

# IDEXX Milk Pregnancy Test— a New Tool for Pregnancy Diagnosis in Cattle

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## Introduction

Accurate and timely detection of pregnancy in dairy cows is an essential component of any bovine reproductive management program. Veterinarians and farmers use detection of nonpregnant (open) cows to make decisions about rebreeding cattle and to manage calving intervals to maximize milk production and revenue for the farm. Increased profits come from decreased average days in milk for the lactating herd, greater milk yield, less culling of good cows because of reproductive failure, and greater consistency in the length of lactation and dry periods.

Currently, pregnancy is determined by rectal palpation, transrectal ultrasound, or laboratory-based blood tests. IDEXX has recently developed an enzyme-linked immunosorbent assay (ELISA) for the detection of pregnancy-associated glycoproteins (PAGs) in milk samples. The new test provides an additional laboratory-based method for the accurate detection of bovine pregnancy. The IDEXX Milk Pregnancy Test can be an important tool for the confirmation of pregnancy status in the dairy herd.

## Materials/Methods

The IDEXX Milk Pregnancy Test, for use with bovine milk samples, detects the presence of PAGs in bovine milk as a marker for pregnancy. The assay is performed in a microtiter plate coated with an anti-PAG antibody. After the test sample is incubated in the coated wells, captured PAG is detected with a PAG-specific antibody (detector solution) and horseradish peroxidase (HRPO) conjugate. Unbound conjugate is washed away, and TMB substrate is added to the wells. Color development is proportional to the amount of PAG in the sample.

This study was conducted to evaluate the sensitivity and specificity of the IDEXX Milk Pregnancy Test in dairy cows. Milk samples were obtained from cows on farms located throughout the United States, the Netherlands, and the United Kingdom. In total, 1,315 milk samples were tested, including samples from inseminated cows at 35 or more days of gestation and samples from open cows that were at least 60 days postcalving and had not been bred. Postcalving PAG decline was measured in samples taken from 327 cows at 1–60 days postcalving. Additionally, 12 cows from a herd in Beijing, China, were sampled on the day of insemination and at regular intervals throughout their gestation period to observe the temporal variation of PAG levels throughout pregnancy.

The pregnancy status of bred cows was confirmed by transrectal ultrasound performed at least 28 days after insemination or by palpation performed after 50 days of gestation. Whenever possible, the confirmation of pregnancy was performed at or near the time of milk sample collection. All samples were tested on the IDEXX Milk Pregnancy Test following instructions in the package insert. Blood samples were also obtained from 216 animals, and these were tested for PAGs using the IDEXX Bovine Pregnancy Test (a serum-based test) following instructions in the package insert.

## Results

### Diagnostic Sensitivity/Specificity

The overall sensitivity and specificity results from 1,315 field samples demonstrate that when samples are taken at least 35 days after breeding or from cows that have not been bred and are at least 60 days postcalving the sensitivity of the IDEXX test is 98.8% and the specificity is 97.4% (figure 1). Further analysis of these results showed that 35–55 days after breeding, the sensitivity of the IDEXX Milk Pregnancy Test is 97.5% and the specificity is 92.2%. When samples are taken 56–75 days after breeding, the sensitivity is 97.8% and the specificity is 100%. After 75 days, the sensitivity is 99.4% and the specificity is 95.5% (figure 2).

**Figure 1: Overall Diagnostic Sensitivity and Specificity of the IDEXX Milk Pregnancy Test**

		Ultrasound/Palpation	
		Pregnant	Open
IDEXX Milk Pregnancy Test	Pregnant	872	10
	Recheck	40	9
	Open	11	373

Sensitivity : 98.8% (95% CI, 97.7%–99.3%)\*

Specificity: 97.4% (95% CI, 95.2%–98.6%)\*

Rechecks: 4% of total tested (3% pregnant and 1% open cows)\*

\*Excluding milk test recheck results

**Figure 2: Diagnostic Sensitivity and Specificity by Gestation Periods**

Pregnant Cows		IDEXX Test Results				Performance	
		Total	Preg	Recheck	Open	Sensitivity*	Recheck
Days After Breeding	35–55	177	157	16	4	97.5%	9.0%
	56–75	204	174	26	4	97.8%	13.0%
	>75	542	538	1	3	99.4%	0.2%

\*Excluding milk test recheck results

### Agreement between IDEXX Milk- and Serum-Based Pregnancy Tests

A portion of the field population was also tested for pregnancy on the IDEXX Bovine Pregnancy Test (a serum-based test), using a serum sample obtained on the same day as the milk sample. A total of 216 samples (47 confirmed open and 169 pregnant) were tested by both methods. The overall agreement between the tests was 98.4% (figure 3). The 47 confirmed open cows were considered open by both the serum and milk test methods. A total of 148 samples from confirmed pregnant cows were considered pregnant by both the serum and milk tests. Eighteen samples from the confirmed pregnant population were considered pregnant on the serum test and “recheck” on the milk assay; these were not included in the determination of agreement between methods. Three samples from the confirmed pregnant population were classified as open by the milk test and pregnant by the serum test. Each of these samples yielded a weak positive on the serum test.

