

TPB 23



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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20852

Animal Feed Rule Hearing
Docket No. 01N-0423

Dear Sir or Madam:

We would like to address question 13 to be discussed at the upcoming Animal Feed Rule Hearing. What new information is available on potential efficient, accurate analytical methods that may be used in detecting mammalian proteins, especially the prohibited mammalian proteins, in feed and what should the sampling parameter of such a program be?

Due to the concern that Bovine spongiform encephalopathy may be introduced to the United States or worldwide by contaminated animal feeds, screening of feed products is essential. Many feed blenders and manufacturers use animal plasma or plasma products as a protein source for ruminant feed. Current FDA regulations prohibit feeding of protein derived from mammalian tissue excluding blood and blood products, and any products whose only mammalian protein consists entirely of porcine or equine protein. While bovine serum is currently acceptable for use, its future is uncertain due to changing attitudes of producers, blenders and consumers who would like to have more products that are "free" of bovine plasma and plasma products.

Toward this end, a novel lateral flow immunoassay has been developed which can quickly and qualitatively determine the presence of bovine Immunoglobulin G, a major component in plasma, at very low concentrations. It is being used to test for bovine IgG contamination in porcine plasma used in the feed industry. Producers and consumers alike can use this device to verify product content at threshold levels. It is an inexpensive, disposable test device, is stable at room temperature, has a long shelf life, requires minimal sample preparation and laboratory equipment or expertise to use and can be performed on site. In addition, this test has the potential to be expanded to include equine serum product screening.

Please find the enclosed manuscript, which describes this assay in detail. The manuscript also describes the sensitivity of the device as compared to current, laboratory diagnostic tests used to monitor bovine IgG in feed additives.

Sincerely,


Jerry McVicker


Jason Newgard

01N-0423

CH2

1 **A Novel Method for Detecting Bovine Immunoglobulin G in Dried**
2 **Porcine Plasma as an Indicator of Bovine Plasma Contamination**

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1 **ABSTRACT**

2 **Objective** - To develop a lateral flow immunoassay device that quickly and qualitatively
3 detects very low levels of bovine Immunoglobulin G (IgG) in spray-dried porcine plasma
4 products used in the feed industry.

5 **Procedure** - A polyclonal antibody to detect Bovine IgG was prepared and cross
6 reacting antibodies to porcine IgG were removed by adsorption chromatography. A
7 lateral flow immunoassay device was prepared using the absorbed antibody. The
8 optimization of test parameters was performed using porcine serum with a known
9 concentration of bovine serum added. The detection limit of the device was evaluated and
10 compared to a commercially available turbidimetric immunoassay (TIA)⁵ method.

11 **Results** -The lateral flow immunoassay device was able to qualitatively detect bovine
12 IgG levels in normal swine serum at concentrations as low as 0.01% (v/v). Using
13 rehydrated spray-dried porcine plasma, the device was able to detect bovine IgG
14 concentrations of 0.001% (w/v).

15 **Conclusion** -The device provides qualitative detection of bovine IgG, which is indicative
16 of bovine protein present in porcine plasma used for feed. With a detection level 0.01%
17 (v/v), the device is more sensitive than a commercially available TIA method (detection
18 limit of 2.5% [v/v]). The device provides a simple and sensitive method for detecting
19 contaminating bovine IgG in porcine plasma products.

INTRODUCTION

1
2 In recent years the threat to foreign and domestic ruminant herds from neuralgic
3 diseases acquired from animal feeds has become a serious issue for consumers, producers
4 and government regulatory agencies¹. Transmissible spongiform encephalopathies (TSE)
5 such as scrapie, bovine spongiform encephalopathy (BSE), and its human form,
6 Creutzfeld-Jakob disease (CJD), have come to the forefront as a serious economic and
7 health risk worldwide². Under current United States Code of Federal Regulations (CFR),
8 any ruminant feed derived from mammalian tissues is forbidden³. This CFR, however,
9 allows for the use of mammalian blood and blood products, and any products whose only
10 protein is of porcine or equine origin to be used in feed products³.

11 Feed blenders and manufacturers usually acquire these products from renderers
12 who process or transport products from ruminants (cattle, buffalo, sheep, goats, deer, elk,
13 antelopes) and non-ruminants (porcine, equine)⁴. Because of this, some care must be
14 taken to insure that cross contamination does not take place in the manufacturing
15 process⁴. While bovine serum is currently acceptable for use, its future is uncertain due
16 to changing attitudes of producers, blenders and consumers who would like to have more
17 products that are "free" of bovine serum and serum products⁴.

18 Currently, there are only a limited number of commercially available assays that
19 can successfully determine if these products contain protein from one or more species⁵.
20 Toward this end, a novel lateral flow immunoassay device has been developed which can
21 quickly and qualitatively determine the presence of bovine Immunoglobulin G, a major
22 component in plasma, at very low concentrations in porcine plasma products used in the
23 feed industry.

