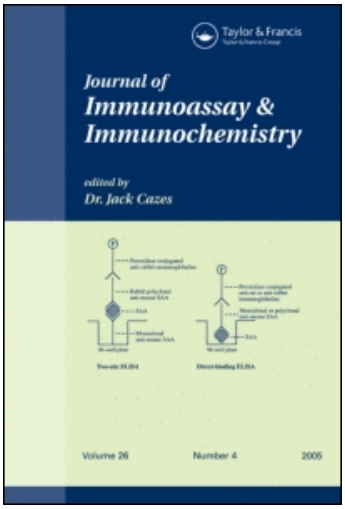

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Prevalence of Antibodies Reactive to Pathogenic and Nonpathogenic Bacteria in Preimmune Serum of New Zealand White Rabbits

Amanda A. Lathrop[a]; Karleigh Huff[a]; Arun K. Bhunia[a]
[a] Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, Indiana, USA

To cite this Article Lathrop, Amanda A. , Huff, Karleigh and Bhunia, Arun K.(2006) 'Prevalence of Antibodies Reactive to Pathogenic and Nonpathogenic Bacteria in Preimmune Serum of New Zealand White Rabbits', Journal of Immunoassay and Immunochemistry, 27: 4, 351 — 361
To link to this Article: DOI: 10.1080/15321810600862223
URL: http://dx.doi.org/10.1080/15321810600862223

Journal of Immunoassay & Immunochemistry, 27: 351–361, 2006

ISSN 1532-1819 print/1532-4230 online
DOI: 10.1080/15321810600862223

Prevalence of Antibodies Reactive to Pathogenic and Nonpathogenic Bacteria in Preimmune Serum of New Zealand White Rabbits

Amanda A. Lathrop, Karleigh Huff, and Arun K. Bhunia
Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, Indiana, USA

Abstract: Polyclonal antibodies are typically produced in rabbits. The rabbit's health plays an important role in the quality of antibodies produced. Therefore, recommendations have been made by organizations on which bacteria and how frequently to test rabbit colonies. Since it is well known that rabbits may contain cross-reactive antibodies in their preimmune serum, it is common to test the rabbits for reactivity prior to immunization. Here preimmune sera from 19 different rabbits were tested with ELISA against 27 pathogenic and nonpathogenic bacterial cultures. ELISA results showed that *Salmonella enterica* serovar Typhimurium and *Bacillus cereus* AS4-12 had the highest average absorbance values (0.60 and 0.54, respectively) and the most preimmune serum samples testing positive was 17. *Pseudomonas putrefaciens* and *B. subtilis* had the lowest absorbance values (<0.1) and did not test positive in any of the preimmune serum samples. Fourteen of the 27 cultures showed positive reactions with 50% or more of the preimmune serum samples tested. Fifty-three percent of the rabbit preimmune sera showed positive reactions with 10 or more bacterial cultures. In Western blot analyses, selected serum samples showing the highest ELISA values reacted with bands in the 97, 36, and 29 kDa regions or with bands in the 63 kDa and 32 kDa regions. Data suggest that the presence of cross-reactive antibodies in the preimmune serum is a common problem amongst the samples tested. Extensive preimmune serum testing should be implemented when polyclonal antibodies are intended for diagnostic testing.

Keywords: Preimmune serum, Antibody, Bacteria, New Zealand White rabbits, ELISA, Western blotting

Address correspondence to Prof. Arun K. Bhunia, Molecular Food Microbiology Laboratory, Department of Food Science, 745 Agriculture Mall Drive, Purdue University, West Lafayette, IN 47907-2009, USA. E-mail: bhunia@purdue.edu

INTRODUCTION

New Zealand White (NZW) rabbits are among the most commonly used animals for polyclonal antibody (PAb) production.[1] They are widely used because they can produce ample serum volumes and are docile in nature, thus making them easier to handle than other animals. Most importantly, rabbits have been shown to respond to a wide variety of immunogens. NZW rabbits are also used in toxicity and safety testing, infectious disease, and other medical research.[2]

