

# COMPARISON OF TEST PERFORMANCE AND EVALUATION OF NOVEL IMMUNOASSAYS FOR TUBERCULOSIS IN A CAPTIVE HERD OF WOOD BISON NATURALLY INFECTED WITH *MYCOBACTERIUM BOVIS*

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**ABSTRACT:** In 1996, the Hook Lake Wood Bison Recovery Project was initiated to establish a small, disease-free, captive, bison-breeding herd. Founders originated from wild bison herds in the Slave River Lowlands in northern Canada, which, like other bison herds in and around Wood Buffalo National Park, are endemically infected with bovine tuberculosis (caused by *Mycobacterium bovis*) and brucellosis (caused by *Brucella abortus*). After 9 yr of apparent disease freedom, tuberculosis was detected within the captive herd, leading to complete depopulation. This study examined the performance of antemortem tuberculosis diagnostic tests used during the project. Performances of the caudal-fold test, fluorescent polarization assay, multiantigen print immunoassay (MAPIA), and the rapid test (RT) were assessed by estimating sensitivity, specificity, positive predictive value, and negative predictive value for each test. Kappa values measuring agreement between tests were calculated. Overall, the tests did not differ with respect to sensitivities and specificities, which ranged from 50% to 92% and from 34% to 100%, respectively. The MAPIA tended to show high sensitivity, and there was significant agreement only between the MAPIA and RT. Serum collected from infected animals at slaughter produced highly variable results on the different assays, and one infected bison was negative on all antemortem tests. The results of this analysis suggest use of multiple antemortem tests in parallel, particularly those incorporating multiple antigens, to optimize sensitivity in detecting bovine tuberculosis in bison. However, as demonstrated in this herd, even a seemingly optimal antemortem testing regimen can fail to detect *M. bovis*-infected individuals.

**Key words:** Antemortem, bison, diagnostic, *Mycobacterium bovis*, testing, tuberculosis.

## INTRODUCTION

Bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*) were introduced into free-ranging wood bison (*Bison bison athabascae*) populations in and around Wood Buffalo National Park (WBNP), Canada, in the 1920s, most likely through the translocation of more than 6,000 plains bison

(*Bison bison bison*) from Buffalo National Park near Wainwright, Alberta (Connely et al., 1990; Brower, 2008). Since then, these diseases have posed an important health risk to wood bison, as well as to domestic livestock and Canadian First Nation communities in areas around WBNP (Gates et al., 2001; Nishi et al., 2006). In 1996, the Hook Lake Wood Bison Recovery Project (HLWBRP) was

initiated cooperatively among the Government of the Northwest Territories (NWT), the Deninu Kue' First Nation, and the Aboriginal Wildlife Harvester's Committee (Gates et al., 1998; Nishi et al., 2001, 2002a, b). The main goals of the project were to establish a tuberculosis- and brucellosis-free, captive, bison-breeding herd, using calves captured from infected wild herds, and to preserve the genetic material from these populations. It was hoped that the project would demonstrate a feasible technique for salvaging healthy bison from known infected herds and that captive-bred animals could eventually be used to replace infected herds in the Slave River Lowlands (Reynolds and Hawley, 1987) or to genetically augment existing conservation herds of wood bison in Canada.

Between 1996 and 1998, 62 newborn bison calves were captured from infected herds in the Slave River Lowlands (Wilson et al., 2005) and transported to an isolation facility near Fort Resolution, Northwest Territories, Canada (61°11'N, 113°41'W). The calves were isolated in pairs, hand-reared, and given antimicrobial prophylaxis to treat any undetected *M. bovis* or *B. abortus* infections (Gates et al., 1998; Nishi et al., 2001, 2002a). When founder animals reached sexual maturity, they were used for breeding and herd establishment. Bison were culled periodically to maintain herd size and genetic diversity (Wilson et al., 2005), with the project's disease surveillance protocol ultimately calling for the cull and postmortem disease screening of all founders. Each animal in the herd was tested, on average, twice annually for brucellosis, using the buffered plate antigen test, standard tube agglutination test, complement fixation test (Stemshorn et al., 1985), competitive enzyme-linked immunosorbant assay (ELISA; Nielsen et al., 1996), and fluorescent polarization assay (FPA; Stemshorn et al., 1985; Nielsen et al., 1996; Gall et al., 2000), and for tuberculosis, using the caudal-fold test (CFT; CFIA, 1987)

and FPA (Lin et al., 1996; Nishi et al., 2002a).

