VALIDATION OF A BRUCELLA ABORTUS COMPETITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR USE IN ROCKY MOUNTAIN ELK (CERVUS ELAPHUS NELSONI)

Authors: Van Houten, Clayton K., Belden, E. Lee, Kreeger, Terry J., Williams, Elizabeth S., Edwards, William H., et al.

Source: Journal of Wildlife Diseases, 39(2) : 316-322

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-39.2.316

Journal of Wildlife Diseases, 39(2), 2003, pp. 316–322

VALIDATION OF A *BRUCELLA ABORTUS* COMPETITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR USE IN ROCKY MOUNTAIN ELK (*CERVUS ELAPHUS NELSONI*)

Clayton K. Van Houten, Jr.,[1,5] E. Lee Belden,[1] Terry J. Kreeger,[2] Elizabeth S. Williams,[1] William H. Edwards,[3] E. Tom Thorne,[4] Walter E. Cook,[3] and Kenneth W. Mills[1]

[1] Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82071, USA
[2] Wyoming Game and Fish Department, 2362 Highway 34, Wheatland, Wyoming 82201, USA
[3] Wyoming Game and Fish Department Wildlife Disease Laboratory, 1174 Snowy Range Road, Laramie, Wyoming 82070, USA
[4] Wyoming Game and Fish Department, 5400 Bishop Boulevard, Cheyenne, Wyoming 82006, USA
[5] Corresponding author (email: ckvhouten@aol.com)

ABSTRACT: Brucellosis caused by infection with *Brucella abortus* is present in some elk (*Cervus elaphus nelsoni*) of the Greater Yellowstone Area (parts of Wyoming, Montana, and Idaho, USA). Since 1985, the Wyoming Game and Fish Department has vaccinated elk on elk feedgrounds in northwestern Wyoming during the winter months using *B. abortus* strain 19 (strain 19). Analysis of this vaccination program is hampered by the inability of standard serologic tests to differentiate between strain 19 vaccinated elk and those exposed to field strain *B. abortus*. In 1993, a competitive enzyme-linked immunosorbent assay (cELISA) was licensed to serologically differentiate between strain 19 vaccinated cattle and cattle exposed to field strain *B. abortus*. Seven groups of elk sera representing various *B. abortus* exposure histories were used to validate the cELISA test for elk. The cELISA test differentiated strain 19 vaccinated elk from elk that were challenged with *B. abortus* strain 2308, a pathogenic laboratory strain. The specificity of the cELISA was 96.8% for elk vaccinated with strain 19 only and sampled between 6 mo and 2 yr post vaccination, or with no *B. abortus* exposure. The sensitivity of the cELISA was 100%. The cELISA test will be useful in evaluating sera collected from elk in vaccinated, brucellosis endemic herds in the Greater Yellowstone Area.

Key words: *Brucella abortus*, brucellosis, cELISA, *Cervus elaphus nelsoni*, Rocky Mountain elk, serologic tests.

INTRODUCTION

Brucellosis was first diagnosed in elk (*Cervus elaphus nelsoni*) on the National Elk Refuge (NER; Teton County, Wyoming, USA) in 1930 (Murie, 1951). Murie, in response to aborted elk fetuses on feedlines of the NER, had nine elk serum samples tested for antibodies against *Brucella* and three were positive. Tunicliff and Marsh (1935) tested elk sera from Yellowstone National Park, Wyoming from 1931 to 1933, and found 8% of 105 elk were positive and 14% were suspects for *Brucella* antibodies. Thorne et al. (1978) tested elk sera from the NER and the Wyoming Game and Fish Department (WGFD) Alpine feedground during the winters of 1970–76. Two hundred-six (43%) of 479 mature females from the NER, and 85 (53%) of 161 mature females from the Alpine feedground were seropositive. Four tests were used to test elk sera in the Thorne study: the standard plate agglutination test (SPT), the buffered *Brucella* antigen rapid card test (BBA), the rivanol precipitation-plate agglutination test (RIV), and the complement fixation test (CF). Any sera that produced a reaction on two or more of the tests were considered positive (Thorne et al., 1978). Brucellosis is found on all 22 WGFD feedgrounds (Smith et al., 1997).