PAb production, like all other research involving animals, relies heavily on the animal's health. Subclinical manifestations of disease can yield animals unusable for research or cause interference with experimental results.[2] Research complications due to the presence of infective agents in rabbits are hard to find because results are rarely published. However, the Gesellschaft Für Versuchstierkunde–Society for Laboratory Animal Science (GV-SOLAS) working group, in 1999, addressed some of these issues by publishing a report summarizing the existing literature.[3] GV-SOLAS looked at the effects of viral, bacterial, and parasitic infections, where most of their findings were focused on mice or rats. For example, mice infected with *Clostridium piliforme* can show a lower susceptibility to experimental arthritis caused by *Yersinia enterocolitica*. Another infection normally found subclinically in rats, *Corynebacterium kutscheri*, may enhance cytokine production, inducing nonspecific resistance. Rabbits that were naturally infected with *Encephalitozoon cuniculi* had an altered immune response to *Brucella abortus*, which led to an elevated amount of IgM and a depressed amount of IgG. This report illustrates the need for rigorous health monitoring of animal colonies.

Organizations such as the Federation of European Laboratory Animal Science Associations (FELAS) recommend routine health monitoring of rabbit colonies for microorganisms (viruses, bacteria, fungi, and parasites) including bacterial species such as *Bordetella bronchiseptica*, *Clostridium piliforme*, *Pasteurella multocida*, and *Salmonella* spp. every 3 months.[4] Other organisms included are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus* spp. Since these organisms normally cause disease in immunodeficient animals, they are not as frequently monitored in immunocompetent animals. Bacterial agents are normally detected using culturing methods. However, serological methods, such as agglutination, immunofluorescence, and enzyme-linked immunosorbent assay (ELISA) are frequently used for hard-to-culture bacteria.[5] A positive serology test does not definitively mean the animal is infected with the organism tested and, therefore, further testing is recommended before taking drastic measures such as culling.[6] Although the presence of antibodies in the serum of rabbits may not be a top concern for all research involving rabbits; these pre-existing antibodies can have a detrimental effect on antibody development for specific applications. In research settings, antibodies to a target protein

are used to determine the presence/absence or level of expression in a wild type or mutant strain in a very specific application. Therefore, its reaction to other proteins from other microorganisms is not important. However, for diagnostic applications in food or clinical samples, antibody specific for a pathogen must not show cross-reaction with other pathogens or non-target organisms. If rabbits carry background antibodies against common microbial pathogens, then these would interfere with assay performance of antibodies generated for a specific target pathogen (antigen).

It is generally accepted that animals may contain cross-reactive antibodies in their serum prior to immunization with a specific antigen; it is common practice to test preimmune serum for reactivity.[7] But, to what extent should serum be tested and how much of a problem are these pre-existing antibodies? Despite the presence of pre-existing cross-reactive antibodies, they can be removed through processes such as cross-absorption and affinity purification. However, these methods can be time consuming, costly, and may not yield satisfactory results.[8] This research focuses on determining the prevalence of pre-existing antibodies that react with bacteria commonly associated with food. The aim is to highlight the scope of the problem and serve as a warning to other researchers developing PAbs for diagnostic purposes.

EXPERIMENTAL

Serum Purification

Serum samples were obtained from 19 rabbits, from three vendors, over a four-year period (Table 1). Samples were collected at different times between February 2001 and June 2004. The ages of the rabbits ranged from 3 months to over 18 months. None of the rabbits from company A were classified as specific pathogen free (SPF), while all the rabbits from company B and C were SPF. Eleven samples were collected from company A, and 4 each from company B and C. Samples were stored at -40°C until use. Antibodies in serum samples were purified by Protein A chromatography using the ÄKTA prime unit (Amersham Pharmacia Biotech AB, Uppsalla, Sweden). Briefly, undiluted samples (2 mL) were loaded onto the column with 20 mM phosphate buffered saline, pH 7.4 (PBS), and eluted with 0.1 M glycine, pH 2.4. The elution peak was collected, pH adjusted to 7.0 with 1 M Tris, and antibody concentrations determined to be from 0.26–2.03 mg/mL. Antibodies were stored at 4°C with 1% bovine serum albumin (BSA) and 0.01% Thimerosal (Sigma).