MATERIALS AND METHODS

2 **Reagents –**

3 Equine serum, porcine serum and bovine serum used as immunoelectrophoresis
4 references and rabbit anti-goat IgG were obtained from Jackson ImmunoResearch
5 Laboratories (West Grove, PA). Immunoelectrophoresis gels, buffers and chambers were
6 purchased from Sebia (Norcross, GA). Goat anti-equine IgG, goat anti-porcine IgG and
7 goat anti-bovine IgG antibodies, as well as dilution buffer were obtained from Midland
8 BioProducts (Boone, IA). Bovine and porcine plasma samples were obtained by jugular
9 venipuncture and collected into EDTA anticoagulant tubes. Plasma was promptly
10 centrifuged and stored at –20 °C. Fourteen spray-dried plasma products (porcine plasma,
11 serum, flavor and stock; bovine whole blood, plasma, serum, flavor and stock) were
12 provided by American Protein Corporation (Ames, IA).

13 **Goat anti-bovine IgG absorption-**

14 The bovine/porcine antibody cross reactivity was compared with that of other
15 inter-species cross reactivity by immunoelectrophoresis (IEP) as per manufacturer's
16 instructions. All cross reactivity to porcine IgG was removed from the goat anti-bovine
17 IgG antibody by affinity chromatography. Purity was and verified by IEP.

18 **Gold colloid production and antibody conjugation-**

19 A 40 nm gold colloid was manufactured using standard colloid growth
20 techniques^{6,7,8,9}. The processed antibody was conjugated to the gold colloid at 1.0 mg/ ml
21 using general conjugation techniques for immunoglobulins^{6,7}.

22

23

1 **Lateral flow device development-**

2 The goat anti-bovine IgG (test line reagent), rabbit anti-goat IgG (control line
3 reagent) and the gold conjugate were incorporated into a patented lateral flow
4 immunoassay device format and a number of prototype devices were assembled for
5 testing and evaluation¹⁰.

6 **Cassette testing-**

7 Bovine serum was added to porcine serum as described in Table 1. Using the
8 dilution buffer, each sample was diluted 1/10. After adding 150µl of each diluted solution,
9 the device was left undisturbed on a level surface for 10 to 25 minutes.
10 Fourteen spray-dried protein products containing bovine and/or porcine plasma were
11 rehydrated, at 10 % (w/v) with de-ionized water, for testing in the same method as the
12 plasma standards.

13 **Results**

14 **Anti-bovine IgG absorption-**

15 Cross reactivity was observed by immunoelectrophoresis (Figure 1) between goat
16 anti-equine IgG and porcine serum (Table 2). Goat anti-porcine IgG cross-reacted with
17 equine serum and bovine serum (Table 2). Goat anti-bovine IgG cross-reacted with
18 equine serum only (Table 2). Verification by IEP indicated that all bovine to porcine
19 cross reactivity was removed from the anti-bovine IgG antibody by affinity
20 chromatography.

21 **Cassette testing-**

22 The six standards prepared by introducing bovine plasma to porcine serum at set
23 concentrations (Table 1) were run in the prototype cassettes and evaluated (Figure 3).

1 Tests of the standards in replicate (Table 3) indicated, by visual evaluation, that the
2 device was able to qualitatively detect bovine IgG concentration to 0.01% (v/v) in
3 porcine serum.
4

5 **Device performance -**

6 Device testing with spray dried porcine products demonstrated visible control
7 lines with all products. Rehydrated spray-dried porcine products (plasma or serum) with
8 low concentrations of rehydrated spray-dried bovine products (plasma, serum or whole
9 blood) introduced, produced visible test lines. The device was able to differentiate
10 between “all pork” (a negative result) samples and “all” or partial beef samples (a
11 positive result). The device tested with mixed pork and beef flavor or stock product
12 samples did not develop test lines (a positive result).

13 **DISCUSSION**

14 Many feed manufactures use animal plasma or plasma products as a protein
15 source for ruminant feed. FDA regulations prohibit feeding of protein derived from
16 mammalian tissue, excluding blood and blood products and any products whose only
17 mammalian protein consists entirely of porcine or equine protein¹. The assay device
18 described here provides a rapid, (10-min) qualitative detection of Bovine IgG (plasma)
19 contamination in porcine serum products used for feed. With a detection level 0.01%
20 (v/v), the device is more sensitive than quantitative commercial automated turbidimetric
21 immunoassay (TIA) method of bovine IgG concentration determination, which is
22 accurate to 2.5% (v/v)⁵. The device provides a disposable test, which requires minimal
23 storage requirements, sample preparation, laboratory equipment and expertise to operate,

1 and may be performed at the field site. With simple, fast and reliable data, producers and
2 consumers alike can use this device to detect bovine IgG content at threshold levels. This
3 test also, has the potential to be expanded to include equine serum products if so desired.