In March 2005, 9 yr into the project, a culled 2-yr-old bull was found, on postmortem examination, to have multiple abscessed lymph nodes from which *M. bovis* was cultured (Lutze-Wallace et al., 2006). Up to that time, *M. bovis* infection was not diagnosed in any animal on antemortem or postmortem testing. The spoligotype of the isolated strain was identical to that found in bison in and around WBNP (Lutze-Wallace et al., 2006). The presence of tuberculosis led to depopulation of the entire captive herd, and slaughtered animals underwent a postmortem tuberculosis-testing protocol that resulted in the detection of 11 additional infected (positive *M. bovis* culture) bison. Investigation into the source of the outbreak revealed that *M. bovis* was most likely brought into the herd by a wild-caught, female calf (Y45), which, on postmortem examination at 9 yr of age, was found to have chronic mycobacterial omphalophlebitis (Himsworth et al., 2009). *Brucella abortus* was not detected in any of the bison clinically or on postmortem examination (including culture of multiple tissues).

Antemortem testing for *M. bovis* infection was continued throughout the 11-mo depopulation with the addition of the multiantigen print immunoassay (MAPIA) and rapid test (RT; Waters et al., 2006).

The objective of the current study was to evaluate retrospectively the performance of the CFT, FPA, MAPIA, and RT assays in this herd of bison.

## MATERIALS AND METHODS

### Animals

The captive herd, which contained 119 animals at the time the index case was detected, was kept in an isolation facility near Ft. Resolution, NWT, Canada. The animals were separated into one 4-ha and two 18-ha pens, although they were frequently moved among pens after an initial period of isolation and disease testing, which resulted in inter-

mingling of most of the bison during the course of the project. Culling of selected animals was initiated in 2002 to maintain herd size and genetic diversity within the constraints of a small, closed population (Wilson et al., 2005). Depopulation, following the detection of tuberculosis, was initiated in March 2005 and completed in March 2006.

### Testing protocol

All animals in the herd were tested, on average, twice a year, with the CFT performed according to methods outlined in the Canadian Food Inspection Agency (CFIA) *Disease Control Manual of Procedures for Tuberculosis* (CFIA, 1987) using purified protein derivative (PDD) tuberculin obtained from the CFIA Mycobacterial Diseases Center of Expertise (MDCE).

The FPA, which was used in 2001, 2002, 2005, and 2006, was performed at the CFIA MDCE. For the FPA, blood was allowed to clot for 12 hr before serum was collected, stored in cryovials, and frozen at  $-20^{\circ}\text{C}$ . The FPA was performed according to methods previously described (Lin et al., 1996). In this herd, FPA scores (calculated by subtracting the milipolarization [mP] value of the negative control from the mP value of the sample)  $<10$  mP were considered negative, and scores  $\geq 10$  mP were considered positive.

In 2005 and 2006, the MAPIA and RT were performed on blindly coded serum samples collected at the time of slaughter. Retrospective analysis of banked serum samples collected before detection of *M. bovis* in the herd was also performed. Samples, collected and handled as outlined for the FPA, were shipped to Chembio Diagnostic Systems, Inc. (Melford, New York, USA) for analysis. The MAPIA and RT were performed as previously described (Waters et al., 2006).

### Postmortem examination

All 158 bison slaughtered during the course of the project (1996–2006) underwent standardized necropsy and gross examination for lesions consistent with bovine tuberculosis. Toward the end of the depopulation, the number of bison being slaughtered exceeded the capacity of personnel to perform full postmortem tuberculosis testing (necropsy, histopathology, and culture) on all animals. Therefore, 37 of 158 animals (23%) were screened only by necropsy and macroscopic tissue examination, 39 (25%) were screened by macroscopic examination and culture, and 82 (52%) were screened by macroscopic examination, histopathology, and culture.

Tissues, including lymph nodes (retropharyngeal, parotid, mandibular, bronchial, mediastinal, superficial cervical, prefemoral, popliteal, supramammary, internal iliac, hepatic, mesenteric, and ileocecal), as well as lung, liver, kidney, spleen, male and female reproductive organs, mammary gland, and any lesions compatible with bovine tuberculosis, were collected from 121 of 158 bison (77%). One set of the aforementioned tissues was frozen, and another was fixed in 10% neutral-buffered formalin. Both sets of tissues were sent to the CFIA MCDE for further examination.