Cultures Used

Frozen cultures from our collection (Table 2) were subcultured in brain heart infusion (BHI) broth, maintained on BHI agar plates, and stored at

Table 1. Rabbit preimmune serum used in the study

Rabbit ID	Serum collection date	Rabbits age (approx.)	Specific pathogen free (SPF) status	Company[a]	Antibody concentration after purification (mg/mL)
1	2/7/01	3 mo	No	A	0.73
2	2/14/01	3 mo	No	A	0.64
3	4/24/02	3 mo	No	A	0.26
4	4/24/02	3 mo	No	A	0.66
5	6/5/02	3 mo	No	A	1.14
6	6/11/02	3 mo	No	A	1.27
7	6/11/02	3 mo	No	A	1.34
8	6/11/02	3 mo	No	A	0.60
9	6/5/02	3 mo	No	A	1.05
10	11/8/02	3–4 mo	Yes	B	0.68
11	11/8/02	3–4 mo	Yes	B	0.72
12	11/8/02	3–4 mo	Yes	B	0.76
13	11/8/02	3–4 mo	Yes	B	0.92
14	6/21/04	>18 mo	Yes	C	2.03
15	6/21/04	>18 mo	Yes	C	1.78
16	2/11/04	3 mo	No	A	0.61
17	2/11/04	3 mo	No	A	0.60
18	6/21/04	8 mo	Yes	C	1.08
19	6/21/04	8 mo	Yes	C	0.89

[a]Company names are withheld.

4°C. Fresh cultures were grown in BHI for 16–18 h and then prepared for use. Cultures chosen are commonly associated with food and many are pathogenic.

Evaluation of Preimmune Serum Reactivity with Bacteria, using ELISA

Indirect ELISA was performed, as described previously, with some modifications.[9] Briefly, 5 mL of bacterial cells were harvested by centrifugation (2,200 × g, 15 min) and resuspended in 0.1 M carbonate coating buffer (pH 9.6) to yield a final concentration of approximately 1×10^9 CFU/mL. One hundred microliters of the cell suspension was dispensed into the wells of a 96-well microtiter plate (1B, Dynex, Chantilly, VA), sealed, and stored at 4°C overnight. The cell suspension was removed from the well by using a vacuum setup and washed three times with PBST (PBS with 0.5% Tween 20). Protein A affinity purified antibodies were diluted to 0.08 mg/mL and

Table 2. ELISA reaction profiles and number of positive preimmune serum samples for 27 different bacterial cultures

