

AntelBio

Johne's Milk ELISA

Highlights

- AntelBio Milk ELISA detects the antibody response to *M. paratuberculosis* in fresh, frozen or DHIA preserved milk samples.
- Test sensitivity is 51% and specificity is 99%.
- The use of preservative (via the DHIA collection process) does not reduce the utility of AntelBio's Milk ELISA.
- Preserved milk samples were unaffected by the storage times and temperatures tested in internal experiments. (*Tests were conducted at refrigeration temperature, room temperature and at an elevated temperature for two weeks.*)
- Test accepted in the management levels of the Michigan Voluntary Johne's Disease Program.

Introduction

The AntelBio Milk ELISA is a highly effective tool for producers and veterinarians to determine Johne's prevalence in their dairy cattle operations. This assay can be conducted on fresh or DHIA preserved milk samples and is directly comparable to a standard serum ELISA in sensitivity and specificity. In addition, the AntelBio Milk ELISA is less costly than other Johne's diagnostic tests, as sample collection can be incorporated into routine DHIA sampling, providing for Johne's monitoring with little or no additional labor or expense. Results of the Milk ELISA are reported five business days after samples are received in the testing center, allowing for immediate management decisions.

Test Description

AntelBio's Milk ELISA is designed to detect an antibody response to *M. paratuberculosis* in fresh, frozen or DHIA preserved milk samples.

Assay Validity

In addition to comparisons to the "gold standard" fecal culture, the Milk ELISA was studied for its effectiveness in DHIA samples. The following studies were conducted to determine the performance of the AntelBio Johne's Milk ELISA.

AntelBio Milk ELISA sensitivity and specificity

Sensitivity is defined as the ability of a test to correctly identify diseased animals as test positive. To determine the sensitivity of the AntelBio Johne's Milk ELISA, milk samples were collected from a total of 124 animals with Johne's disease as determined by a positive fecal culture. Table 1 shows the Milk ELISA correctly identified 63 or 51% of the animals with Johne's disease. The performance of a commercial serum ELISA in the same animal population is included for comparison.

Table 1. Sensitivity and specificity of the AntelBio Johne's Milk ELISA

	Johne's Status	
	Positive (n=124)	Negative (n=687)
AntelBio Johne's Milk ELISA	63	684
Commercial Serum ELISA	68	686

Milk ELISA sensitivity: $63/124=51\%$
Milk ELISA specificity: $684/687=99\%$

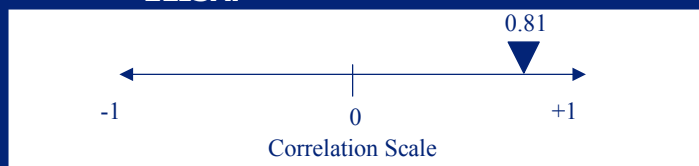
Specificity is defined as the ability of a test to correctly identify non-diseased animals as test negative. To determine the specificity of the AntelBio Johne's Milk ELISA, milk samples were collected from a total of 687 animals in herds with no previous evidence of Johne's disease. Table 1 shows the Milk ELISA correctly identified 684 or 99% of the animals from herds of negative Johne's status. The performance of a commercial serum ELISA in the same animal population is included for comparison.

Correlation is defined as the degree of similarity between two random variables. To determine the correlation between the AntelBio Johne's Milk ELISA and a commercial serum ELISA, milk and serum samples were simultaneously drawn from 2,098

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animals. Figure 1 shows the correlation between the two assays expressed on the correlation scale of -1 (complete negative correlation) to 0 (no correlation) to +1 (complete positive correlation).

Figure 1. Correlation between AntelBio Johne's Milk ELISA and a commercial serum ELISA.



Significant correlation indicates that as the level of Johne's antibody in serum increases, the level of Johne's antibody in milk increases.

Detection of varying prevalence levels

Whole-herd analysis of Johne's disease and prevalence was conducted separately from milk and serum ELISA data and compared. Table 2 summarizes the apparent prevalence of Johne's disease as a percentage in individual herds.

