JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1987, p. 350–354
0095-1137/87/020350-05$02.00/0

Vol. 25, No. 2

Comparison of TRACK XI Fluorometric Immunoassay System with Other Serologic Tests for Detection of Serum Antibody to *Brucella abortus* in Cattle†

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The TRACK XI system (TRACK) is a commercially available fluorometric immunoassay system that has been adapted for the detection of antibodies to several antigens in various animal species. Serum antibodies to *Brucella abortus* were detected by TRACK and compared with those obtained from two primary binding assays (a fluorometric immunoassay [FIAX] and an enzyme-linked immunosorbent assay) and three standard serological tests (complement fixation, Rivanol precipitation, and CARD tests). A total of 298 serum samples were tested by each serological test. Of these serum samples, 134 were negative controls, 43 were from cattle 1 month after vaccination with *B. abortus* 19, and 121 were from cattle 10 to 12 weeks after a midgestational, intraconjunctival challenge with *B. abortus* 2308. The results of this study indicated that TRACK is both reproducible and accurate. The results compared favorably with those of other serological methods. TRACK is more rapid than either the enzyme-linked immunosorbent assay or FIAX system. TRACK was the most sensitive (96.3%) test, with a specificity of 100%.

Bovine brucellosis caused by *Brucella abortus* is an economically important disease associated with abortion and infertility. Despite numerous methods of eradication, including vaccination, testing, and slaughter, the disease has remained prevalent in many areas of the world (8, 12).

Most eradication schemes involve the use of serological tests on live or slaughtered cattle to indicate the presence of *B. abortus* infection within cattle herds. Several of the more commonly used serological tests include the complement fixation (CF), Rivanol precipitation (RIV), and buffered antigen (CARD) tests (11, 12, 15). Several authors describe the use of an enzyme-linked immunosorbent assay (ELISA) for the detection of serum antibodies to *B. abortus* (3, 5, 16). We recently described a fluorometric immunoassay (FIAX) that detects serum antibodies to *B. abortus* in cattle (9).

The TRACK XI system (TRACK) is a commercially available fluorometric immunoassay system (Daryl Laboratories, Santa Clara, Calif.) adapted for the detection of antibodies to several antigens in various animal species (10). This manuscript presents the results obtained with TRACK for detection of serum antibodies to *B. abortus* in cattle. Antibody responses detected by TRACK were compared with those obtained from two primary binding assays, FIAX and ELISA, as well as three standard serological tests (CF, RIV, and CARD).

MATERIALS AND METHODS

TRACK test. TRACK is an immunofluorescence system in which serum antibrucella antibodies are bound to *B. abortus* antigens and detected with specific fluorescein isothiocyanate-conjugated (FITC) antiglobulin. Antibrucella antibodies are quantitated with the TRACK fluorescence reader.

Tests were run on plastic disposable test tracks. Each track (Daryl) contains 12 individual wells coated with a three-dimensional colloid-phase polymer. The antigen, which is applied to the wells by the manufacturer, is a soluble extract of *B. abortus* 1119. The antigen (Richard A. Harte, personal communication) was prepared as follows. Standard tube test antigen (S-1119; U.S. Department of Agriculture, Ames, Iowa) was diluted 1:75 in 0.066 M carbonate-bicarbonate buffer (pH 9.0), sonicated, and extracted with 1% sodium deoxycholate in phosphate-buffered saline (PBS). The material was centrifuged, and the pellet was suspended in PBS, heated to 100°C for 20 min, and recentrifuged at 10,000 × *g* for 30 min. The supernatant (30 μl) was applied to each track well and allowed to dry.

Tests were conducted at room temperature as follows. The tracks were soaked for 10 min in distilled water and then patted dry. Undiluted test serum (20 μl) was applied to a test well containing the colloid-bound antigen. High, medium, and low TRACK test control sera were supplied with the test and were run on each track. After a 10-min incubation at room temperature and a brisk rinse in distilled water, 20 μl of undiluted FITC goat anti-bovine immunoglobulin G (IgG) (Daryl) was then applied to each well and incubated for 10 min. The track was briskly washed in distilled water, patted dry, and read in the TRACK fluorometer. A microprocessor within the fluorometer utilizes the TRACK test control sera from each track to calculate a linear-linear standard curve of fluorescence signal versus a known value, referred to as a TRACK titer. The titers of the nine remaining test samples on the track were extrapolated from the standard curve. In this laboratory, TRACK titers of <29 were determined to be negative with a discriminate analysis method (14).

