadian origin and were oldenough to have consumed ruminant-derived protein before the ban on such protein in ruminant feed was implemented in Canada. None of the three animals were slaughtered for human food. There also has been a ban on the importation into the U.S. of live ruminants and ruminant meat and bone meal from the UK since 1989 and all of Europe since 1997, so no potentially infected cattle could be blood sources for ImmunoLin.

The primary concern with BSE was and continues to be the transmission of a variant of the BSE disease to humans. Following the outbreak of BSE in cattle in the United Kingdom, 149 cases of variant Creutzfeldt-Jakob Disease (vCJD) appeared in people, presumably as the result of having consumed BSE-infective meats. vCJD is a fatal brain disease in humans. There is now substantial evidence that BSE infectivity and vCJD carry the same molecular "fingerprint," and it is generally accepted by scientists that BSE-contaminated meat caused the human cases of vCJD.

2. <u>BSE-Related Food Safety Measures are Effective</u>

In 1989, Great Britain instituted a ban on the domestic consumption of specified bovine offals.[1] By 1996, the UK and the EU had taken a broad series of measures aimed at limiting the spread of BSE to animals. These included the banning the feeding of any mammalian meat and bone meal to farm livestock and the slaughtering of infected cattle and those more than 30 months old. In May 2001, the EU Community introduced harmonized EU-wide controls for BSE and other transmissible spongiform encephalopathies. (The Community TSE Regulation: EC-999/2001).

In January 2004, FDA published an Interim Final Rule prohibiting the use of various cattle materials in FDA-regulated human food, food supplements and cosmetics to address the

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[1] Specified bovine offals are the brain, spinal cord, thymus, tonsils, spleen, and intestines from cattle more than 6 months old. **(K&H to insert citation.)**

potential risk of BSE.[2] In July 2004, FDA issued a revised Interim Final Rule that established a blanket prohibition on the use of the following substances in human food or animal feed: specified risk materials (SRMs), all material from non-ambulatory disabled (i.e., "downer") cattle, all material from cattle not inspected and passed for human consumption, and mechanically separated beef.[3] Specified risk materials are certain food-animal tissues that are known to harbor high titers of BSE-infectious material in infected cattle, and which have been found to have the highest degree of BSE infectivity in both field and laboratory tests.[4] According to the Scientific Steering Committee of the European Commission, in an animal with clinical disease, approximately 64% of the infectivity is in the brain, 26% is in the spinal cord, 4% is in the dorsal root ganglia, and 3% is in the distal ileum.[5] The eyes (retina) are estimated to contain less than 1% of the infectivity. Id. Other tissues, muscle, milk, and blood did not demonstrate any infectivity at the level of detection used. Id. Bovine blood is specifically not considered an SRM either for human food or for animal feed.[6, 7]

These efforts to eliminate entirely specified cattle tissues from the diet were taken because there was no known practical test known then to determine which products contained infectious material and which do not, and because the infectious dose of the material was unknown. Despite widespread exposure to BSE-contaminated meat in the UK, only a very small percentage of the exposed human population has been diagnosed with vCJD to date. Also,

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2 Interim Final Rule; "Prohibition of the Use of Specified Risk Materials for Human Food and Requirements for the Disposition of Non-Ambulatory Disabled Cattle;" 69 Fed. Reg. 1862 (January 12, 2004).

3 Interim Final Rule; "Use of Materials Derived From Cattle in Human Food and Cosmetics;" 69 Fed. Reg. 42256 (July 14, 2004).

4 *Id.* SRMs are the brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, the transverse processes of the thoracic and lumbar vertebrae and the wings of the sacrum), and dorsal root ganglia of cattle 30 months and older; and the tonsils and distal ileum of the small intestine of all cattle regardless of age. 9 C.F.R. § 310.22.

5 *Id.* at p. 42258 (July 14, 2004), citing "Opinion of the Scientific Steering Committee on the Human Exposure Risk (HER) Via Food with Respect to BSE," published by the Scientific Steering Committee, European Commission, and accessible at http://europa.eu.int/comm/food/fs/bse/scientific_padvice08_en.html.