**Figure 3: Agreement between Milk- and Serum-Based Tests**

		IDEXX Serum-Based Test	
		Pregnant	Open
IDEXX Milk-Based Test	Pregnant	148	0
	Recheck	18	0
	Open	3	47

Overall Agreement = 98.4%\*

\*Excluding milk test recheck results

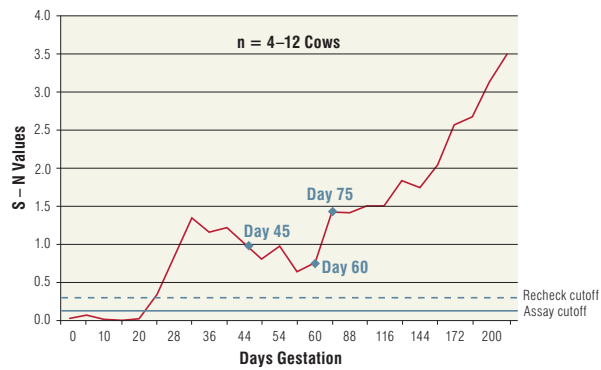
Open Cows		IDEXX Test Results				Performance	
		Total	Preg	Recheck	Open	Specificity*	Recheck
Days After Breeding	35–55	80	6	3	71	92.2%	4.0%
	56–75	25	0	1	24	100.0%	4.0%
	>75	23	1	1	21	95.5%	4.0%

\*Excluding milk test recheck results

### Temporal Detection of PAGs throughout Gestation

Twelve cows from a dairy herd in Beijing, China, were sampled on the day of insemination and at regular intervals throughout gestation in order to observe variation in PAG levels in milk. Milk samples were taken at intervals of 2–5 days for the first 60 days of pregnancy and at 14-day intervals thereafter, with 4–12 cows sampled at each time point. Each of the cows was confirmed to be pregnant by ultrasound at or around 29 days of pregnancy. Figure 4 shows the average S – N value for all the cows tested at each time point. PAG levels were well below the assay cutoff, until approximately day 20 of gestation, when they rose rapidly. By day 28 of pregnancy, each of the cows was considered pregnant by the IDEXX Milk Pregnancy Test. Around day 45, the PAG levels began to decline slightly until approximately 60 days postbreeding, when they began to rise again. This increase in PAGs continued throughout the remainder of the pregnancy, reaching its highest levels just before calving.

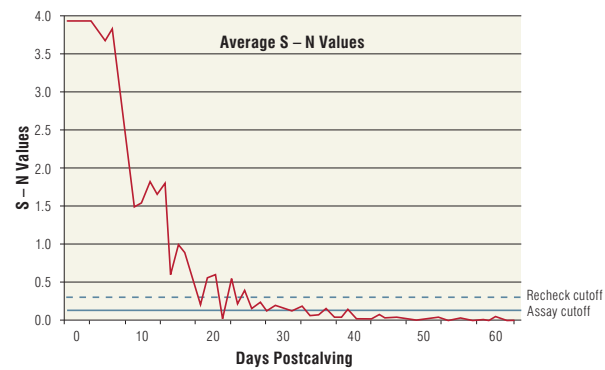
Figure 4: PAG Levels in Milk throughout Gestation



### Postcalving Decline of PAGs in Milk

To determine the average rate at which PAG levels in milk decline after the birth of a calf, milk samples were obtained from cows on farms located throughout the United States, the Netherlands, and the United Kingdom. Samples were collected from a total of 327 cows that were 1–60 days in milk. Figure 5 shows the average S – N values of all samples tested by days postcalving. By 30 days postcalving, the PAG levels in milk were typically below the “recheck” cutoff for the assay, and after day 60, the specificity of the IDEXX Milk Pregnancy Test was 100%. This indicates that PAGs from a previous pregnancy will not interfere with later testing.

Figure 5: Postcalving PAG Decline



### Significance

This evaluation of the IDEXX Milk Pregnancy Test indicates that a milk-based pregnancy test used as part of routine herd monitoring could provide important additional information to customers and help them maximize the use of milk samples as a herd-management resource. The test could be a useful addition to existing reproductive management programs. As with any diagnostic test, the IDEXX Milk Pregnancy Test should be used under the guidance of a veterinarian as part of the farm's overall health and reproductive management program.