The reproducibility of the TRACK system was determined by using replicates of the TRACK test control sera. Seven tracks were used to test the variation among tracks. Each track had high, medium, and low TRACK test control sera as controls and as test samples for determining variability. Variation within a track was measured by using high, medium, and low TRACK test control sera for the standard curve on each of three tracks; the remaining nine wells on

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each of the three tracks had one of the three types of TRACK test control sera (high, medium, or low).

The variability of the TRACK system with different antigen preparations and FITC conjugates was compared by using replicates of the high and low control sera. Non-antigen-coated blank tracks were prepared in our laboratory with a soluble extract of *B. abortus* 1119 (BASA-d) (2, 17). Thirty microliters of PBS (pH 7.2) containing 0.625 μg of BASA-d was applied to each well of the blank tracks and allowed to dry overnight at 37°C. The conjugate used was a 1:2 dilution of FITC rabbit anti-bovine IgG (heavy and light chain specific) (Cappel Laboratories, Cochranville, Pa.) with PBS. The assays were performed as outlined above, and the results obtained were compared with those obtained from manufactured antigen-coated tracks and the supplied conjugate (Daryl).

FIAX test. The FIAX (International Diagnostic Technology, Inc., Santa Clara, Calif.) system, like the TRACK system, is a fluorometric immunoassay. In the FIAX test, 25 μl of PBS (pH 7.2) containing 25 μg of BASA-d antigen (17) per ml was applied to a nitrocellulose disk attached to a plastic carrier (StiQ; International Diagnostic Technology). All StiQs were incubated at room temperature for 30 min with 0.51 ml of a 1:51 dilution of test serum, washed for 10 min in PBS containing 0.15% Tween 20, and then incubated with 0.5 ml of a 1:800 dilution of FITC rabbit anti-bovine IgG (heavy and light chain specific) (Cappel). The StiQ-associated fluorescein was determined by using a fluorometer, and FIAX titers, expressed in nanograms of immunoglobulin binding, were extrapolated from an IgG standard curve. The mean of duplicate or triplicate samples was used to calculate FIAX titers for each test serum. For this study, FIAX titers of <36 ng of immunoglobulin bound per StiQ, based on discriminate analysis, were negative.

ELISA. The ELISA was performed as previously described (5). One hundred microliters of BASA-d in carbonate buffer (pH 9.6) was used to coat the wells of a polystyrene microtiter plate (Nunc, Roskilde, Denmark) overnight at room temperature. After three washes in PBS with 0.05% Tween 20, 100 μl of serum diluted 1:250 in PBS-Tween 20 buffer containing 1.0% bovine serum albumin was added to duplicate wells of the plate and incubated for 1.5 h at room temperature. After three additional washes, 100 μl of a 1:400 dilution of horseradish peroxidase-conjugated, affinity-purified rabbit anti-bovine IgG (Pel Freeze, Rodgers, Ark.) was added to each well and incubated for 45 min at room temperature. After six washes in PBS-Tween 20 buffer, 100 μl of the substrate containing *o*-phenylenediamine (0.4 mg/ml) and hydrogen peroxide (0.04% of a 30% solution) in phosphate-citric acid buffer was added to each well. Plates were incubated in the dark for 30 min at room temperature. The reaction was stopped with 40 μl of 0.25 M sulfuric acid. An average optical density at 490 nm was determined on duplicate samples, and the amount of IgG (nanograms per well) was determined from a standard curve. Values of <20 ng of immunoglobulin bound per well were considered negative.