In controlled studies, *Brucella abortus* strain 19 (strain 19) vaccination was shown to reduce abortion rates in elk (Thorne et al., 1981a). Field vaccination trials began on feedground elk in 1985 using methylcellulose strain 19 vaccine-loaded biobullets (Angus, 1989; Herriges et al., 1989a) and continue to the present. Analysis of the vaccination program is hampered by the inability of standard serologic tests to differentiate between vaccinated animals and those naturally exposed to *B. abortus*.

Conventional *Brucella* serologic tests measure antibody to the smooth lipopolysaccharide (sLPS), which is present on both field strain *B. abortus* and strain 19. Therefore, positive results due to strain 19 are often obtained on sera from vaccinated animals.

A competitive enzyme-linked immunosorbent assay (cELISA) to serologically differentiate between strain 19 vaccinated cattle and cattle exposed to field strain *B. abortus* was validated in 1991 (Adams and Mia, 1991). This test (D-Tec® Brucella A, Synbiotics Corporation, San Diego, California, USA) was licensed in 1993 and was distributed commercially until 1996. This cELISA is based on a mouse monoclonal antibody that differentially competes with antibodies against strain 19 and field-strain *B. abortus* for a specific antigenic determinant on the *B. abortus* sLPS. Antibodies produced in response to strain 19 vaccination compete poorly with the monoclonal antibody, while antibodies produced following field strain *B. abortus* infection compete strongly with the monoclonal antibody. The amount of monoclonal antibody bound to the sLPS is measured by reacting streptavidin-peroxidase followed by a substrate chromagen. The optical density signal produced by the reaction is proportional to the amount of monoclonal antibody bound to the LPS and inversely proportional to the amount of competition between the monoclonal antibody and the sample antibody.

The purpose of the present study was to validate use of the cELISA to differentiate strain 19 vaccinated elk from those exposed to virulent *B. abortus.*

MATERIALS AND METHODS

Sera from seven elk groups of known vaccination and *B. abortus* exposure status were used to determine the validity of the cELISA test. Details about the groups are listed in Table 1. Samples were obtained from banked elk sera from WGFD vaccination studies between 1979 and 2000 (Thorne and Anderson, 1979; Thorne et al., 1980, 1981b, 1982a, b, 1983, 1984, 1986, 1987; Herriges et al., 1988, 1989b;

TABLE 1. Vaccination and *Brucella abortus* challenge history of elk used to validate the cELISA.

Group	Location[a]	Number of elk	Sex and age[b]	Strain 19 vaccination		Strain 2308 challenge		Sampled
				Dose (cfu)[c]	Route[d]	DPV[e]	Dose (cfu)[c]	
1	Sybille, CDW	72	PF, MC	NA[f]	NA	NA	4.5×10^6–1.1×10^7	27–85 DPC[g]
2	Sybille	41	PF, MC	4×10^7–7.65×10^9	C, Biobullet	NA	NA	15–43 DPV
3	Sybille	47	PF, MC, FC	9×10^8–10.8×10^9	C, Biobullet	NA	NA	90–162 DPV
4	Sybille	66	PF, MC, FC	4×10^7–10.8×10^9	C, Biobullet	NA	NA	251–565 DPV
5	TRNP	46	mixed	NA	NA	NA	NA	NA
6	Sybille	12	PF	4×10^7	Biobullet	90	4.5×10^6	90 DPC
7	Sybille	64	PF, MC	4×10^7–10.8×10^9	C, Biobullet	82–755	4.5×10^6–1.1×10^7	13–85 DPC

[a] Sybille=Wyoming Game and Fish Sybille Wildlife Research Unit; CDW=Colorado Division of Wildlife Foothills Research Facility; TRNP=Theodore Roosevelt National Park.
[b] PF=pregnant female; MC=male calf; FC=female calf.
[c] Vaccination dose in colony forming units.
[d] C=subcutaneously at base of neck.
[e] Days post vaccination with strain 19.
[f] NA=not applicable, not vaccinated or challenged.
[g] Days post challenge with strain 2308.

Cook, 1999; Kreeger et al., 2000). The elk from these studies were necropsied, and various tissues, as well as aborted fetuses, were cultured for *B. abortus. Brucella abortus* strain 2308 (strain 2308) a virulent laboratory strain was used as a challenge in groups 1, 6, and 7. Strain 2308 exposure is used in vaccination studies to mimic exposure to field strain *B. abortus* (Elzer et al., 1998).