6 *Id.* at p. 42256. Moreover, if blood was deemed to be an SRM, all beef would have to be placed under the SRM ban since both muscle meat as well as organ meat contain blood.

7 Proposed Rule; "Substances Prohibited From Use in Animal Food or Feed;" 70 Fed. Reg. 58670, 58595 (October 6, 2005).

because of the species barrier, there is thought to be a 10-10,000-fold increase in the amount of infectious material needed to cause illness in humans, as compared with cattle.[8]

3. Blood and Blood Products are Safe

There is currently little concern over the safety of bovine blood, bovine plasma or protein derived from bovine plasma. Bovine blood is not regarded as an SRM, and bovine blood has no recorded infectivity in experimental animal trials. Indeed, as stated in the Harvard Center for Risk Analysis Report on BSE in the U.S., "*No detectable infectivity has been found in blood or blood components of cattle infected with BSE.*"[9]

In its January 2004 and July 2004 Interim Final Rules, FDA specifically considered blood and blood products and determined them not to be a tissue of concern.[10] Indeed, FDA specifically considered and rejected an alternative that would have banned the use of blood and blood products in ruminant feed, stating that they could not show at the time that there was any BSE risk from the animal consumption of these products.[11]

Even if it is deemed possible that bovine blood from a BSE-infected cow has the potential to transmit disease, the most likely scenario is one where the blood has been contaminated from improper slaughtering practices involving other tissues rather than from infective prions in the blood of a diseased cow. Such a potential contamination was considered by an EU Scientific Steering Committee that was evaluating slaughtering practices that could enhance the titer of infectivity in collected blood by causing infected brain tissue to enter the blood stream.[12] The Committee noted that the use of pneumatic stunning procedures in the

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8 Interim Final Rule; "Use of Materials Derived From Cattle in Human Food and Cosmetics." (July 14, 2004), *supra,* at p. 42263, citing Scientific Steering Committee, European Commission, "Oral Exposure of Humans to BSE Agent: Infective Dose and Species Barrier," accessible at http://europa.eu.int/comm/food/fs/sc/ssc/out79_en.pdf. Conversely, studies have shown that the intraspecies infectious dose (*e.g.,* from cow to cow) is quite low. As little as 0.01 gram of ingested, infected brain homogenate was sufficient to cause a 6.7 % incidence of BSE in calves. *Id.* at p. 42263.

9 Cohen J, Duggar K, et al. (Harvard Center For Risk Analysis, Harvard School of Public Health) and Abdelrahman H, HabteMariam T,et al. (Center for Computational Epidemiology, Center of Veterinary Medicine, Tuskegee University). (2001). "Evaluation of the Potential for Bovine Spongiform Encephalophy in the United States," at p. 34. Research conducted per 1998 request by United States Department of Agriculture, and published on November 26, 2001.

10 Interim Final Rule, *supra* at fn. 3; pp. 42256 - 42285.

11 Proposed Rule, *supra* at fn. 7, p. 58595.

12 European Commission Scientific Steering Committee. (2000). "Opinion on the Safety of Ruminant Blood With Respect to TSE Risks." Adopted 13-14 April 2000.

slaughter of cattle can cause brain-tissue particles to be injected into the blood stream.[13] Current USDA practices forbid the use of pneumatic stun guns in the slaughter of cattle, and all bovine blood used to produce ImmunoLin™ is collected from cattle slaughtered using methods that prevent such contamination.