Conventional tests. The standard serological tests (CARD, CF, and RIV) were performed with standard protocols and reagents (NADL Diagnostic Reagents Manuals 65d and 65e), at the State-Federal Brucellosis Laboratory, Oklahoma City, Okla. The standard procedures were described previously (1, 11). These standard serological tests were evaluated by the criteria of Deyoe et al. (6). The CARD test was negative if no agglutination was observed. A negative CF test was defined by a reaction of <3+ at a serum dilution of 1:10. The RIV test was considered negative if no precipitation was observed at a serum dilution of <1:25.

Serum samples. A total of 298 serum samples from cattle used in four separate field trial experiments were tested by each of the serological tests. Of these sera, 134 were negative controls because they were from heifers that were neither vaccinated with *B. abortus* S-19 nor challenged with a virulent field strain (S-2308) of *B. abortus* but were from dams that were seronegative. Forty-three serum samples were from the same heifers that had been used as controls 1 month after vaccination with 10^9 or 10^{10} CFU of S-19. A total of 121 serum samples (54 of these samples were from animals that had been used as vaccinates or controls) were taken from cattle 10 to 12 weeks after a midgestational, intraconjunctival challenge with approximately 10^7 CFU of virulent *B. abortus* biotype 1 S-2308 (a standard challenge strain supplied by the National Animal Disease Laboratory, Ames, Iowa) (6).

Culture techniques. Standard culture techniques (1) were used to detect *B. abortus* in the 121 challenged animals, either at the time of abortion or 14 to 18 weeks after challenge. Tissues from the fetus (spleen, stomach contents, lung, mesenteric lymph nodes, and placenta) and the dam (milk samples, mammary gland, supramammary lymph node, mandibular lymph node, internal iliac lymph node, and spleen) were cultured for *B. abortus*. Animals were considered culture negative if *B. abortus* could not be isolated from any of the tissues.

Abortions. Cows were abortion positive if they delivered a dead or weak, premature calf. Cows that delivered healthy calves were abortion negative. Abortions were classified as indeterminate if either no calf was delivered or abortion could not be definitively determined.

Sensitivity and specificity. The sensitivity and specificity (4) were determined by using 188 serum samples; 134 were negative controls (disease negative), and 54 were samples from cattle that had been challenged and aborted and were culture positive (disease positive). A true-positive was defined as a serum sample that was disease positive and seropositive; a true-negative was a serum sample that was disease negative and seronegative. A false-negative was defined as a serum sample that was seronegative and disease positive; a false-positive was a serum sample that was seropositive and disease negative. Sensitivity was defined as [(number of true-positives)/(number of true-positives + number of false-negatives)] × 100%. Specificity was defined as [(number of true-negatives)/(number of true-negatives + number of false-positives)] × 100%.

Statistical analysis. The means for the TRACK reproducibility study were compared by using multiple two-tailed Student's *t* tests (14). A *P* value of <0.05 was considered statistically significant.

RESULTS

Variation of TRACK. The means, standard deviations of the means, and coefficients of variation of TRACK, with the TRACK test control sera as test samples, are presented in Table 1. A significant difference was observed between high and medium TRACK test control sera and medium and low TRACK test control sera both among tracks and within tracks. When the means for the among-track samples were compared with their respective within-track means, only the means of the middle TRACK test control sera were not significantly different. The highest coefficient of variation observed was 16.1% among tracks for the low TRACK test control sera.

TABLE 1. Variation of TRACK test control sera within the same track and among different tracks

TRACK test control sera (*n*)	TRACK titer		
	Mean	SD	CV[a]
Within tracks			
High (9)	225.0	24.8	11.0
Middle (9)	37.4	4.3	11.5
Low (9)	8.2	0.7	8.1
Among tracks			
High (7)	263.4	18.2	6.9
Middle (7)	39.7	4.3	10.8
Low (7)	11.7	1.9	16.1

[a] CV, Coefficient of variation.

The mean titers and standard deviations are presented (Table 2) for replicates of high and low test sera with a different antigen preparation and different conjugate than those supplied by the manufacturer. When the mean titers within the high-serum group were compared, no significant difference was observed for values obtained with the Daryl conjugate or the Cappel conjugate. No significant difference was observed in comparing the mean titers of the commercially prepared antigen-coated tracks with those of the BASA-d antigen-coated tracks prepared in our laboratory. Within the low-serum group, only one combination (TRACK antigen and Daryl conjugate) was significantly different from the other combinations of antigen and conjugate.

