A rapid serologic test for the detection of antibodies to *Mycobacterium avium* subsp. *paratuberculosis* with applications for bovine practitioners

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**Keywords:** *Mycobacterium avium* subsp. *paratuberculosis*, antibodies, SNAP™ device, veterinarian, dairy cattle.

**SUMMARY**

An ELISA test has been developed on the IDEXX SNAP™ device to detect antibodies to *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), the causative organism of Johne’s disease in ruminants. Initial validation studies have been completed utilizing bovine serum as the specimen type. This SNAP test format has potential applications as an in-clinic assay for large animal veterinarians who require a rapid test result (22 minutes) for symptomatic or suspicious animals. The purpose of the study in this report was to evaluate the performance of this new *Map* ELISA by testing populations of dairy cattle (*n* = 1276). Data collected utilizing the SNAP test device were compared to data obtained in parallel testing with an established microtiter-plate ELISA kit. Observed proportional agreement between tests for all sera tested was 96.6 % (kappa value = 0.75). The microtiter plate test detected a slightly higher proportion of positive sera (15.6 %) from herds with a positive test history for paratuberculosis versus the number of sera detected as antibody-positive by the SNAP test (11.7 %) from these same herds. Specificity performance of the two tests in assays of sera from herds presumed to be paratuberculosis-negative was near equivalent (> 99 %). These data suggest that the SNAP test format may be a useful tool for veterinarians in obtaining a preliminary paratuberculosis antibody disposition on high-risk cattle (symptomatic, previously untested or potentially exposed cows).

**INTRODUCTION**

Johne’s disease is a bacterial infection that occurs in ruminants as a result of environmental or in-utero exposure to *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). It is a progressive, lifelong disease characterized by weight loss, diarrhea and granulomatous gastrointestinal and lymphatic lesions in severely-affected individuals (4,11). In dairy cattle, reduced milk production has been associated with both clinical and subclinical presentations of the disease (7,9,12).

Diagnosis of paratuberculosis is complicated by the slow progression of the infection, the intermittent occurrence of bacterial shedding, a protracted incubation period required for in-vitro culture, and a delayed onset of host humoral antibody response. For this reason, test and control programs must rely upon a comprehensive approach to diagnostic method and animal management strategies (2). The use of a serologic test by veterinarians has a place in providing timely information to producers in assessing high-risk animals and making any needed changes in managing these animals. In this report, we compare the performance of a test designed for practitioners to a laboratory antibody-ELISA in order to evaluate the relative sensitivity and specificity of this alternative antibody detection test platform.

**MATERIALS AND METHODS**

Bovine serum specimens were collected from eleven different dairy herds and two sire service facilities of varying geographic origin. Herd status was investigated with regard to previous diagnostic test histories (serology and culture); differential observations of herd veterinarians (evidence of symptomatic animals); and with regard to the introduction of replacement cattle into the herds (open vs. closed herd).
Sera were tested with the prototype test system (Figures 1 & 2) and with a USDA-licensed microtiter plate antibody test kit. Both of these tests are indirect-ELISA test formats that incorporate a pre-adsorption treatment of test specimens with Mycobacterium phlei extracts (14). Quantitative data were recorded for the SNAP test platform by taking densitometric readings of the diagnostic spot at the completion of the test protocol. These values were compared to the S/P ratios yielded by the microtiter-plate technique. Regression analysis of these data was performed.

SNAP test results recorded by visual observation were compared to plate test S/P result interpretations. Blue color detected in the Map antigen spot in the SNAP device indicates a positive reaction. The absence of color in the antigen spot is interpreted as a negative result. Trace blue color in the antigen spot that might not be detected by all users was scored as an equivocal reaction in this study. Equivocal results were considered discordant with both positive and negative plate kit results for the purposes of comparison. Plate test S/P values are derived by a calculation which compares plate test optical densities measured for unknown specimens to optical densities measured for positive and negative controls provided in the test kit. S/P values equal to or greater than 0.25 are considered as positive. Statistical test agreement analysis of specimen dispositions determined by these two methods was performed (10).

RESULTS

Investigation of the presumptive disease status with regard to evidence of Map exposure for each of the herds studied resulted in the following herd classifications: known infected herds - six (6) dairy herds with a history of repeated positive culture and serology results, two (2) of the six (6) herds presented a low percentage of symptomatic animals (n = 643); presumed negative herds – three (3) dairy herds and two (2) sire service facilities with a history of repeated negative culture and serology results (n = 538); herds of unknown status – two (2) dairy herds with no previous test history for Map and no apparent clinical disease (n = 95).