4. Current Status of the Transmission of vCJD by Blood

Early epidemiological evidence of BSE transmission was based on Creuztfeldt-Jakob Disease (CJD), the original human form of the prion disease. The epidemiological evidence does not suggest that sporadic CJD is transmitted from person to person via blood transfusion, but this evidence may not apply to vCJD.[14, 15] More recent infectivity studies have shown that the blood carries vCJD prions in both symptomatic and presymptomatic stages of the disease in animals and that blood transfusions can cause disease. (Gregori *et al*, 2006, citing Houston *et al*, 2000).[16] Llewelyn *et al* (2004) and Peden *et al* (2004) cite two separate cases in which it appears that vCJD infection may have been transmitted between humans by blood transfusion.[17] This suggests that infective prions exist in relatively elevated quantities in the blood of individuals already incubating vCJD. A recent report by Saa *et al* (2006) has shown that prions can be detected at exquisitely low levels in the blood of infected - but asymptomatic - animals by means of Protein Misfolding Cyclic Amplification (PMCA) technology.[18] These new findings suggest the theoretical possibility that asymptomatic, BSE-infected animals could harbor enough infective PrP^{SC} prions (the main infectious agent) in blood to transmit the disease to humans.

Importantly, while these studies indicate that transmission of vCJD to human from BSE-infected cattle via blood is a theoretical possibility, the reality is that the potential for

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13 *Id.* at p. 38.

14 Llewelyn CA, Hewitt PE, Knight RS et al. (2004). "Possible Transmission of variant Creutzfeldt-Jakob Disease by Blood Transfusion." Lancet; 363: 417-421.

15 Esmonde T, Will R, Slattery J *et al.* (1993). "Creutzfeldt-Jakob Disease and Blood Transfusion." Lancet; 341: 205-207.

16 Gregori L, Gurgel P, Lathrop J, *et al.* (2006). "Reduction of Infectivity of Endogenous Transmissible Spongiform Encephalopathies Present in Blood by Adsorption to Selective Affinity Resins." Lancet 368: 2223-30; *see also* Houston F, Foster ID, Chong A, Hunter A, Bostock CJ. (2000). Transmission of BSE by Blood Transfusion in Sheep. Lancet; 356: 999-1000.

17 Peden AH, Head MW, Ritchie DL, Bell JE, and Ironside JW. (2004). "Preclinical vCJD After Blood Transfusion in a PRNP Codon 129 Heterozygous Patient." Lancet; 364: 527-529.

18 Saa P, Castilla J, and Soto C. (2006). "Presymptomatic Detection of Prions in Blood." Science; 313: 92-94.

transmissibility is substantially reduced by the cross-species barrier. Furthermore, blood as a mode of vCJD transmission has been tested for and remains unobserved.[19] The implications of the Houston *et al* paper were considered by the Scientific Steering Committee of the European Commission in September of 2002, and the Committee concluded that although the transmission of infectivity through blood vCJD urgently needs further study, the new data did not justify nor add arguments for the introduction of new methods or approaches to the assessment of blood safety.[20]

a. Any Hypothetical Concentration of Prions in ImmunoLin™ During Manufacture To Be Offset by Dilution Caused by Plasma Pooling

If the blood of an infected but asymptomatic animal is used for food production, its potential infectivity will depend on the titer of the prion in the blood. As discussed above, the prion titer will ordinarily be very small and the blood is unlikely to be infective as a consequence. No animal ever processed for food use in the United States has yet been found to be infected with BSE. The pooling of the plasma would further reduce any hypothetical prion titer by a factor of at least 1000.[21] This substantially reduces the BSE risk from the blood of any single animal that might have been infected. It is theoretically possible that some concentration of prions could occur during the process of extracting the globulin from the plasma, if they were present in the plasma. The following worst-case scenario suggests that while such a concentration effect could theoretically occur, it would be more than compensated for by the dilution obtained from the plasma pooling.

For example, assume that prions are initially distributed in the blood mixture at a concentration of 1.0 ppb (1.0 ng/kg), and during the initial centrifugation process (separating cells from plasma), prions remain in the plasma fraction. As indicated the Table 4, the albumins constitute from 3.5-5% of the mass of the plasma, the total globulins (α, β and the immunoglobulins) from 2.95-7.5% of the plasma, and the immunoglobins from 0.95 - 2.5% of the plasma.