Mean titers. Means, standard errors of the means, and ranges of titers for serum groups for the TRACK, FIAX, ELISA, CF, and RIV tests are shown in Table 3. With all five tests, the lowest antibody values were in the control group. The mean titer of the S-19-vaccinated group was higher than that of the control group and lower than that of the challenged group by all five serological tests. Within the challenged group, sera from culture-positive or abortion-positive animals had a higher mean titer than did sera from culture-negative or abortion-negative animals, respectively.

The percentages of serum samples that were positive by the six serological tests are presented in Table 4. The TRACK, RIV, and CARD tests all had no false-positive reactions in the control group. ELISA had the highest number of positive reactions (11.2%) in the control group. In the vaccinated group, all tests had a similar number of positive sera; ELISA had the highest (62.8%), and the CF test had the lowest (44.2%). TRACK detected the highest number (76.9%) of positive sera in the challenged group, whereas the CF and RIV tests detected the lowest (49.6%). Of the sera from animals that were challenged and subsequently determined to be culture positive, TRACK detected the highest number (90.8%); results from the other five tests were similar, with the CF value (72.4%) being slightly lower than the others. The RIV test (8.9%) detected the least number of positive sera for the group that was challenged and found to be culture negative; TRACK (53.3%) detected the highest number of positive samples in that group. For the group that was challenged and later aborted, all tests detected >80% of the sera as positive; TRACK (96.4%) had the highest number of positives, and ELISA (82.1%) had the lowest. Of the sera from the cattle that were challenged but did not abort, the CF test (9.1%) detected the lowest number of positives, and TRACK (54.5%) detected the highest number. Of the animals that were challenged, 42 were both culture negative and abortion negative. Of these 42, 27 were seropositive by at least one serological test. TRACK detected the highest number of positive samples from this group (21/42), whereas the CF and RIV tests detected the lowest number of seropositive samples (2/42). Of the 121 animals that were challenged, 54 were both culture positive and abortion positive. Of these 54, 14 were seronegative by at least one serological test. TRACK detected the lowest number of seronegative samples (2/14), whereas ELISA detected the highest (10/14). Thirteen animals were challenged and subsequently found to be culture positive but did not abort. TRACK detected the highest number (9/13) of these as positive; CF detected the lowest (3/13). There were two animals that were challenged and were abortion positive but culture negative; both of these animals were positive by all five serological tests.

Sensitivity and specificity. The sensitivity and specificity of the six serological tests are presented in Table 5. TRACK had the highest sensitivity (96.3%) and specificity (100%). ELISA had the lowest sensitivity (81.5%) and specificity (88.8%).

DISCUSSION

The TRACK system is reproducible and has a variance small enough to allow for clear and repeatable distinctions between the high, middle, and low ranges of TRACK test control sera. A statistical difference was observed in both the high and low TRACK test control sera when among-track means were compared with within-track means (<15% difference for the high TRACK test control sera), but its practical significance is questionable. The coefficients of variation are all below 17%, which compared favorably with results obtained for other serological assays within this laboratory. The variation among antigen preparations or conjugates is not thought to a major problem in use of the TRACK system. The various combinations of antigen and conjugate appear to perform similarly, probably because the standard curve is calculated each time the test is performed. This fitting of a standard curve was used in the FIAX and ELISA systems and is a reliable method of correcting for small differences in antigen or conjugate preparations. A preliminary study showed that a 1:2 dilution of the Cappel conjugate was required to give results similar to those of the Daryl conjugate. It should be noted that one of the low sera was significantly lower than the other three. These lower values would still be classified as negative and would not affect the interpretation of the test.