Formalin-fixed tissues were processed routinely, embedded in paraffin, sectioned at  $5\ \mu\text{m}$ , and stained with hematoxylin and eosin (HE) and Ziehl-Neelsen stains (Luna, 2008). Eighty-two of 121 sets (68%) of fixed, embedded tissues were examined microscopically at the CFIA MCDE. Of these, 29 sets contained at least one tissue with lesions potentially consistent with tuberculosis (Caswell and Williams, 2007). These 29 sets of fixed, embedded tissues were sent to the Western College of Veterinary Medicine (Saskatoon, Saskatchewan, Canada) for further examination using  $4\text{-}\mu\text{m}$  sections stained with HE and Fite's method for acid-fast organisms (Luna, 2008).

A histopathologic lesion was considered consistent with bovine tuberculosis if it contained a core of necrotic material or degenerate neutrophils with or without areas of mineralization, and a mantle of epithelioid macrophages, multinucleated giant cells, lymphocytes, and plasma cells (Caswell and Williams, 2007). A fibrous capsule or acid-fast bacteria were additional supporting, but not obligate, criteria. Any animal with histopathologic lesions consistent with this case definition was classified as histopathology-positive.

Frozen lymph nodes from 121 bison were thawed, trimmed, and examined grossly for lesions potentially consistent with tuberculosis. For each bison, tissues with no visible lesions were, in general, pooled (1–3 g/pool, three pools/animal) according to anatomic location (head or neck, thorax, abdomen or other carcass nodes), and tissues with lesions were cultured separately. All tissue samples were processed as previously described (Sangster et al., 2007) and inoculated on special media (including Herrold's with mycobactin, naladixic acid, fungizone, and vancomycin; Lowenstein-Jensen with and without sodium pyruvate; Stonebrink; 7H9 broth with naladixic acid, fungizone and vancomycin; and modified 7H10 [Allied Monitor, Fayette, Missouri, USA]). Inoculated media were incubated at

37 C and examined once a week for 2 wk and then every 2 wk for up to 10 wk. Isolates were identified based on colony morphology, pigmentation, temperature of growth, biochemical and molecular assays (AccuProbe® Culture Identification Tests, Gen-Probe Inc., San Diego, California, USA). Any animal from which *M. bovis* was isolated was classified as culture-positive.

#### Data analysis

Only results from antemortem tests performed within 1 mo of slaughter were used for analysis of test performance, to increase the likelihood that the disease status of an animal, as determined at postmortem examination, was reflective of its status at the time that blood was collected for testing (Caswell and Williams, 2007).

Sensitivity (Se) and specificity (Sp) for each antemortem test were calculated twice—once in comparison to culture results (culture-positive vs. culture-negative) and once in comparison to histopathology results (histopathology-positive vs. histopathology-negative) (Dohoo et al., 2003). This provided a range of values for each parameter, increasing the likelihood that the true values for Se and Sp were within that range. Previous studies have used a similar technique to measure the performance of tuberculosis diagnostic tests (Whipple et al., 1995).

Positive predictive value (PPV) and negative predictive value (NPV) for each antemortem test were calculated using test results only from animals that received a complete postmortem examination. This was done to avoid biases that would be introduced through inclusion of animals selected for culture or histopathology based on observed lesions consistent with tuberculosis. Once these prescreened animals were excluded, there was insufficient histopathology data to permit analysis. Therefore, only bacterial culture results were used to calculate PPV and NPV (Dohoo et al., 2003).

Values for Se, Sp, PPV, and NPV were not considered to be significantly different if there was overlap of the 95% confidence intervals (CI); the chi-square test (Dohoo et al., 2003) was used to compare values when the 95% CI did not overlap. For the FPA, scores, in mP units, were compared between culture-positive and culture-negative results, as well as between histopathology-positive and histopathology-negative animals using the Wilcoxon rank-sum test (Dohoo et al., 2003), with a significance level of  $P=0.05$ .

Agreement among antemortem tests was evaluated using the kappa statistic (Dohoo et

al., 2003), with a significance level of  $P=0.05$ . All calculations were performed using the program STATA/IC version 10.0 (StatCorp LP, College Station, Texas, USA). Se, Sp, PPV, and NPV were determined using the additional STATA function “diagt” (Juil, 2006).