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[19] European Commission Scientific Steering Committee. (2000). "Preliminary Opinion: Oral Exposure of Humans To the BSE Agent: Infective Dose and Species Barrier." Adopted 2-3 March 2000.

[20] European Commission Scientific Steering Committee. (2002). "Opinion on the Implications of the Recent Papers on Transmission of BSE by blood Transfusion in Sheep (Houston et al, 2000; Hunter et al, 2002)." Adopted on 12-13 September 2002.

[21] Current practice actually involves the pooling of 1800 animals before the plasma collection is completed, so the factor of 1000 is roughly ½ that which actually would occur. Given that only three BSE infected animals have been found in the U.S. out of the billions and billions slaughtered, the odds are astronomically high against having two infected animals in the pool of 1800.

Table 4
Protein Composition of Plasma

Component	% in Plasma
Albumin	**3.5-5.0**
Globulins (total)	**2.0-5.0**
α	1.0-2.0
β	0.3-0.5
Immuno (total)	**0.95- 2.5**
IgG	0.80 –1.8
IgA	0.09- 0.45
IgM	0.06- 0.25
Fibrinogen	**0.2-0.45**
Complement	**0.1-0.2**
Total Protein	**6.9 -10.7**

During the separation of these protein fractions, the prions may distribute equally among them, or preferentially with any of them. Clearly the worst-case is that the prions migrate preferentially with the immunoglobins during separation and are retained with the final product. Therefore as a worst-case, they could be concentrated as much a 100-fold over their plasma concentration. So if a person were to consume this plasma-derived immunoglobulin as a protein concentrate (~50 grams), he could ingest 0.050 kg x 100 ng/kg = 5.0 ng of prions.

How does this assessment compare with the prion content of a steak from the same animal that contaminated the plasma pool above? Muscle meat has never been found to be infective, even from BSE animals in the later stages of infection.[22] We will assume as a worst-case that the prion titer in the muscle meat comes only from the small amount of contaminated residual blood that is not drained from the tissue after slaughter. Animals are drained of blood fairly efficiently at slaughter, so an assumption that steak contains at most 1% blood is

[22] European Commission Scientific Steering Committee. (1999). "Opinion on the Human Exposure Risk (HER) via Food with Respect to BSE." Preliminary opinion adopted 10 December 1999, p. 11, citing the Committee's "Opinion on the Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency to the French Government on the Draft Decree amending the Decree of 28 October 1998 Establishing Specific Measures Applicable to Certain Products of Bovine Origin Exported from the United Kingdom." Adopted 29 October 1999.

reasonable. The prion content of this blood is assumed to be 1000 ppb.[23] The total amount of prion ingested from a 500 g steak can be calculated as 0.5 kg x 0.01 x 1000 ng/kg = 5.0 ng, which is the same as above. These calculations show that the effect of concentrating the prions during ImmunoLin™ manufacture is compensated by the dilution of the plasma during pooling. Importantly, the pooling is a real occurrence, while the concentration is conjectural. Whether this concentration occurs or not depends on the actual partitioning of the prions between the plasma, plasma protein, and formed elements in the blood.

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[23] Since the prion concentration in the pooled plasma is reduced by 1000, the blood concentration of the infected animal must be proportionately increased since it is not diluted by pooling. Neither muscle or blood are considered SRMs and the concentration of prions in both blood and muscle in infected cattle are orders of magnitude less than that of brain and probably comparable. For purposes of the calculation we assume that only the blood is contaminated.

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ATTACHMENT 19

Summary of Studies

Summary of Animal Safety and Nutrition Studies on Plasma Proteins

Introduction

Blood and plasma proteins have long been recognized as high quality feed ingredients for farm animals, including swine, cattle, and poultry. Blood proteins are readily available and have an excellent amino acid profile and high digestibility. Indeed, plasma has been recommended for use in animal diets for hundreds of years – not only as a source of protein, but also as a source of immunological support. Prior to the development and widespread use of antibiotics in animal agriculture, proteins such as egg yolk, blood and colostrum were used as a source of immunologically active proteins to help animals recover from disease. Further, use of blood proteins in animal diets removes this by-product stream from environmental contamination and can increase the value of the animal to the farmer.