A majority of the elk were housed at the WGFD Sybille Wildlife Research Unit (41°45′N, 105°45′W) near Wheatland, Wyoming in 0.4 ha corrals and fed alfalfa hay supplemented with a pelleted ration. Water and trace mineral block were provided ad libitum. A few of the elk were housed at the Colorado Division of Wildlife (CDW) Foothills Wildlife Research Facility, Fort Collins, Colorado (USA; 40°35′N, 105°10′W) (Kreeger et al., 2000). Elk calves were fed alfalfa hay cubes supplemented with grass hay and a high-energy pelleted supplement, while cows were fed alfalfa hay. Water and trace mineral block were provided ad libitum. *Brucella abortus* infected elk were intraconjunctivally exposed to strain 2308.

Sera were also obtained from elk from Theodore Roosevelt National Park, North Dakota (USA). These animals were negative on the standard serologic tests and were from a herd outside of *B. abortus* endemic areas.

Hybridomas were generously supplied by Dr. Garry Adams (Texas A & M University, College Station, Texas, USA). The hybridomas were grown in Dulbecco's modified Eagle's medium-10% fetal bovine serum (Sigma-Aldrich, St. Louis, Missouri, USA) and injected intraperitoneally into Balb-c mice for ascites fluid production (Yokoyama, 1994). Monoclonal antibodies (mAb) were derived via salt split from the ascites fluid. Protein concentration was adjusted to 1 mg/ml. N-hydroxysuccinimidobiotin (Sigma-Aldrich) was rapidly dissolved into dimethyl sulfoxide (Sigma-Aldrich) at a concentration of 1 mg/ml. This mixture was added to the mAb at a ratio of 120 μl/mg. This was left at room temperature for 4 hr and then dialyzed overnight at 4 C against phosphate buffered saline (PBS).

Competitive ELISA plates were prepared using purified *Brucella* sLPS antigen (0.448 mg/ml; Synbiotics Corporation). The sLPS was diluted 1:1,000 in double distilled water (ddH_2O) and 100 μl of diluted sLPS was added to each well of a 96-well microtiter plate (Immulon 2 Flat Bottom plate, Dynex Technologies, Chantilly, Virginia, USA). The antigen was incubated for 24 hr in an incubator at 37 C and then washed three times with ddH_2O to remove all unbound antigen. Plates were blocked by adding 100 μl of 0.05% bovine serum albumin (BSA Fraction V, Sigma-Aldrich) in PBS + 0.05% Tween 20 (PBS/Tween) to each well. The plates were incubated for 2 hr at room temperature on a rotating plate and washed three times with PBS/Tween. Test serum and controls were diluted by adding 50 μl of serum to 250 μl of PBS/Tween in glass test tubes and vortexed. Then 250 μl of 1:1,000 PBS/Tween-diluted mAb was added to each tube. Negative control serum was from an elk negative on the standard serologic tests, while positive control serum was from the D-TEC® commercial kit and consisted of bovine anti *B. abortus* antibodies with protein stabilizers and preservatives. Serum samples were tested in duplicate by adding 100 μl of each sample into two wells. One well on the plate was left as a blank. The plates were incubated at room temperature on a rotating plate for 15 min and washed three times with PBS/Tween. Then 100 μl of 1:500 diluted avidin-hydrogen peroxidase (1mg/ml; Sigma-Aldrich) was added to each well and incubated at room temperature for 30 min on a rotating plate. The plates were washed three times with PBS/Tween and two times with ddH_2O. Fifty μl of substrate chromagen 3,3′,5,5′-tetramethyl benzidine (Sigma-Aldrich) was added to each well. The plates were incubated at room temperature for 5 min, after which the reaction was stopped by adding 50 μl of 2M H_2SO_4. Plates were read on a plate reader (Bio-Rad Model 3550; Bio-Rad, Hercules, California, USA) at 450 nm. The results were calculated using the following equation, where S is the average sample optical density, and N is the average negative optical density:

$$\text{Percent inhibition} = (1 - S/N) \times 100$$

If the optical density of the two negative control wells differed by more than 10% from their average optical density, then results from the entire plate were discarded and the samples retested. Individual samples were also retested if the two optical density readings differed by more than 10% from the sample's average optical density.

Several of the samples used to validate the cELISA were from elk sampled just over 20 yr previously. In order to verify that older samples were maintaining antibody titers, 70 samples from groups 1, 2, 4, 5, and 7 were randomly selected for testing on the SPT, RIV, CF, CARD, and BAPA tests by the USDA-APHIS laboratory in Laramie, Wyoming. These results were compared to the original standard serologic test results to determine if antibody titers had changed.