## RESULTS

A total of 158 bison were slaughtered during the course of the project. Culture results were available from 121 animals, of which 12 were culture-positive. Histopathology was available from 82 animals, of which 17 were histopathology-positive.

Overall, there was no significant difference between Se and Sp values generated through comparison of culture versus histopathology (Table 1). There was no significant difference in Se among any of the antemortem tests (Table 1), although the MAPIA appeared to have a higher range of potential Se values than the other tests. There was no significant difference between the Sp of the CFT, RT, and MAPIA (Table 1); however, the FPA was significantly less specific than the CFT, RT, and MAPIA ( $P\leq 0.05$  for all comparisons, using values generated both through culture and histology).

There was no significant difference between the distribution of FPA scores in culture-positive and culture-negative animals, or in histopathology-positive and histopathology-negative animals ( $P=0.70$ ,  $n=46$  and  $P=1.00$ ,  $n=21$ , respectively).

There was no significant difference between the NPV of any of the tests (Table 2). Additionally, there was no significant difference between the PPV of the FPA and CFT, or between the PPV of the CFT, RT, and MAPIA (Table 2). However, the PPV for the FPA was significantly lower than those of the MAPIA and RT were ( $P<0.05$  for both comparisons). Using the kappa statistic, there was significant agreement in test results only between the MAPIA and RT (Table 3).

For the 12 MAPIA-reactive bison, the antigen to which the greatest number of

TABLE 1. Sensitivity (Se) and specificity (Sp) of antemortem tuberculosis tests in a captive herd of bison using culture and histopathology as gold standards. Upper and lower limits of 95% confidence interval (CI) provided in parentheses.

Test <sup>c</sup>	Culture <sup>a</sup>			Histopathology <sup>b</sup>		
	n <sup>d</sup>	Se (95% CI)	Sp (95% CI)	n <sup>d</sup>	Se (95% CI)	Sp (95% CI)
FPA	56	67 (30, 93)	34 (20, 49)	32	56 (21, 86)	48 (27, 69)
CFT	26	50 (1, 99)	88 (68, 97)	26	67 (9, 99)	91 (72, 99)
MAPIA	82	92 (62, 100)	97 (90, 100)	43	77 (46, 95)	97 (83, 100)
RT	82	67 (35, 90)	99 (92, 100)	43	62 (31, 86)	100 (88, 100)

<sup>a</sup> Calculated using *Mycobacterium bovis* culture as the gold standard.

<sup>b</sup> Calculated using histopathology as the gold standard.

<sup>c</sup> FPA = fluorescent polarization assay; CFT = caudal-fold test; MAPIA = multiantigen print immunoassay; RT = rapid test.

<sup>d</sup> n = No. bison included in each calculation (one test result per bison).

test-positive animals developed antibody was ESAT6 protein (eight of 12 animals), followed by the ESAT6/CFP10 fusion (seven of 12), CFP10 antigen (five of 12), and MPB83 protein (four of 12). Antibody against the MPB70 antigen (included in the MAPIA and the single antigen used in the FPA) was found in one animal.

Table 4 summarizes data for antemortem testing performed at slaughter for bison that were positive for tuberculosis on culture or histopathology. Use of the MAPIA on banked serum samples from bison Y45 (believed to have been infected throughout the duration of the project [Himsworth et al., 2009]) revealed the presence of anti-*M. bovis* antibody since

TABLE 2. Positive predictive value (PPV) and negative predictive value (NPV) of antemortem tuberculosis tests in a captive herd of bison using culture as the gold standard. Upper and lower limits of 95% confidence interval (CI) provided in parentheses.

Test <sup>a</sup>	n <sup>b</sup>	PPV (95% CI)	NPV (95% CI)
FPA	52	11 (3, 27)	94 (71, 100)
CFT	26	25 (1, 81)	96 (77, 100)
MAPIA	78	85 (55, 98)	99 (92, 100)
Rapid	78	89 (52, 100)	95 (87, 99)

<sup>a</sup> FPA = fluorescent polarization assay; CFT = caudal-fold test; MAPIA = multiantigen print immunoassay; RT = rapid test.

<sup>b</sup> n = No. of bison included in each calculation (one test result per bison).