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Table 1. Standard concentrations

(1)	1.0%	bovine serum in porcine serum
(2)	0.1%	bovine serum in porcine serum
(3)	0.01%	bovine serum in porcine serum
(4)	0.001%	bovine serum in porcine serum
(5)	Negative control	100 % porcine serum
(6)	Positive control	100% bovine serum

Table 2. Inter-species cross reactivity

	Equine Serum	Bovine Serum	Porcine Serum
Anti-Equine IgG	X		X
Anti-Porcine IgG	X	X	X
Anti-Bovine IgG	X	X	

X indicates inter-species cross reactivity

Table 3. Cassette results

	Std. Concentration		Device Result
(1)	1.0%	bovine serum in porcine serum	Positive
(2)	0.1%	bovine serum in porcine serum	Positive
(3)	0.01%	bovine serum in porcine serum	Positive
(4)	0.001%	bovine serum in porcine serum	Negative
(5)	Negative control	100 % porcine serum	Negative
(6)	Positive control	100% bovine serum	Positive

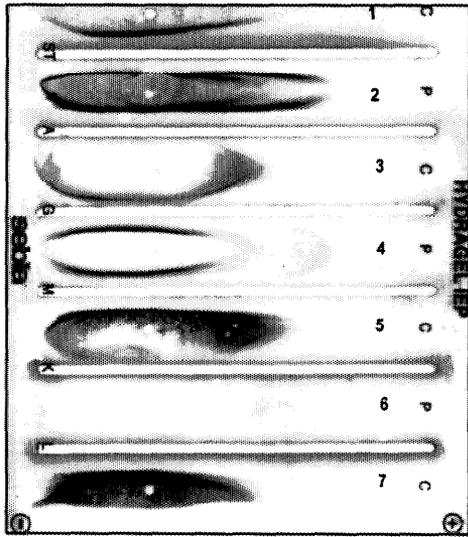


Figure 1. IEP of inter-species cross reactivity. Numbered wells contain: (1&5) porcine serum, (2,4&6) equine serum, (3&7) bovine serum. Troughs contain: (ST&A) anti-equine IgG, (G&M) anti-porcine IgG, (K&L) anti-bovine IgG.

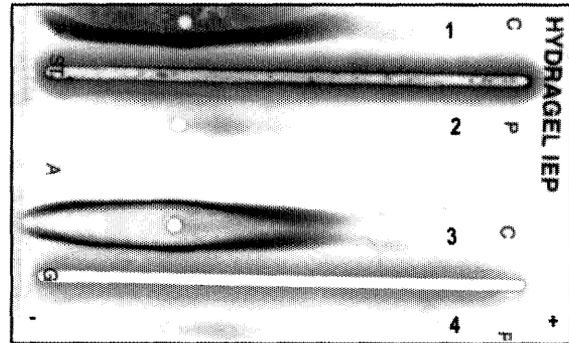


Figure 2. IEP of intra-species cross reactivity removed. Numbered wells contain: (1&3) bovine serum, (2&4) porcine serum. Troughs contain: (ST) anti-bovine IgG with porcine reactivity, (A) anti-bovine IgG without porcine reactivity, (G) anti-bovine IgG control.

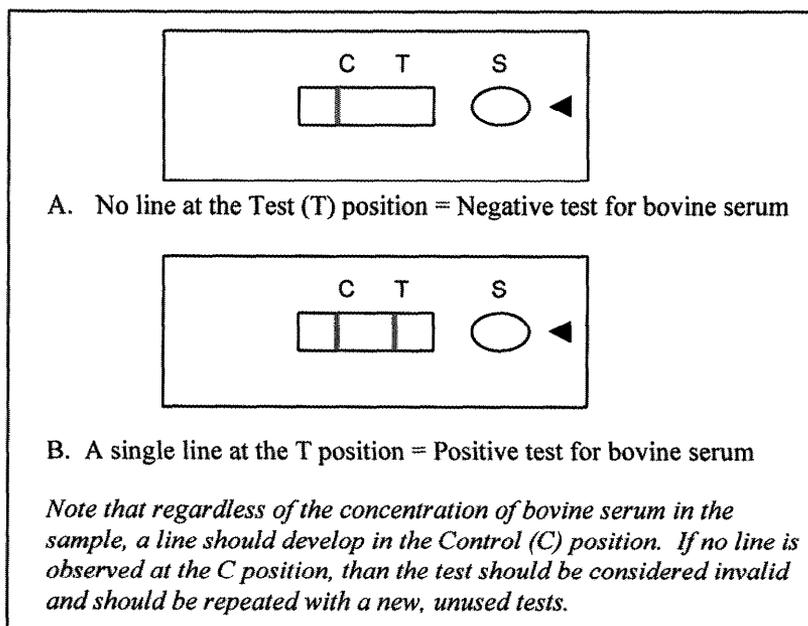
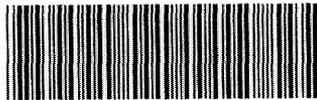


Figure 3. Lateral flow device interpretation.

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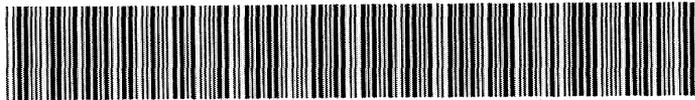


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