The cutoff values for the assay were determined using receiver operating characteristic (ROC) curve analysis (MedCalc Version 6.12, MedCalc, Mariakerke, Belgium) for groups 1,

3, and 4. Receiver operating characteristic curve analysis is used to determine the ability of a laboratory test to discriminate positive cases from negative cases (Metz, 1978). This analysis determines the optimal cutoff value based on achieving the highest possible sensitivity and specificity without a suspect range (Zweig and Campbell, 1993). This is especially useful for free-ranging populations for which re-sampling of suspect animals is impossible.

RESULTS AND DISCUSSION

The results of the cELISA are shown in Table 2. The cut-off value for the cELISA was ≥52% inhibition, giving an optimal sensitivity of 100% and a specificity of 96.5%. The groups of elk used in this analysis were chosen because the populations of elk that will be tested are elk on WGFD feedgrounds and are assumed to be either vaccinated or exposed to field strain *B. abortus.* Receiver operating characteristic curve analysis was also used to determine that 99.2% of the time a randomly selected individual animal from a positive population will have a test value greater than a randomly selected individual animal from a negative population (Hanley and McNeil, 1982).

The results from group 2 elk (strain 19 vaccinated, serum collected 15–43 days post vaccination [DPV]) were not used in the calculations for the cutoff value. The mean percent inhibition of this group was 29 (SD 33%). When this group was added to the ROC analysis, the cutoff value only rose to 54% inhibition, but the test specificity dropped to 88%. Based on the 52% inhibition cut-off value, 34% of the samples from this group were false positive. Because of the high variability, the results of the cELISA in these elk demonstrate a limitation for the use of this test within 43 DPV. During this period of time the test is not able to differentiate antibodies against strain 19 and strain 2308. This could be the result of high anti-*Brucella* antibody titers in elk following vaccination. The antigenic determinant to which the monoclonal antibody is specific may be present on the surface of strain 19 in very

TABLE 2. Results of cELISA on elk vaccinated, challenged, or not exposed to *Brucella.* Percent inhibition greater than 52% was considered positive.

Group[a]	Exposure status	Number	Mean percent inhibition (SD)	Maximum percent inhibition	Minimum percent inhibition	Number positive	Number negative	Sensitivity	Specificity
1	Strain 2308[b] not vaccinated	72	81.1 (8.7)	100	58	72	0	100%	NA
2	Strain 19 vaccinated no strain 2308	41	29.3 (33.4)	82	0	14	27	NA	66%
3	Strain 19 vaccinated no strain 2308	47	11.5 (23.7)	82	0	5	42	NA	89%
4	Strain 19 vaccinated no strain 2308	66	10.9 (12.4)	41	0	0	66	NA	100%
5	No exposure to strain 19 or strain 2308	46	0.7 (2.3)	10	0	0	46	NA	100%
6	Strain 19 vaccinated Strain 2308 inoculated	12	44.4 (32.4)	90	64	12	0	100%	NA
7	Strain 19 vaccinated Strain 2308 inoculated	64	77.6 (8.4)	100	0	22	42	91%	90%

[a] See Table 1 for details.
[b] Strain 2308, virulent laboratory strain of *Brucella abortus.*

low concentrations. But, during the first 2 mo following vaccination, antibody titers to that antigenic determinant may be high enough to compete with the monoclonal antibody, resulting in decreased test specificity.

Group 3 consisted of sera from animals sampled between 90 and 162 DPV. This group had a maximum percent inhibition of 82. The sample that gave this high percent inhibition was collected 92 DPV. Four elk from this group were cELISA positive, and three of the four were sampled between 92 and 123 DPV. The cELISA appears to be most accurate when elk are sampled more than 123 DPV.

Group 5 contained elk samples from a herd of free-ranging elk free of brucellosis. All of the samples from this group were negative on the cELISA, giving a specificity of 100% using the 52% inhibition cutoff value. Elk with no exposure to *B. abortus* antigens should test negative on the cELISA.

Group 6 contained samples from elk that were vaccinated with strain 19, challenged with strain 2308, and sampled 3 mo post-challenge. All of the samples in this group were positive on the cELISA, and all of the pregnant elk aborted, and/or were culture positive at necropsy. Thus, though the elk were vaccinated with strain 19, they had a serologic response to strain 2308, which was detected by the cELISA.