Table 2. Johne's prevalence as a percentage in individual herds

Farm	Animals	Prevalence (%)	
		Serum	Milk
1	77	7.8	6.5
2	179	1.7	0.6
3	90	0	0
4	127	6.3	6.3
5	28	3.6	3.6
6	73	8.1	5.4
7	87	0	0
8	157	1.3	1.3
9	182	0	1.1
10	48	0	0
11	217	0.4	0
12	137	1.5	2.9
13	64	0	0
14	249	4.8	3.2
15	55	10.9	16.4
Total	1770	3.1	3.2

The AntelBio Milk ELISA and the serum ELISA each detected Johne's disease in 10 of the 15 herds analyzed at equal prevalence levels.

Each assay found evidence of Johne's in 10 of 15 herds with an average level of prevalence of 3.1%. A paired t-test statistic did **not** detect a difference in prevalence estimates between the serum and milk analyses ($P > 0.9$). Given the fact that currently available Johne's serological tests are only approved as whole-herd tests, AntelBio's Milk ELISA represents an economical and labor-efficient tool to evaluate and monitor Johne's status in dairy herds.

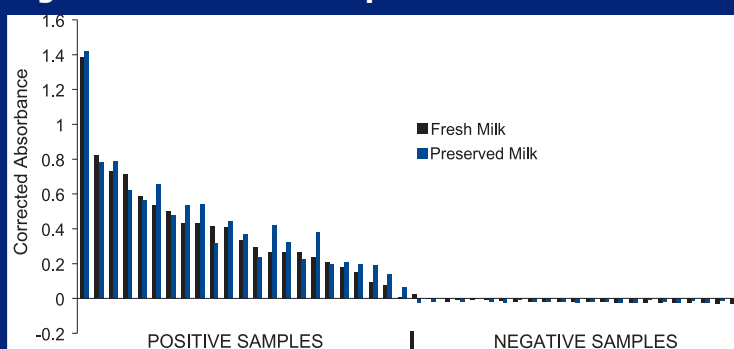
Use of DHIA preserved milk samples

The DHIA system collects and analyzes individual milk samples on a whole-herd (milking) basis at regular intervals. The data below addresses several issues regarding DHIA practices that

AntelBio's Milk ELISA is an exceptional tool in determining the presence of these antibodies for the diagnosis of herd level prevalence of Johne's disease.

could potentially interfere with the interpretation of data from Johne's testing on DHIA milk samples. A sample bank was constructed of 26 positive milk samples and 26 negative milk samples which were divided into replicates for repeated experimentation. The positive samples were chosen to represent the normal range of ELISA values (0.1 – 2.0) experienced in whole-herd assays.

Figure 2. Effect of milk preservative

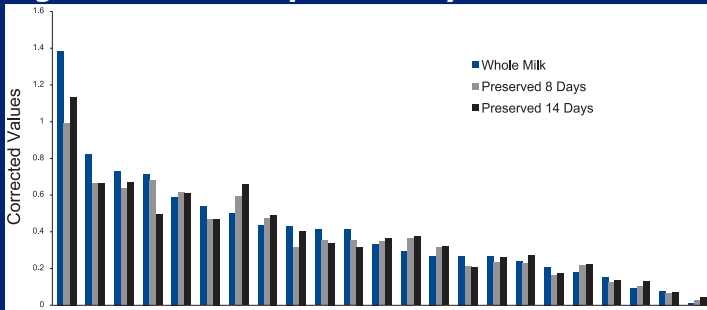


The preservative does not reduce the utility of the AntelBio Milk ELISA in milk samples.

DHIA samples are often preserved with bronopol and natamycin (D & F Control Systems, Inc., Broad Spectrum Microtabs). Figure 2 shows the effect of Broad Spectrum Microtabs on AntelBio's Milk ELISA when the preservative is used according to manufacturers instructions.