Traditionally, proteins were considered simply sources of amino acids for the animal. However, increasingly, scientists recognize that some proteins retain biological activity in the animal – these are called *functional proteins*. Functional proteins are recognized as having some function in the animal that elicits a physiological response. Spray-dried animal plasma (SDAP) is an ingredient widely used in animal feed applications. Several important functional proteins are retained in SDAP that can improve animal survival, health and performance of animals.

Method of collection

Blood proteins have traditionally been collected and heated to high temperatures in the manufacture of blood meal, which destroys the functional components. Conversely, spray-drying preserves the functional characteristics of the proteins, including biologically active peptides, such as albumin and IgG. In this process, blood is collected into stainless steel tanks, troughs or other devices containing an anticoagulant in abattoirs under government inspection. Blood is only collected from animals determined to be fit for slaughter by veterinary inspection. The blood is then transferred to stainless steel tanks, followed by centrifugation to separate plasma from the cellular fraction and chilling to 4-5°C. Chilled plasma is then transported by dedicated equipment to separate and dedicated facilities where it is spray-dried to produce a light brown, freely flowing powder that may be used in feed applications. Recently, the North American Spray-Dried Blood and Plasma Producers Association was formed to establish policies and GMP procedures for the manufacture of high quality and safe products (Russell, 2001).

Characteristics of Plasma

Functionality. The method of processing will influence the degree of biological activity that a protein will retain. Spray-drying plasma maintains the functionality of important proteins in plasma, including protease inhibitors, hormones, growth factors and immunoglobulins. Because plasma contains approximately 15% IgG, the functionality of this fraction is biologically meaningful to the animal consuming SDAP. Further fractionation can be used to increase IgG concentration to greater than 90%. Of course, the method of processing and preparation have important effects on the degree of functionality of the proteins.

The IgG in plasma are maintained and are biologically active. The Ig titers reflect the combined immunological history of the animals from which blood was collected and typically reflect both

the vaccination and exposure history of the animal. Plasma is collected into large lots, therefore, wide swings in Ig titer are avoided. It is of interest that the immunological history of animals (swine and cattle) will change over time; therefore the Ig titers in plasma will change also. Spray-dried animal plasma typically contains a wide array of specific antibodies that can be measured by standard serology tests. Plasma as a source of IgG will always reflect the immunogenic history of cattle.

Much research has been done to determine whether Ig from one species of animal may provide immunological support in another species of animal. In the feed industry, bovine, porcine and mixed plasma are available. In several studies, improvements in animal performance have been reported in animals fed swine or bovine plasma (Campbell et al., 2003; Quigley et al., 2003). Additionally, a tremendous number of published literature is available that documents the use of IgG (primarily bovine IgG or chicken IgY) as oral therapy in humans. More recent studies have evaluated the use of bovine colostral IgG as a means of improving athletic performance.

Role of IgG in intestinal immunity

Oral IgG are partially resistant to digestion (Roos et al., 1995) and can be measured in the feces of animals consuming SDAP. This suggests that intact and immunologically active IgG from SDAP may support the intestinal immune response of animals.

In addition to IgG provided by SDAP in the diet, researchers have shown that circulating IgG move from the circulation into the lumen of the intestine, where they can also support the intestinal immune system. Besser et al., (1988a) injected I^{125} labeled IgG into the jugular vein of calves. The researchers monitored production of I^{125} (total and protein-bound) in urine and feces following the injection. Approximately 2.5% of the I^{125} was excreted daily in the urine, and only about 3% of this was bound to protein. In addition, about 1.5% of the total I^{125} injected was excreted in the feces; however, 82% was still bound to protein. The researchers concluded that a portion of the IgG injected into the jugular vein moved into the intestine, where it was then excreted in the feces. In a subsequent study, Besser et al., (1988b) reported that the subcutaneous injection of colostral whey containing high titers of rotavirus antibody protected colostrum deprived calves from an oral rotavirus infection. Again, the authors concluded that circulating IgG were an important source of IgG to support intestinal immunity.