TABLE 2. Variation of TRACK titers with different conjugates and antigen preparations

Antigen[a]	Conjugate source[b]	Sera (*n*)	Titer	SD
BASA-d	Cappel	High (5)	193.6	14.3
BASA-d	Daryl	High (8)	199.7	6.9
TRACK	Cappel	High (8)	208.3	14.6
TRACK	Daryl	High (5)	198.8	12.0
BASA-d	Cappel	Low (5)	17.5	7.0
BASA-d	Daryl	Low (8)	17.1	2.2
TRACK	Cappel	Low (8)	11.6	6.3
TRACK	Daryl	Low (5)	2.1	4.2

[a] BASA-d, *B. abortus* soluble antigen (as used in FIAX and ELISA) with 0.625 μg per track well; TRACK, commercially prepared and applied antigen.
[b] Cappel, a 1:2 dilution with PBS of Cappel rabbit anti-bovine IgG (heavy and light chains); Daryl, nondiluted goat anti-bovine IgG (heavy and light chains) supplied with kit.

TABLE 3. Means, standard errors of the means, and ranges of antibody to *B. abortus*

Group (*n*)	Titer														
	TRACK			FIAX[a]			ELISA[a]			CF			RIV		
	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM	Range
Controls (134)	2.2	0.4	25.0	1.4	0.5	51.5	12.9	0.6	43.7	1.0	0.3	20.0	0.0	0.0	0.0
Vaccinated with S-19 (43)	39.7	4.1	125.0	65.9	11.5	318.4	47.0	6.2	190.7	13.5	3.0	80.0	44.8	9.1	200.0
Challenged with S-2308 (121)	105.5	6.6	289.0	170.4	17.4	891.0	92.1	7.7	361.0	92.8	15.4	640.0	91.5	9.9	400.0
Culture positive (76)	141.1	7.0	288.0	241.1	22.9	891.0	118.9	9.4	347.0	144.6	22.5	640.0	137.2	12.4	400.0
Culture negative (45)	45.4	7.0	229.0	51.0	14.0	389.4	46.9	10.5	360.0	5.3	2.7	80.0	14.4	7.6	200.0
Abortion positive (56)	162.7	6.3	284.0	305.1	25.7	891.0	131.0	10.4	345.0	192.0	27.9	640.0	180.8	12.2	400.0
Abortion negative (55)	47.0	6.2	202.0	45.5	11.0	342.5	54.9	10.5	361.0	6.2	3.4	160.0	13.6	6.3	200.0
Abortion indeterminate (10)	106.9	17.8	164.0	103.0	29.7	237.7	79.3	23.1	201.5	14.0	8.5	80.0	20.0	11.1	100.0

[a] Measured in nanograms of bound immunoglobulin.

In this study, the mean titers were lower in the vaccinate group than in the challenged group. However, on the basis of the percentage of positive sera, TRACK was no more advantageous than the other assays in discriminating between vaccinal titers and those resulting from field strain infection.

The serological results from challenged cattle were examined by subgroups. By all five serological tests, the mean titers for the challenged, culture-negative cattle were similar to the mean titers for the challenged, abortion-negative cattle. These titers do not represent exact duplication of the same serum samples, as 13 of the 55 cattle that did not abort were culture positive, and 2 of the 56 cattle that aborted were culture negative. Both of these cattle were positive by all five serological tests. By all five serological tests, the culture-positive cattle had lower mean titers than the abortion-positive cattle did. Also, there was a lower percentage of seropositive animals by all five tests in the culture-positive group than in the abortion-positive group. Therefore, the abortion-negative animals lowered the percent positive and the mean titer of the culture-positive group. Isolation of *B. abortus* from a heifer should, in most cases, represent infection, but the above serological data indicate that cattle may asymptomatically or latently carry *B. abortus* without pathological or serological reactions, as previously described (7, 12); this possibility should be considered when evaluating serological data.