Paired t-tests between preserved and fresh (unpreserved) milk samples found that while the preservative has no effect ($P > 0.41$) on corrected values in negative milk samples, it potentially enhances the corrected values ($P < .05$) in positive milk samples. In conclusion, the preservative does not reduce the utility of the preserved milk samples in AntelBio's Milk ELISA.

Figure 3. Milk sample stability at 99°F



Milk samples preserved with bronopol and natamycin were unaffected by the storage times and temperatures tested in these experiments.

Stability of DHIA collected milk samples

Preserved milk samples were tested at refrigeration temperature (4°C/39°F), room temperature (25°C/77°F) and at an elevated temperature (37°C/99°F) for two weeks. Figure 3 shows the stability of 23 positive preserved milk samples at elevated temperatures, the most extreme storage conditions studied.

Paired t-tests did not detect ($P > 0.1$) a difference between fresh milk and preserved milk that had been stored for eight or 14 days at 99°F. Although not shown, no significant differences were found for the remaining storage times and temperatures tested in these experiments. Likewise, negative milk samples preserved with bronopol and natamycin were unaffected by the storage times and temperatures tested.

Effects of carry-over contamination

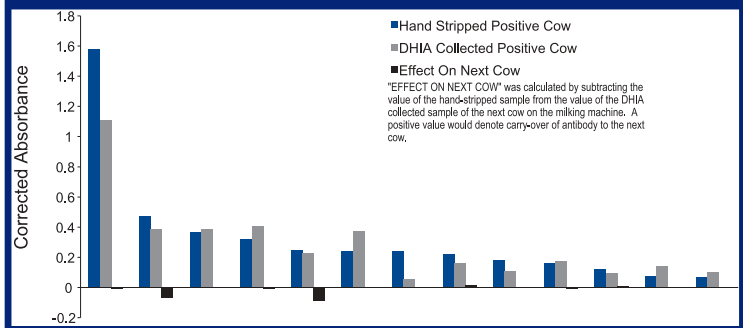
Two areas regarding carry-over contamination of milk samples during the DHIA collection and analysis process were studied. The first area of concern occurs during the collection of milk via

the beaker used to collect a representative sample of the milk and therefore, to some extent contaminates the following sample. The second area of concern occurs during analysis; as sample stirrers and collection needles are not rinsed between samples and thus present another potential route of contamination. Although this contamination is not likely to exceed 1%, several comparisons have been made to address these issues.

AntelBio's Milk ELISA represents an economical and labor efficient tool to evaluate and monitor Johne's status in dairy herds.

the metering machines. Milk from the previous cow on the milking machine remains in the milk line and on the sides of

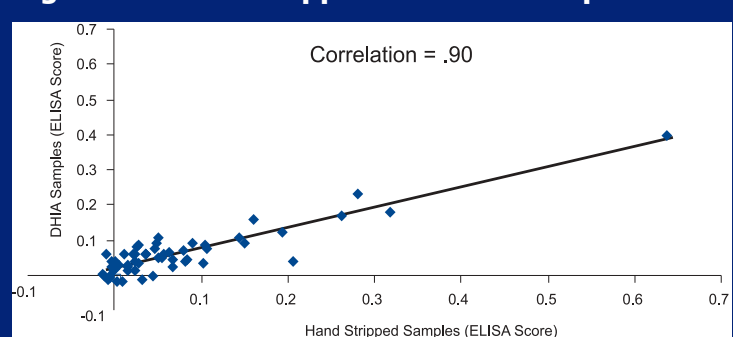
Figure 4. Carry-over from milk meter



Metered milk did not contain higher levels of Johne's antibody than hand-stripped milk from cows following positive cows on the same metering machine.

Figure 4 shows the effect of milk from positive cows on the difference in ELISA values between hand-stripped milk and metered milk from the cow immediately following the positive cow on the same metering machine. Metered milk did not ($P > 0.21$) contain higher levels of the Johne's antibody than hand-stripped milk in cows following positive cows on the same metering machine. The lack of carry-over is particularly evident in the milk samples collected from the animal immediately following the cow with the highest level of milk antibodies (1.6).