Use of plasma in animal diets

Spray-dried animal plasma has been included in the diets of commercially reared early weaned pigs for at about 20 years. Today, the vast majority of all feeds fed to pigs immediately after weaning (pig starters) in the U.S. contain SDAP at the rate of 2 to 7% of the formulation. Most research has reported improved intake, animal growth, feed efficiency and animal health in animals (including pigs and calves) when animals were fed SDAP (Gatnau et al., 2000; Morrill et al., 1995; Kats et al., 1994; Quigley and Bernard, 1996; Quigley et al., 2002, 2003; Van der Peet-Schwering, 1995, 1997). Others have compared SDAP to antimicrobials in diets of pigs (Coffey and Cromwell, 1995; Conde et al., 2000; Torrallardona et al., 2002, 2003) and calves (Quigley and Drew, 2000). We have summarized 47 research trials that used plasma in diets of pigs immediately after weaning – the average improvement in body weight gain, feed intake and

feed efficiency were 34, 23 and 9%, respectively. Pigs are very susceptible to anorexia immediately after weaning, therefore, a highly palatable ingredient could make a tremendous difference in animal performance (van Dijk et al., 2001a). The availability of SDAP in weanling pig diets allowed growers to reduce the age at weaning, and although the overall cost of the weaning ration increased with the inclusion of SDAP, overall return to the producer was improved.

Animals fed SDAP under low antigenic conditions (excellent hygiene and low exposure of the pigs to enteric pathogens) showed little improvement in performance when plasma was fed to pigs. On the other hand, when pigs were raised in a more "conventional" environment with typical exposure to enteric pathogens (indicated in the study by greater incidence of scours), animals fed plasma grew much faster than those fed the control ration. Coffey and Cromwell (1995) confirmed this finding when they fed pigs diets without or with plasma in "low antigenic" or "high antigenic" conditions. Pigs exposed to greater environmental stressors responded more dramatically to the inclusion of SDAP in the diet. These findings suggest that components in SDAP reduced effects of environmental stress. The authors suggested that the IgG fraction was critical to the overall response.

The value of animal plasma in the diets of herd replacement calves has been evaluated experimentally (Morrill et al., 1995; Quigley and Bernard, 1996). Morrill et al. (1995) reported improved body weight gain in calves fed plasma (25% of protein) compared to control (whey protein concentrate). All diets in this study were medicated with neomycin / oxytetracycline. Animals in this study were purchased from sale barns and transported to a commercial calf ranch in Kansas. Amount of stress in these calves was significant, as was shown by loss of body weight for the first two weeks of the study. Under these conditions, SDAP (bovine or porcine) resulted in significantly greater BW at 6 wk of age compared to calves fed control. By the end of 6 wk, calves fed milk replacer containing plasma had consumed 4.15 kg more calf starter than calves on control.

Quigley and Bernard (1996) reported no significant effect of bovine plasma (25% of protein) on animal growth, intake, or efficiency. Animals in this study were derived from dairy farms, raised under excellent management conditions and exposed to little stress. Rates of body weight gain in this study were greater than the study by Morrill et al. (1995) and were indicative of excellent management conditions.

Quigley et al. (2002, 2003) reported that the inclusion of SDAP or bovine serum in calf milk replacers improved the health and survival of Holstein bull calves (n = 360) purchased from dairy farms or sale barns. Bovine serum was used as an additive to milk replacer for the first 15 days of the study; bovine or porcine plasma was blended into the milk replacer formula and fed throughout the milk feeding period. Calves were not challenged with a specific pathogen, but incidence of diarrhea was significant, due to exposure to pathogens after birth and during transport. In all experiments, most calves had low concentrations of circulating IgG and had been exposed to significant immunological challenge from transport and commingling. The authors reported lower mortality, reduced incidence and/or severity of diarrhea and reduced use of therapeutic antibiotics during the first four weeks of each study when SDAP or bovine serum were included in the diet (Quigley et al., 2002, 2003).