Figure 1. The Map SNAP ELISA test can be performed without customary laboratory equipment. Total assay time required is 22 minutes.

Figure 2. Completed Map SNAP tests. Diagnostic antigen spot (top position); positive control spot (bottom position). Results are interpreted as positive: (left and center devices) – and negative: (device at right).

Figure 3. Bovine Population Study (n = 1276): Agreement in specimen dispositions between the two Map antibody detection methods utilized.

Figure 4. Regression analysis comparing SNAP test densitometry values to plate kit S/P values recorded for bovine population specimens.

\[ \text{S/P} = 0.25 + 0.75 \times \text{SNAP test optical density} \]

\[ \chi^2 = 6.73, p = 0.009 \]

\[ \text{Cohen's kappa} = 0.75 \]

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\* Densitometer, Model RCP, Tobias Associates, Inc., Ivyland, Pa. 18974 USA
The observed proportional agreement in test results between the two assay methods evaluated for each herd classification were as follows: known infected herds – 94.4%; presumed negative herds – 99.1%; herds of unknown status – 96.8% (Table 1). The overall observed proportional agreement in test results for this population was 96.6% (1232 of 1276; \( \kappa = 0.75 \)) (Figure 3). Regression analysis of quantitative data shows a significant correlation between the two techniques utilized; \( (R^2 = 0.73, p < 0.0001, 95\% \text{ CI}) \) (Figure 4).

Table 1. Summary of \( Map \) serology comparison between two ELISA methods.

<table>
<thead>
<tr>
<th>Herd Group</th>
<th>Total n</th>
<th>No. of herds</th>
<th>% positive - SNAP</th>
<th>% positive - microtiter plate</th>
<th>Obs. Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known infected</td>
<td>643</td>
<td>6</td>
<td>11.66</td>
<td>15.55</td>
<td>94.40%</td>
</tr>
<tr>
<td>Presumed negative</td>
<td>538</td>
<td>5</td>
<td>0.19</td>
<td>0.93</td>
<td>99.07%</td>
</tr>
<tr>
<td>Unknown status</td>
<td>95</td>
<td>2</td>
<td>1.05</td>
<td>2.10</td>
<td>96.84%</td>
</tr>
<tr>
<td>All sera</td>
<td>1276</td>
<td>13</td>
<td>6.03</td>
<td>8.39</td>
<td>96.55%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The use of the microtiter-plate ELISA technique for detecting antibodies to \( Map \) has been a customary laboratory method for herd screening for many years (1,8,13). The USDA-licensed commercial microtiter-plate test kit utilized for assay performance comparisons in this study has been widely used and evaluated (3,5,6). The development of an ELISA for \( Map \) antibody detection using the SNAP test platform incorporates assay parameters that differ considerably in comparison to microtiter-plate tests. Evaluation of this new SNAP test versus an established method is therefore warranted.

Good to excellent agreement in assay results (kappa = 0.75) was observed between these two test formats for the populations examined. For the presumed negative herd group, 532 of the 533 plate-test negative sera were interpreted as negative by the SNAP test (relative specificity 99.8%). The greatest differences in test results were observed for certain plate-test positive sera from the known infected herd group: the SNAP test detected 72 positive sera and 11 equivocal sera among the 100 specimens interpreted as positive by the plate-test kit (relative sensitivity 72%). This difference was not unexpected given the shorter incubation steps employed in the SNAP test. The high relative specificity performance observed for the SNAP test is viewed as essential in assuring the validity of positive test results however.

The SNAP ELISA format provides the large animal clinician with a test for \( Map \) antibody detection that can be performed without extensive laboratory facilities. This test may be a practical tool for obtaining a preliminary antibody disposition on high-risk individuals, (symptomatic, previously untested or potentially exposed cows). A positive test result may add value by indicating to the veterinarian and herd owner the need for immediate preventive management measures, and may help to justify the need for further diagnostic investigation. The SNAP test will be best applied as a part of a more comprehensive herd-level serology and organism detection program.

**CONCLUSIONS**

The relative sensitivity and specificity of a new ELISA test was measured against an established technique. These data provide evidence of a significant correlation in performance between the new SNAP test and the microtiter-plate format. Further, the prototype SNAP test yields specimen dispositions which are consistent with the known source-herd histories for the bovine populations studied.

**ACKNOWLEDGEMENTS**

Thanks to Lori Plourde, John Santoro, J.P. Benoit, Kevin Fahrman and Joni McNutt for assistance in the preparation of this manuscript. We are especially indebted to the many herd owners and veterinarians who kindly provided specimens from the herds under their care and willingly offered information regarding the health histories of these animals.
REFERENCES