In *B. abortus* infections, sensitivity and specificity are difficult to define in a manner that is meaningful and beyond reproach. Because of the possibility that animals asymptomatically carry *B. abortus* rather than have a pathological infection, the disease-positive sera used in calculating sensitivity were strictly defined to be the sera from cattle that had been challenged, were culture positive, and had aborted. When evaluating sensitivity and specificity, we are always defining it in terms of another test(s). Some tests are generally regarded as incorrigible evidence, such as a bacteriological or histopathological diagnosis (13), but even these are not infallible, especially in the case of negative results. Animals that aborted and were culture positive could well be classified as disease positive. It is also possible for an animal to be infected and even diseased, and yet for numerous reasons the organism may not be isolated. It is for this reason that challenged animals were excluded from the disease-negative group, regardless of culture or abortion status. Disease-negative animals were strictly defined as cattle that had been neither challenged nor vaccinated. The values obtained for sensitivity and specificity should be used for accurately comparing the relative sensitivity and specificity among the various tests. It is questionable whether the absolute values obtained from such strictly defined populations would extrapolate to the population(s) of cattle at large. With these criteria, TRACK had good sensitivity and excellent specificity. Only two serum samples from cattle that were challenged, culture positive, and abortion positive were seronegative by the TRACK test, and both of these were negative by all other serological tests. There would have been a loss of specificity with TRACK if the

TABLE 4. Comparison of serum samples by six serological tests

Group (*n*)	% Positive					
	TRACK	FIAX	ELISA	CF	RIV	CARD
Controls (134)	0.0	0.7	11.2	6.0	0.0	0.0
Vaccinated with S-19 (43)	62.8	55.8	67.4	44.2	58.1	58.1
Challenged with S-2308 (121)	76.9	59.5	62.8	49.6	49.6	53.7
Culture positive (76)	90.8	77.6	76.3	72.4	73.7	77.6
Culture negative (45)	53.3	28.9	40.0	11.1	8.9	13.3
Abortion positive (56)	96.4	89.3	82.1	92.9	91.1	91.1
Abortion negative (55)	54.5	27.3	40.0	9.1	10.9	14.5
Abortion indeterminate (10)	90.0	70.0	80.0	30.0	30.0	60.0

TABLE 5. Sensitivity and specificity of six serological tests[a]

Test	Sensitivity[b] (%)	Specificity[c] (%)
TRACK	96.3	100.0
FIAX	88.9	99.3
ELISA	81.5	88.8
CF	92.6	94.0
RIV	90.7	100.0
CARD	90.7	100.0

[a] Of the 188 serum samples (1 sample per animal), 54 were from cattle that were challenged and culture positive and had aborted, and 134 were controls from heifers.

[b] Sensitivity = [(number of true-positives)/(number of true-positives and false-negatives)] × 100%.

[c] Specificity = [(number of true-negatives)/(number of true-negatives and false-positives)] × 100%.

seropositive cattle that were culture negative (53.3%) and abortion negative (54.5%) (Table 4) were considered disease negative. The specificity of TRACK was excellent for the 134 control samples that were neither challenged nor vaccinated; thus, the hypothesized loss of specificity may well be explained by the ability of TRACK to detect small amounts of *Brucella*-specific antibody. TRACK probably was detecting residual titers from the initial challenge or possibly titers resulting from levels of infection below the threshold of detection by standard serological or culture techniques.

TRACK is a rapid, simple, and relatively economical system that is available commercially for use in the detection and quantitation of serum antibodies to several diseases. Although the test for brucellosis is not currently available commercially, tracks without antigens can be supplied by the manufacturer, and the system can be adapted to other *B. abortus* antigen preparations and conjugates. The results indicate that TRACK is both reproducible and accurate. The results compare favorably with other serological methods. TRACK is faster than either the ELISA or FIAX system. TRACK is simple because no dilution of serum is needed. TRACK appears to be more sensitive than the FIAX, ELISA, CF, CARD, and RIV tests and more specific than the ELISA and CF tests.

ACKNOWLEDGMENTS

We thank Chad Faulkner and Sharon Oltjen for technical assistance, Rick Palmer and Daryl Laboratories for providing supplies and equipment, and the State-Federal Brucellosis Laboratory, Oklahoma City, for assistance.

This research was supported in part by cooperative research agreement no. 58-519B-2-1190 from the College of Veterinary Medicine, The Robert S. Kerr Foundation, and the Agricultural Research Service of the U.S. Department of Agriculture.

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