Plasma reduces effect of enteric challenge

Numerous studies have been conducted in many classes of animals that indicate that plasma reduces mortality and morbidity associated with enteric challenge.

Pig challenge studies. Studies have evaluated the use of SDAP in the diet of pigs challenged with enteric pathogens, particularly *Escherichia coli* and rotavirus, which are economically important pathogens to the swine industry. While a thorough review of all of these studies is outside the scope of this presentation, several trials are particularly instructive. Researchers in the U.S. reported that the administration of water-soluble SDAP reduced the effects of oral challenge with *E. coli* (Borg et al., 1999). Italian researchers (Bosi et al., 2001) challenged pigs with an enteropathogenic strain of *E. coli* at weaning. Pigs were fed for 15 days and intake, gain, feed efficiency and mortality were determined. Feeding SDAP reduced mortality, improved growth and efficiency following oral challenge.

A follow-up study by the same researchers (Bosi, et al., 2004) also reported improved performance in pigs fed 6% SDAP or antibiotic (250 mg/kg colistin + 500 mg/kg amoxycycline) in the diet and challenged with *E. coli* (10^{10} CFU *E. coli* K88 on d 3 after weaning). These researchers also measured the amount of specific K88 antibody in both the plasma and saliva of the pigs on d 15 after challenge. Interestingly, production of K88 specific *E. coli* antibodies in plasma and saliva were reduced when either the antibiotic or SDAP was included in the diet. These data suggest that animals were not exposed to the challenge organism, and therefore, did not produce antibody.

Calf challenge studies. Quigley and Drew (2000) challenged 36 colostrum-deprived Holstein bull calves with *E. coli* K99 at 3 d of age. Calves were fed commercial calf milk replacers containing no additive, an antibiotic (neomycin and oxytetracycline) or bovine serum at 3.3% of the formula. All calves showed signs of enteric infection following oral challenge; however, calves fed either antibiotic or bovine serum had lower mortality, morbidity (number of days with diarrhea) than calves fed the control milk replacer.

Arthington et al. (2002) fed Holstein bull calves milk replacer containing 0 or 80 g/d of bovine serum as a therapy following oral challenge with bovine coronavirus on d 0. Feeding bovine serum improved average packed cell volume and respiration rate compared to calves fed diets without serum. The authors concluded that supplementation of milk replacer with bovine serum improved the rate of recovery in calves following coronavirus challenge.

Hunt et al. (2002) challenged 24 calves orally with 10^8 cfu of *Cryptosporidium parvum* at 8 days of age. Calves were fed milk replacer with added bovine serum or soy protein concentrate. Oral challenge caused significant fecal shedding of *C. parvum* oocysts, diarrhea, increased intestinal permeability, reduced villus surface area and reduced intestinal lactase activity. Calves fed bovine serum had a 33% reduction in oocyst shedding and a 33% reduction volume of diarrhea produced, improved intestinal morphology and more rapid recovery following challenge. The authors (Hunt et al., 2002) recommended the use of bovine serum as a possible therapy in human cryptosporidiosis.