Figure 5. Hand-stripped vs. DHIA samples



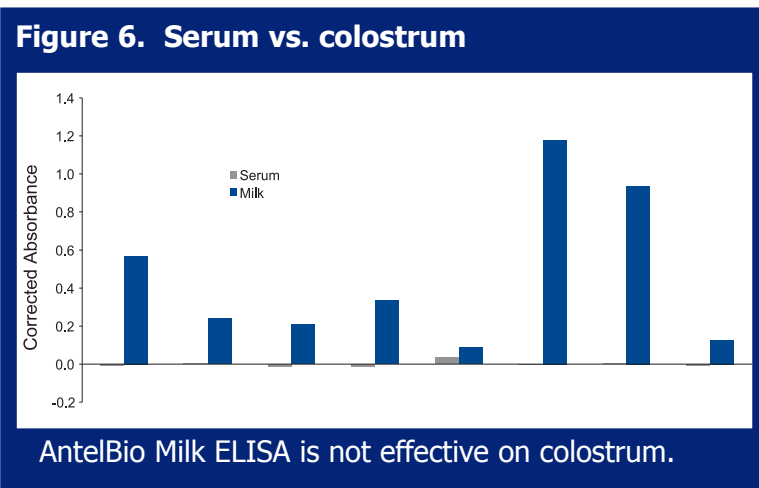
The AntelBio Milk ELISA is negligibly affected by normal carry-over that occurs in the DHIA system.

Figure 5 shows the effect of the entire DHIA collection, transportation and analysis system on the performance of

AntelBio's Milk ELISA for an entire herd. Milk ELISA values were compared between hand-stripped milk samples and DHIA samples after SCC, protein, butterfat and MUN determinations. The correlation between the two sets of data was 0.90. A paired t-test did not detect ($P > 0.38$) a difference in milk ELISA values between hand-stripped milk and milk from the DHIA collection system. The AntelBio Milk ELISA is negligibly affected by normal carry over that occurs in the current DHIA system.

Efficacy of test on colostrum

ELISA assays designed to measure specific antibodies, such as Johne's antibodies, generally suffer from interference due to the presence of large amounts of related antibodies naturally present in serum. It would be expected that colostrum, a



concentrated source of antibodies for passive immunity, would also lead to interference in AntelBio's Milk ELISA. Figure 6 shows serum and milk comparisons in eight cows within three days of freshening. Clearly, the use of colostrum in AntelBio's Milk ELISA must be avoided. Since cows are normally reintroduced into the milking herd after this time frame, these cows would not be sampled during DHIA testing and therefore, colostrum does not present a problem during whole-herd tests on DHIA samples.

Conclusions

AntelBio's Milk ELISA is a cost-effective and labor efficient tool to screen and detect Johne's disease in dairy operations. Screening results can be used by producers and their veterinarians to develop additional testing and management strategies for the control of Johne's disease.

The AntelBio Milk ELISA is effective on fresh, frozen or DHIA preserved samples with a sensitivity of 51% and a specificity of 99%. Various studies on DHIA preserved samples show that the preservative used in DHIA samples has little or no effect on AntelBio's Milk ELISA to detect the presence of *M. paratuberculosis*. Additionally, there is little effect of carry-over contamination using the DHIA system and samples can be stored at varying temperatures for up to two weeks without any interference in the detection of Johne's disease.

AntelBio...

Complete Johne's Testing Center

AntelBio is a full service Johne's disease testing center offering the AntelBio Milk ELISA and AntelBio Rapid Fecal Test, as well as traditional fecal culture and serum ELISA. AntelBio is a USDA accredited Johne's disease testing center. In an effort to make Johne's disease testing quick and accurate, AntelBio has pioneered on-farm sample collection kits that provide high quality, consistent samples that maximize the reliability of test results. To help ensure the integrity of results, AntelBio is incorporating a quality system, based upon ISO and FDA guidelines.



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