Bacteria	Source	ELISA ($A_{490} \pm SD^a$)	Number of positive preimmune serum out of 19 serums tested
Salmonella enterica subsp. Typhimurium	ATCC	0.60 ± 0.36^{a}	17
Bacillus cereus AS4-12	J. Handelsman, PU	0.54 ± 0.34^{a}	17
Enterobacter aerogenes	Our collection	0.41 ± 0.29^{b}	13
Salmonella enterica subsp. Enteritidis 13096	M.F. Slavik, UA	0.39 ± 0.26^{b}	15
Proteus vulgaris	D. Fung, KSU	$0.38 \pm 0.29^{c,b}$	14
Escherichia coli ATCC 51739	ATCC	$0.35 \pm 0.23^{c,b,d}$	13
E. coli EDL 933 (O157:H7)	USDA-ARS ERRC	$0.34 \pm 0.26^{c,b,d}$	10
Pseudomonas aeruginosa ATCC 10145	ATCC	$0.30 \pm 0.23^{c,e,d}$	9
Corynebacterium glutamicum ATCC 31834	ATCC	$0.27 \pm 0.25^{f,e,d}$	10
Enterococcus faecalis	D. Fung, KSU	$0.25 \pm 0.19^{f,e}$	10
Bacillus cereus T	A. Aronson, PU	$0.25 \pm 0.11^{f,e}$	13
Hafnia alvei	D. Fung, KSU	$0.24 \pm 0.19^{f,e,g}$	6
Streptococcus mutans ATCC 25175	ATCC	$0.24 \pm 0.18^{f,h,e,g}$	9
Citrobacter freundii	ATCC	$0.24 \pm 0.15^{f,e,g}$	9
Listeria monocytogenes F4244	CDC	$0.20 \pm 0.18^{f,h,i,g}$	9
Lactococcus lactis ATCC 11454	ATCC	$0.16 \pm 0.15^{j,h,i,g}$	4
Listeria monocytogenes V7	USFDA	$0.15 \pm 0.13^{j,h,i}$	5
Bacillus cereus F837	A. Wong, UW	$0.15 \pm 0.11^{j,i,k}$	2
Listeria grayi ATCC 19120	ATCC	$0.14 \pm 0.12^{j,i,k}$	5
Listeria welshimeri ATCC 35897	ATCC	$0.12 \pm 0.10^{j,i,k}$	3
Listeria seeligeri SE 31	FDA	$0.12 \pm 0.10^{j,i,k}$	2
Bacillus thuringiensis H073	A. Aronson, PU	$0.12 \pm 0.08^{j,i,k}$	3
Listeria ivanovii V12	Our collection	$0.11 \pm 0.07^{j,k}$	1

(*continued*)

Table 2. Continued

Bacteria	Source	ELISA ($A_{490} \pm SD^a$)	Number of positive preimmune serum out of 19 serums tested
Listeria innocua ATCC 33090	ATCC	$0.10 \pm 0.08^{j,k}$	1
Listeria monocytogenes SLCC 2373	Our collection	$0.09 \pm 0.09^{j,k}$	2
Pseudomonas putrefaciens	ATCC	$0.08 \pm 0.06^{j,k}$	0
Bacillus subtilis	Our collection	$0.07 \pm 0.05^{j,k}$	0

ATCC, American Type Culture Collection; USDA, United States Department of Agriculture; ARS, Agricultural Research Service; ERRC, Eastern Regional Research Center; CDC, Centers for Disease Control and Prevention; PU, Purdue University; UW, University of Wisconsin; FDA, Food and Drug Administration; KSU, Kansas State University; UA, University of Arkansas.

[a]ELISA values with the same letters (a,b,c, ...) are not significantly different at $P < 0.05$. Values above 0.20 were considered positive.

100 μL was added to each well. Plates were sealed and incubated for 1 h at 37°C on a rotary shaker and washed as above. Peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA) was diluted (1:5,000), dispensed (100 μL/well), incubated for 1 h at 37°C on a rotary shaker, and washed 3 times with PBST. Substrate was prepared by combining 5 mL of phosphate-citrate buffer with sodium perborate (Sigma) and one OPD tablet (O-phenylenediamine, Sigma). One hundred microliters was dispensed per well and incubated at room temperature for 15 min. The reaction was stopped with one hundred microliters of H_2SO_4 (0.1 M) and plates were read at 490 nm using a Microplate Reader (Bio-Rad).

Western Blot Analysis

Approximately 1×10^9 CFU/mL bacterial cells were harvested from 10 mL of broth by centrifugation (10,000 × g, 5 min), resuspended in 200 μL of sample solvent,[10] and incubated at 37°C for 1 h. The suspension was centrifuged and the supernatant was then collected. Twenty microliters of the protein containing supernatant was analyzed on a SDS-PAGE criterion gel (10% acrylamide, Bio-Rad) and transferred to an Immobilon-P membrane (Millipore, Bedford, MA). The membranes were soaked in 5% BSA solution overnight at 4°C, washed with PBST, and then reacted with antibody (0.08 mg/mL) for 2 h at room temperature. The membranes were

washed and goat anti-rabbit HRP (1:5,000, Bio-Rad) was added and incubated for 1 h at room temperature. The membranes were washed and DAB (Sigma Fast™ 3,3′-diaminobenzidine, Sigma) substrate was added. The reaction was stopped after 15 min with tap water and the membranes were then air-dried.[10]

Statistical Analysis

Statistical analysis was performed using SAS 8.02 (SAS Institute, Cary, NC). ELISA data were analyzed using the proc GLM (general linear regression model) procedure and significant differences between the bacterial cultures and rabbits were determined using Tukey's test at $P < 0.05$.