Researchers from Belgium (Nollet et al., 1999) reported that the presence of glycoprotein glycans in bovine plasma may have an antimicrobial role in animals. Calves were assigned to receive no added SDAP or bovine or porcine plasma added to milk replacer to 14 days of age. Plasma powder was manufactured by collection of blood from EU approved slaughterhouses. Blood was centrifuged to collect plasma, which was then pasteurized at 50°C for 15 min to destroy antibodies. Calves were infected with 10^{10} CFU of *E. coli* F5+ or F17+ strains at 12 to 24 h of age. Subsequent evaluation to determine titers to F5+ or F17+ recovered none. After pasteurization, plasma was concentrated and spray dried. The researchers concluded that inclusion of bovine plasma improved survival of challenged calves, reduced incidence and severity of diarrhea, improved overall health of calves and reduced excretion of F5 organism by 2 to 4 logs due to inhibition of adhesion. The concept of carbohydrate moieties (oligosaccharides) to non-reversibly bind bacteria has been shown experimentally (Grizard and Barthomeuf, 1999; Le Blay et al., 1999) and is used in some animal industries (Oli et al., 1998).

Other effects of SDAP

Recent research suggests that the functional proteins in SDAP may play important roles in other biological functions of the animal.

Researchers in Spain fed weanling pigs (21 days age weaning) diets containing 0 or 7% SDAP and measured concentrations of caecal microflora following challenge with *E. coli* (Torradellona, 2001). Counts of Clostridium were reduced from 1.56 to 0 log CFU/g of caecal contents when pigs were fed SDAP. Further, concentrations of Lactobacilli were increased with SDAP in the diet (8.83 vs. 8.01 log CFU/g).

Modification of bone mass in pigs fed SDAP after weaning has also been reported (Jiang et al., 2000), as well as a significant reduction in intestinal weight in rats (Moreto, 2001, unpublished data) and pigs (Jiang et al., 2000). Jiang et al. (2000) also reported reduced urea N concentrations when pigs were fed SDAP postweaning, and suggested that changes in intestinal mass and cellularity was related to changes in circulating urea N concentrations. Plasma or purified globulin fed to pigs has been shown to reduce the concentration of circulating IgG in pigs (Campbell et al., 2000, unpublished data) and Bosi et al. (2001) reported that the inclusion of SDAP protein or antibiotics reduced concentrations of IL-8, TNF-α and IFN-γ in the jejunum in pigs challenged with *E. coli*.

When taken collectively, these data suggest that dietary SDAP may serve as a "first line of defense" against enteric pathogens, including viruses and bacteria. This "passive enteric immunity" reduces stimulation of the immune system under modern commercial animal production. The animal's immune system is not stimulated, and as a result, pro-inflammatory cytokines are not secreted by macrophages. Cytokines secreted by macrophages affect many tissues and are responsible for liberating nutrients for use in supporting an immune response. Recent reports (Touchette et al., 2002) indicate that feeding SDAP reduced secretion of TNF-α, IFN-γ, and IL-6-β. When the immune system is not overstimulated, more nutrients are available for tissue accretion. According to many researchers, SDAP may serve an antimicrobial role in the intestine (Jiang, et al., 2000), thereby reducing the overall enteric challenge.

Constituents in SDAP have also been reported to improve repair of damaged intestinal epithelial tissue in vitro (Rhoads et al., 2000) and in humans (Lembcke et al., 1997). Reports of improved intestinal integrity in pigs challenged with enteric pathogens (Torrallardona et al., 2002; van Dijk, 2001b) are also consistent with these observations. It is not yet clear which component(s) are responsible for the observed improvements in regrowth of damaged tissue.

Research continues to elucidate an important role for the functional proteins in SDAP in animal agriculture and potentially, in human nutrition and health. The urgent need to reduce the overuse of antibiotics in diets of animals demands that viable alternatives be found. Numerous published research trials have documented the value of SDAP in reducing the effects of experimental and on-farm enteric challenges.

Summary

Use of SDAP is well accepted in animal agriculture. The value of functional proteins, including IgG, to support enteric health, reduce the effects of pathogenic challenge and reduce morbidity and mortality are well documented in both scientific and popular literature. Although not reviewed in this paper, the use of functional proteins (especially IgG) to improve human health is also well documented. Continued availability of SDAP to dairy and beef farmers will improve animal health, reduce the need for antibiotics and improve the efficiency and safety of beef and dairy products.

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