1997, the year after capture and 8 yr before postmortem isolation of *M. bovis* (Table 5). The RT and FPA became positive in 1999 and 2005, respectively, and the CFT was never positive in this animal.

During the project, four of 83 (5%) animals tested with the FPA in 2001 were positive, of which one was histopathology-positive and culture-negative at cull. In 2002, none of the 23 animals tested with the FPA were positive; however, 25 of 89 (28%) and 46 of 59 (78%) bison tested with the FPA were positive in 2005 and 2006, respectively. A total of 697 caudal-fold tests were performed in this herd from 1998 to 2005. The first caudal-fold test reactors were detected during the last

TABLE 3. Kappa ( $\kappa$ ) values for comparison of antemortem tuberculosis test results in a captive herd of bison.

Test <sup>a</sup>	n <sup>b</sup>	$\kappa$	P value
MAPIA vs. RT	82	0.79	<0.001
FPA vs. RT	72	<0.00	0.56
FPA vs. MAPIA	55	<0.00	0.42
FPA vs. CFT	7	<0.00	0.89
MAPIA vs. CFT	11	0.12	0.32
RT vs. CFT	11	<0.00	0.78

<sup>a</sup> FPA = fluorescent polarization assay; CFT = caudal-fold test; MAPIA = multiantigen print immunoassay; RT = rapid test.

<sup>b</sup> n = No. of bison included in each calculation (one result per test per bison).

TABLE 4. Antemortem tuberculosis test results performed in the month before slaughter for all bison that were positive for tuberculosis on culture or histology.<sup>a</sup>

ID <sup>b</sup>	Sex	Age (yr)	Histology	Culture	CFT	FPA	RT	MAPIA
Y36	F	9.2	+	+	n/a	+	+	+
Y45	F	9.2	+	+	n/a	+	+	+
B-21	M	5.8	+	+	n/a	—	+	+
O-13	M	4.9	+	+	n/a	—	—	—
O-28	M	2.8	+	+	—	n/a	+	+
O-30	M	3.2	+	+	n/a	+	+	+
O-29	M	3.3	+	+	+	—	—	+
O-45	M	3.4	+	+	n/a	n/a	+	+
O-47	M	3.5	—	+	n/a	+	—	+
O-68	M	1.0	+	+	n/a	n/a	+	+
O-69	M	1.3	+	+	n/a	+	—	+
O-70	F	1.3	+	+	n/a	+	+	+
Y49	F	9.4	+	—	+	—	—	—
Y124	M	7.8	+	—	n/a	n/a	—	—

<sup>a</sup> ID = bison identification number; FPA = fluorescent polarization assay; CFT = caudal-fold test; MAPIA = multiantigen print immunoassay; RT = rapid test; + = positive test result; — = negative; n/a = test result not available.

<sup>b</sup> Bison identification numbers starting with “Y” indicate the individual was a founder (i.e., captured as a wild calf). Identification numbers starting with “B” or “O” indicate captive-born animals.

year of caudal-fold testing in 2005, during which nine of 109 (8.3%) animals tested were reactors.

## DISCUSSION

These results summarize the performance of four antemortem, tuberculosis diagnostic tests in a captive herd of wood bison. Previously published data on tuberculosis test performance in bison were limited to the CFT (Thoen et al., 1988; Joly and Messier, 2004), which, in this analysis, demonstrated similar Se and Sp to that previously reported (Joly and Messier, 2004).

Overall, there was no significant difference between the Se among the CFT, FPA, MAPIA, and RT. This could, however, be a result of insufficient power, as reflected by wide confidence intervals for many of the Se values generated by this analysis. The MAPIA, however, showed a trend toward higher Se compared with the other tests, which may be associated with the use of multiple *M. bovis* antigens in this assay. Previous studies in *M. bovis*-infected cattle have shown marked differences in antigen recognition patterns among individuals (Fifis et al., 1994), which supports the notion that tests using

TABLE 5. Antemortem tuberculosis test history for bison Y45.<sup>a</sup>

Test	1996 <sup>b</sup>	1997	1998	1999	2000	2001	2002	2003	2004	2005 <sup>c</sup>
CFT	—	—	—	—	—	—	—	n/a	n/a	—
FPA	n/a	n/a	n/a	n/a	n/a	—	—	n/a	n/a	+
MAPIA	—	+	n/a	+	+	n/a	+	n/a	n/a	+
RT	—	—	n/a	+	+	n/a	+	n/a	n/a	+

<sup>a</sup> FPA = fluorescent polarization assay; CFT = caudal-fold test; MAPIA = multiantigen print immunoassay; RT = rapid test; — = negative test result; + = positive test result; n/a = test result not available.