RESULTS AND DISCUSSION

Reaction Profiles of Preimmune Serum to Bacterial Cultures

Average reaction profiles of all 27 bacterial cultures were calculated to determine which bacteria were most reactive amongst the tested preimmune serum samples (Table 2). Results showed that *S. Typhimurium* and *B. cereus* AS4-12 had the highest average reaction values, as well as the highest number of positive ELISA readings (17 out of 19). As mentioned before, rabbits are recommended by FELAS to be tested for *Salmonella* every three months via culture methods. So, even if the organism is not detected because the animal warded off or cleared the infection, antibodies could still persist. *P. putrefaciens* and *B. subtilis* had the lowest average values and none of the preimmune serum samples were positive for those two pathogens. Overall, 14 of the 27 cultures tested showed positive reactions with 50% or more of the preimmune serum tested (Table 2).

To determine which rabbits had the highest pre-existing cross-reactive antibodies, the reaction profiles of 19 rabbit preimmune serum samples against 27 different bacteria were determined (Table 3). ELISA readings from all bacterial cultures have been averaged for each rabbit tested. An ELISA value of above 0.20 was considered the cutoff for a positive reaction. This was used because serum samples that were reacted with unsensitized (no bacteria) wells yielded values below 0.20. Rabbit 6 had the highest average ELISA reading while rabbit 18 had the lowest reaction, 0.46 and 0.07, respectively. Rabbits 1 and 13 also had high average values, 0.42. Rabbit 1 was positive for 23 cultures while rabbit 18 was positive for only one culture. Most (53%) rabbit preimmune serum samples showed positive reactions with 10 or more bacterial cultures (Table 3).

Table 3. ELISA reaction profiles and number of positive bacterial cultures for the 19 different rabbit preimmune serum samples

Rabbit ID	ELISA ($A_{490} \pm SD^a$)	Number of cultures out of 27 cultures positive to the rabbit preimmune serum
6	0.45 ± 0.32^{a}	21
13	$0.42 \pm 0.30^{b,a}$	20
1	$0.41 \pm 0.25^{a,b}$	23
2	$0.38 \pm 0.24^{b,c}$	20
8	$0.34 \pm 0.24^{d,c}$	18
12	$0.31 \pm 0.28^{d,c}$	14
5	$0.29 \pm 0.27^{d,e}$	12
14	$0.23 \pm 0.19^{f,e}$	12
10	$0.21 \pm 0.20^{f,g}$	11
11	$0.21 \pm 0.20^{f,g}$	9
3	$0.19 \pm 0.21^{f,g,h}$	11
16	$0.19 \pm 0.15^{f,g,h}$	7
7	$0.16 \pm 0.21^{f,i,g,h}$	5
9	$0.16 \pm 0.13^{j,i,g,h}$	5
15	$0.14 \pm 0.09^{j,i,k,h}$	5
19	$0.13 \pm 0.12^{j,i,k,h}$	5
17	$0.11 \pm 0.09^{j,i,k}$	3
4	$0.10 \pm 0.08^{j,k}$	3
18	0.07 ± 0.05^{k}	1

[a]ELISA values with the same superscript letters (a,b,c,d. . .) are not significantly different at $P < 0.05$ and the absorbance values above 0.20 were considered positive.