Twenty-two elk were cELISA positive and 42 were negative from group 7 (strain 19 vaccinated, strain 2308 challenged, and sampled between 13 and 85 days). Of the 22 elk that were cELISA positive, 20 (91%) aborted and/or were strongly culture positive at necropsy, two were culture negative at necropsy, and all tested positive on the four standard serological tests. These elk were vaccinated with strain 19, but had serologic responses to strain 2308, which was detected by the cELISA. Of the 42 elk with negative cELISA results, 38 (90%) were culture negative at necropsy and did not abort, and four were culture positive with few *B. abortus* isolated. All were positive on the standard serologic tests. These elk were vaccinated with strain 19 and did not have serologic responses to strain 2308, and were subsequently negative on the cELISA.

Of 70 random samples tested by the standard serologic tests to evaluate stability of antibodies in frozen sera, 67 (96%) of the results were similar (positive or negative) to their original test results (results not shown). *Brucella* antibodies were very stable in sera frozen for nearly 30 yr (in some cases) and we considered the sera valid for use in the evaluation of the cELISA.

The cELISA offers several advantages over the use of standard *B. abortus* serologic tests. The test only takes a few hours to complete and uses a minimal amount of sample serum. The standard serologic tests combined take a minimum of 1 ml of serum and roughly 3 days to complete. Thorne et al. (1978) tested elk samples on the SPT, BBA, RIV, and CF and concluded "no single serological test should be relied on to diagnose brucellosis in elk." This conclusion was based on the fact that standard serologic tests have a relatively low specificity. The cELISA has a high sensitivity, a high specificity for non-exposed elk, and strain 19 vaccinated elk that are sampled after 90 DPV, and could be used as a single diagnostic test for brucellosis in elk.

The WGFD uses strain 19 vaccinations as part of its brucellosis management program on Wyoming elk feedgrounds. Wyoming Game and Fish personnel vaccinate elk calves beginning in December and continue throughout the winter. Monitoring herds for brucellosis is conducted by serologically testing cow elk captured on feedgrounds in the winter. These cows were likely vaccinated $\geq$1 yr prior to testing. Exposure to field strain *B. abortus* may occur on feedgrounds in late winter, thus, in the field, some elk may be both vaccinated and exposed to virulent *B. abortus.* The inability of standard serologic tests to differentiate between vaccinated

elk and elk naturally exposed to field strain *B. abortus,* has hampered evaluation of the vaccination program.

The cELISA is most accurate with elk that were vaccinated 4 mo or more prior to testing. The cELISA also has the ability to distinguish elk that were vaccinated with strain 19 and subsequently exposed to virulent *B. abortus* and seroconverted, from elk that were vaccinated with strain 19 and did not seroconvert to virulent *B. abortus* following exposure. We believe the cELISA could be used to assist in evaluation of the WGFD elk vaccination program.

ACKNOWLEDGMENTS

We thank the Wyoming Game and Fish Department for funding this project and providing elk sera; the employees of the Wyoming State Veterinary Laboratory; E. W. Nelson and R. A. Wills of the USDA-APHIS Laboratory in Laramie, Wyoming; G. Adams of Texas A&M University, College Station, Texas for generously supplying the hybridomas; Synbiotics Corporation; the Colorado Division of Wildlife; and R. Andrascik, Theodore Roosevelt National Park.

LITERATURE CITED

ADAMS, L. G., AND A. S. MIA. 1991. Field evaluation of "D-Tec Brucella A.," a monoclonal antibody-based competitive enzyme-linked immunosorbent assay (cELISA) for serodiagnosis of brucellosis in cattle. Proceedings of the United States Animal Health Association 95: 92–112.

ANGUS, R. D. 1989. Preparation, dosage delivery, and stability of a *Brucella abortus* strain 19 vaccine ballistic implant. Proceedings of the United States Animal Health Association 93: 656–666.

COOK, W. E. 1999. Brucellosis in elk: Studies of epizootiology and control. Ph.D. Dissertation, University of Wyoming, Laramie, Wyoming, 183 pp.

ELZER, P. H., F. M. ENRIGHT, L. COLBY, S. D. HAGIUS, J. V. WALKER, M. B. FATEMI, J. D. KOPEC, V. C. BEAL, AND G. G. SCHURIG. 1998. Protection against infection and abortion induced by virulent challenge exposure after oral vaccination of cattle with *Brucella abortus* strain RB51. American Journal of Veterinary Research 59: 1575–1578.