<sup>b</sup> Year this bison was captured as a calf.

<sup>c</sup> Year this bison was culled.

multiple antigens may be more sensitive than those that use a single antigen.

The potential for increased diagnostic sensitivity using the MAPIA is supported by the antemortem testing history of bison Y45, which was believed to have become infected with *M. bovis* as a calf and was the most likely source of disease for the rest of the herd (Himsworth et al., 2009). Although bison Y45 was negative on multiple CFT and FPA tests during the project, and only positive on the FPA at slaughter, retrospective analysis of banked serum samples using the MAPA and RT revealed that infection might have been detected as early as 1997 (8 yr before culture-based diagnosis) and 1999, respectively, had these tests been available at that time.

In this herd, ESAT6, CFP10, and ESAT6/CFP10 fusion protein were the antigens against which the greatest number of bison produced antibody detected by the MAPIA. Only one of 12 MAPIA-positive bison had antibody against the MBP70 protein, the antigen used in the FPA. The relative serodominance of ESAT6 and CFP10 compared with MPB70 is consistent with findings of previous studies using the MAPIA in cattle (Waters et al., 2006). Its use of a single, nonserodominant antigen may explain why the FPA appeared to have lower sensitivity compared with the MAPIA.

With regard to test specificity, the FPA was significantly less specific compared with the other tests. However, it is not possible to form conclusions about the true specificity of the FPA based on this analysis because of problems associated with evaluating diagnostic test specificity in an infected herd. It may be the case, particularly toward the end of the outbreak, that FPA-positive, culture-negative, and histopathology-negative bison were recently exposed to the bacterium or in a very early stage of infection undetectable by culture or histopathology. Inclusion of these animals in specificity calculations would artificially decrease the apparent

specificity of the test because these animals would be considered “false-positives” on the FPA when using either culture or histopathology as standards. The proportion of FPA-positive bison increased dramatically during the course of the project, particularly during the time when infection is thought to have spread within the herd. This may be consistent with an increase in the number of animals exposed to *M. bovis* because the proportion of nonspecific reactors would not be expected to demonstrate temporal variation.

The results for PPV and NPV are consistent with what would be expected in a herd with a low prevalence of tuberculosis (Dohoo et al., 2003), with NPV being higher, in general, than PPV. The PPV of the FPA was significantly lower than that of the MAPIA and RT, which is a reflection of the low apparent sensitivity of the FPA compared with the other assays.

Based on kappa values, the only antemortem diagnostic tests that demonstrated significant agreement with each other, were the MAPIA and RT. This is not surprising because they are both serologic tests that detect antibody to multiple antigens using sandwich ELISA-type technology, and the antigens used in the RT (MPB83, ESAT-6, and CFP-10) are among the serodominant antigens used in the MAPIA.

Inconsistencies among antemortem tuberculosis diagnostic tests are also evident on review of test results obtained at slaughter from bison that were positive for tuberculosis on culture or histopathology (Table 4). This suggests that when high overall diagnostic sensitivity is desired (as was the case in this herd), it may be beneficial to use multiple tuberculosis diagnostic tests in parallel (Plackett et al., 1989). Even the most thorough antemortem testing protocol, however, cannot guarantee detection of *M. bovis* infection. Bison Y45 was negative on all tests performed during the first year after

capture, despite the fact that she is believed to have been infected in the wild (Himsworth et al., 2009). Additionally, bison O13 was negative on all antemortem tuberculosis tests up to and including those performed at slaughter, but was culture-positive and histopathology-positive. Previous studies in cattle have also identified *M. bovis*-infected animals at postmortem that were negative on multiple antemortem tuberculosis tests used in parallel (Wood et al., 1992).

The results of this study suggest that the sensitivity of antemortem tuberculosis testing in bison can be optimized through use of multiple diagnostic tests in parallel, particularly when the selected assays include tests that use multiple antigens. Negative results, however, must be interpreted with caution because there is significant potential for infected animals to escape detection.

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