Western Blot Analysis with Selected Rabbit Preimmune Serum

To identify specific bacterial proteins that reacted with the preimmune serum, Western blot analysis was performed on selected samples, based on their average ELISA reactions. Antibodies from rabbits 4, 6, 8, 13, and 18 were chosen. Rabbits 6 and 13 showed the highest average reactions in ELISA, 8 showed a medium reaction, and 4 and 18 had the lowest reactions. In ELISA, *L. welshimeri* and *L. monocytogenes* SLCC 2373 showed weak reactions, *Enterococcus faecalis* and *Corynebacterium glutamicum* showed moderate reactions, and *E. coli* ATCC 51739, *B. cereus* AS4-12, *S.* Typhimurium, and *Proteus vulgaris* reacted strongly. Rabbit preimmune sera 4, 8, and 18 showed no reaction with the chosen bacterial surface protein extracts (data not shown). This was as expected for rabbit samples 4 and 18, since overall ELISA values were quite low. Rabbit 6 showed reaction with a protein band in the 97-kDa range in 5 out of the 8 bacteria tested and also showed reaction

with protein bands in the 36 and 29-kDa range in 3 of the 8 bacteria (Fig. 1). Rabbit 13 reacted strongly with protein bands in the 63-kDa range and weakly with the 32-kDa range in all of the bacteria tested (Fig. 1). It was expected that bacterial cultures which showed strong ELISA reaction would also show high reactions in Western (intensity or number of bands). However, this is not what was observed in the preimmune serum from rabbit 6. *B. cereus* AS4-12 showed a high reaction in ELISA but showed a weak reaction in Western blot. This could be due to the fact that the antibodies specific for *B. cereus* are possibly reacting with a non-protein component, such as a lipid or carbohydrate,[11] which was not resolved in SDS-PAGE.

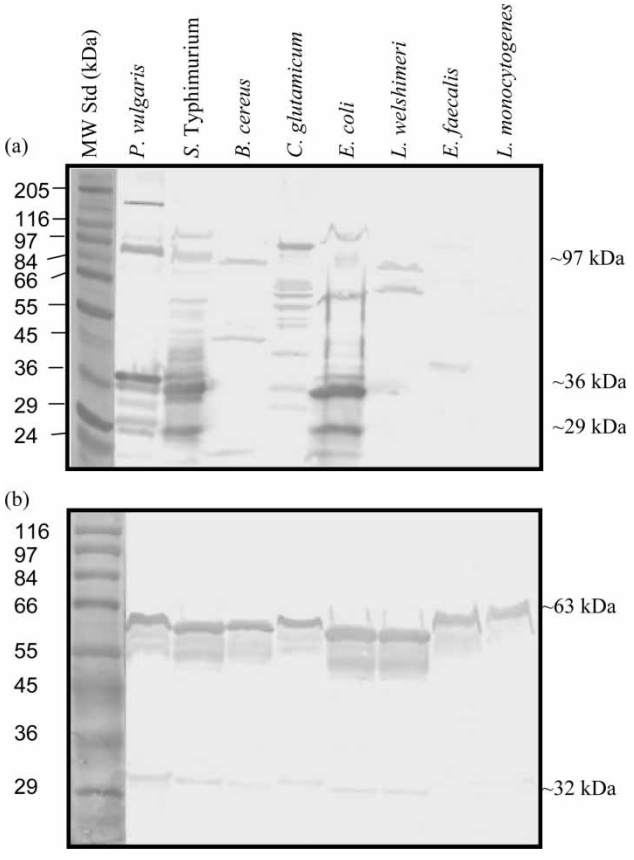

Figure 1. Western blot analysis of surface protein preparations from various bacterial cultures using affinity purified antibodies from preimmune serum from rabbit 6 (a) and rabbit 13 (b). The cultures used in this study were *Proteus vulgaris*, *Salmonella enterica* serovar Typhimurium, *Bacillus cereus* AS4-12, *Corynebacterium glutamicum*, *Escherichia coli* ATCC 51739, *L. welshimeri* ATCC 35897, *Enterococcus faecalis*, and *Listeria monocytogenes* SLCC 2373.

L. welshimeri and *L. monocytogenes* SLCC 2373 with low ELISA values showed a strong reaction in Western blot with antibodies from Rabbit 13. This may be because, during protein extraction and SDS-PAGE analysis, denaturation may have unraveled the antigenic epitopes, which was inaccessible to the antibody during ELISA. Interestingly, rabbit 13 reacted with ~63 and ~32-kDa bands in all test cultures. These cultures may share a common antigenic epitope that the immune system recognizes. The rabbit might have had a response to one of the bacterial cultures and antibodies generated to this may be responsible for cross-reaction with others, since those share common epitopes on the surface.