HANLEY, J. A., AND B. J. MCNEIL. 1982. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 143: 29–36.

HERRIGES, J. D., E. T. THORNE, A. D. REESE, AND S. L. ANDERSON. 1988. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Job Number BDSWCBF551. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 42–54.

———, ———, S. L. ANDERSON, AND H. A. DAWSON. 1989a. Vaccination of elk in Wyoming with reduced dose strain 19 *Brucella*: Controlled studies and ballistic implant field trials. Proceedings of the United States Animal Health Association 93: 640–655.

———, ———, A. D. REESE, AND S. L. ANDERSON. 1989b. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Job Number BDSWCBF551. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 38–67.

KREEGER, T. J., M. W. MILLER, AND S. C. OLSEN. 2000. Safety and efficacy of *Brucella abortus* strain RB51 vaccine in captive pregnant elk. Journal of Wildlife Diseases 36: 477–483.

MEDCALC STATISTICAL SOFTWARE. MedCalc version 6.12. Mariakerke, Belgium, http://www.medcalc.be/index.html.

METZ, C. E. 1978. Basic principles of ROC analysis. Seminars in Nuclear Medicine 8: 283–298.

MURIE, O. J. 1951. The elk of North America. The Stackpole Co. Harrisburg, Pennsylvania, and Wildlife Management Institute, Washington D.C., 376 pp.

SMITH, S. G., T. J. KREEGER, AND E. T. THORNE. 1997. Wyoming's integrated management program for brucellosis and free-ranging elk. Proceedings of the United States Animal Health Association. http://www.usaha.org/speeches/wyelk97.html

THORNE, E. T., AND C. C. ANDERSON. 1979. Immunological response of elk to parenteral vaccination against brucellosis. Job Completion Report, Project Number FW-3-R-24, Work Plan 1, Job 14W. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 34–36.

———, J. K. MORTON, AND G. M. THOMAS. 1978. Brucellosis in elk 1. Serological and bacteriological survey in Wyoming. Journal of Wildlife Diseases 14: 74–81.

———, C. C. ANDERSON, AND T. J. WALTHALL. 1980. Immunological response of elk to parenteral vaccination against brucellosis. Job Completion Report, Project Number FW-3-R-25, Work Plan 1, Job 14W. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 23–34.

———, T. J. WALTHALL, AND H. A. DAWSON. 1981a. Vaccination of elk with strain 19. Proceedings of the United States Animal Health Association 82: 359–374.

———, C. C. ANDERSON, AND T. J. WALTHALL. 1981b. Immunological response of elk to parenteral vaccination against brucellosis. Job Completion Report, Project Number FW-3-R-26,

Work Plan 1, Job 14W. Wyoming Game and Fish Department, Cheyenne, Wyoming pp. 31–45.

———, ———, AND ———. 1982a. Immunological response of elk to parenteral vaccination against brucellosis. Job Completion Report, Project Number FW-3-R-27, Work Plan 1, Job 14W. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 21–37.

———, ———, AND ———. 1982b. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Project Number FW-3-R-27, Work Plan 1, Job 15W. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 38–43.

———, ———, AND ———. 1983. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Project Number FW-3-R-28, Work Plan 1, Job 15W. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 38–43.

———, A. D. REESE, AND S. L. ANDERSON. 1984. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Job Number BDSWCBF551. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 37–47.

———, ———, F. HAMMOND, AND S. L. ANDERSON. 1986. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Job Number BDSWCBF551. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 37–51.

———, ———, ———, AND ———. 1987. Immune response of elk vaccinated with a reduced dose of strain 19 *Brucella* vaccine. Job Completion Report, Job Number BDSWCBF551. Wyoming Game and Fish Department, Cheyenne, Wyoming, pp 37–47.

TUNNICLIFF, E. A., AND H. MARSH. 1935. Bang's disease in bison and elk in the Yellowstone National Park and on the National Bison range. Journal of the American Veterinary Medical Association 86: 745–752.

YOKOYAMA, W. M. 1994. Monoclonal antibody supernatant and ascites fluid production. *In* Current protocols in immunology. J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober (eds.). John Wiley and Sons, Inc. New York, New York, pp. 2.6.1–2.5.7.

ZWEIG, M. H., AND G. CAMPBELL. 1993. Receiver-operating characteristic (ROC) plots: A fundamental evaluation tool in clinical medicine. Clinical Chemistry 39: 561–577.

Received for publication 23 July 2002.