CONCLUSIONS

Results obtained in this study show the prevalence of common pathogenic and nonpathogenic bacteria reactive antibodies in the preimmune serum of NZW rabbits. The presence of *S.* typhimurium and *B. cereus* AS4-12 antibodies was frequently detected in rabbit preimmune serum samples. Antibodies to other *Salmonella* and *E. coli* strains were also commonly detected. Over 50% of the rabbit serum tested showed reaction with 10 or more bacterial cultures. Serum from one of the rabbits, which gave high ELISA values, reacted with protein bands of ~97, 36 and 29-kDa, while another rabbit reacted with ~63 and ~32-kDa bands. Proper and extensive preimmune serum testing is needed prior to initiating PAb production. Otherwise, the quality of antibodies produced in these animals will be compromised. Therefore, it is not only important to test preimmune sera for the agent of interest, but also for other possible agents that could show nonspecific reactions in the final application.

ACKNOWLEDGMENTS

We thank Ms. Yali Liu and Dr. Jun Xie from the Purdue University Statistical Consulting Service for their help with statistical analysis.

This research was supported through the National Cattlemen's Beef Association and a cooperative agreement with the Agricultural Research Service of the USDA project number 1935-035 and the Center for Food Safety and Engineering at Purdue University.

REFERENCES

1. Suckow, M.A.; Douglas, F.A. *The Laboratory Rabbit*; CRC Press: Boca Raton, FL, 1997; 1–102.
2. Manning, P.J.; Ringler, D.H.; Newcomer, C.E. *The Biology of the Laboratory Rabbit*, 2nd Ed.; Academic Press: San Diego, CA, 1994; 131–446.

3. Nicklas, W.; Homberger, F.; Illgen-Wilcke, B.; Jacobi, K.; Kraft, V.; Kunstyr, I.; Mahler, M.; Meyer, H.; Pohleyer-Esch, G. Implications of infectious agents on results of animal experiments. Lab. Anim. **1999**, *33* (Suppl 1), 39–87.
4. Nicklas, W.; Baneux, P.; Boot, R.; Decelle, T.; Deeny, A.A.; Fumanelli, M.; Illgen-Wilcke, B. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. Lab. Anim. **2002**, *36*, 20–42.
5. Hansen, A.K. *Handbook of Laboratory Animal Bacteriology*; CRC Press: Boca Raton, FL, 2000; 55–99.
6. Boot, R. Development and validation of ELISAs for monitoring bacterial and parasitic infections in laboratory rodents and rabbits. Scand. J. Lab. Sci. **2001**, *28*, 44–50.
7. Crowther, J.R. *The ELISA Guidebook. Methods in Molecular Biology*; Humana Press Inc.: Totowa, NJ, 2001; Vol. 149, 57–78.
8. Catty, D. *Antibodies: A Practical Approach*; IRL Press Limited: Oxford, England, 1988; Vol. 1, 19–78.
9. Lathrop, A.A.; Jaradat, Z.W.; Haley, T.; Bhunia, A.K. Characterization and application of a *Listeria monocytogenes* reactive monoclonal antibody C11E9 in a resonant mirror biosensor. J. Immunol. Meth. **2003**, *281*, 119–128.
10. Bhunia, A.K.; Ball, P.H.; Fuad, A.T.; Kurz, B.W.; Emerson, J.W.; Johnson, M.G. Development and characterization of a monoclonal antibody specific for *Listeria monocytogenes* and *Listeria innocua*. Infect. Immun. **1991**, *59* (9), 3176–3184.
11. Whitfield, C. Bacterial extracellular polysaccharides. Can. J. Microbiol. **1988**, *34*, 415–420.

Received May 10, 2005
Accepted August 14, 2005
Manuscript 3168