

IDEXX Milk Pregnancy Test

Validation Data Report

Glossary

Repeatability

Sensitivity (Diagnostic)

Specificity (Diagnostic)

Overall Performance—Bovine Milk Samples

Overall Performance—Caprine Milk Samples

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The IDEXX Milk Pregnancy Test, for use in bovine and caprine milk samples, is an enzyme-linked immunosorbent assay designed to detect the presence of pregnancy-associated glycoproteins (PAGs) in bovine and caprine milk as a marker for pregnancy. A microtiter plate format is configured by coating an anti-PAG antibody onto the plate. After incubation of the test sample in the coated well, captured PAG is detected with a PAG-specific antibody (detector solution) and a 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 and is measured using a spectrophotometer.

Results are calculated from the optical density (OD) of the sample minus the OD of the negative control at 450 nm (with both values corrected by subtraction of the reference wavelength OD), which results in an S-N value. For both bovine and caprine samples, if the S-N value is less than 0.100, the animal is considered not pregnant (open). If the S-N value is equal to or greater than 0.250, the animal is considered pregnant. If the S-N value is equal to or greater than 0.100, but less than 0.250, the animal should be rechecked to confirm pregnancy status. For bovine samples only, cows more than 45 days postbreeding may be considered pregnant if the S-N is greater than or equal to 0.10.

I. Glossary of Terms

The following definitions have been adapted from the Glossary of Terms section of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (World Organization for Animal Health, 2012) and may be used to describe the assay's performance characteristics in this validation report.

Repeatability—Level of agreement between replicates of a sample both within and between runs of the same test method in a given laboratory.

Sensitivity (diagnostic)—Proportion of known pregnant animals that test pregnant in the assay; pregnant animals that test nonpregnant (open) in the assay are considered to have false-negative results.

Specificity (diagnostic)—Proportion of known non-pregnant (open) animals that test open in the assay; nonpregnant animals that test pregnant in the assay are considered to have false-positive results.

II. Repeatability

A. Intraplate Variability

Purpose: To assess the intraplate variability of the IDEXX Milk Pregnancy Test.

Procedure: One sample was tested across three 96-well plates according to the standard assay protocol.

Results/

Conclusions: Microtiter plate OD values and coefficient of variation (%CV) are shown in figure 1 below. The %CVs for the three plates were 4.60%, 3.75% and 5.28%, respectively. These values indicate good intraplate repeatability for the IDEXX Milk Pregnancy Test.

Figure 1. %CV

Wavelength A(450) - A(650)													Rows			
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	S.D.	CV	
A	0.646	0.688	0.641	0.624	0.590	0.620	0.621	0.603	0.599	0.613	0.645	0.657	0.629	0.028	4.41%	
B	0.672	0.683	0.637	0.622	0.603	0.614	0.607	0.607	0.606	0.623	0.640	0.662	0.631	0.028	4.39%	
C	0.653	0.648	0.729	0.581	0.616	0.575	0.607	0.611	0.606	0.620	0.657	0.692	0.633	0.045	7.12%	
D	0.680	0.641	0.642	0.612	0.627	0.576	0.597	0.590	0.612	0.593	0.637	0.671	0.623	0.032	5.20%	
E	0.647	0.641	0.619	0.625	0.632	0.590	0.595	0.602	0.618	0.630	0.647	0.679	0.627	0.025	3.99%	
F	0.670	0.643	0.621	0.609	0.634	0.578	0.604	0.592	0.640	0.649	0.600	0.662	0.625	0.029	4.63%	
G	0.691	0.641	0.626	0.609	0.626	0.611	0.603	0.589	0.613	0.624	0.616	0.637	0.624	0.026	4.10%	
H	0.622	0.633	0.651	0.642	0.641	0.639	0.612	0.623	0.612	0.632	0.640	0.676	0.635	0.018	2.79%	
Columns																
Mean	0.660	0.652	0.646	0.616	0.621	0.600	0.606	0.602	0.613	0.623	0.635	0.667	Overall	0.628	0.029	4.60%
S.D.	0.022	0.021	0.035	0.018	0.017	0.024	0.008	0.012	0.012	0.016	0.018	0.017				
CV	3.37%	3.21%	5.49%	2.88%	2.76%	3.99%	1.36%	1.95%	2.00%	2.59%	2.90%	2.48%				

Wavelength A(450) - A(650)													Rows			
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	S.D.	CV	
A	0.649	0.662	0.629	0.636	0.623	0.626	0.601	0.618	0.619	0.636	0.619	0.646	0.630	0.017	2.62%	
B	0.629	0.652	0.641	0.632	0.603	0.607	0.580	0.608	0.569	0.615	0.604	0.635	0.615	0.025	4.00%	
C	0.627	0.631	0.613	0.608	0.602	0.578	0.619	0.604	0.603	0.588	0.609	0.624	0.609	0.016	2.55%	
D	0.631	0.635	0.615	0.613	0.609	0.587	0.598	0.609	0.611	0.621	0.599	0.643	0.614	0.016	2.64%	
E	0.616	0.633	0.611	0.601	0.608	0.583	0.583	0.597	0.607	0.620	0.607	0.602	0.606	0.014	2.35%	
F	0.636	0.640	0.612	0.588	0.591	0.577	0.568	0.580	0.582	0.593	0.595	0.655	0.601	0.028	4.65%	
G	0.627	0.611	0.607	0.577	0.580	0.569	0.562	0.572	0.582	0.576	0.594	0.612	0.589	0.021	3.50%	
H	0.617	0.617	0.590	0.594	0.584	0.593	0.567	0.597	0.579	0.583	0.573	0.582	0.580	0.015	2.62%	
Columns																
Mean	0.629	0.635	0.615	0.608	0.600	0.590	0.585	0.598	0.594	0.604	0.600	0.625	Overall	0.607	0.023	3.75%
S.D.	0.011	0.017	0.015	0.021	0.014	0.019	0.020	0.015	0.018	0.022	0.014	0.025				
CV	1.67%	2.64%	2.45%	3.39%	2.38%	3.14%	3.39%	2.57%	3.05%	3.59%	2.27%	3.97%				

Wavelength A(450) - A(650)													Rows			
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	S.D.	CV	
A	0.679	0.710	0.634	0.655	0.647	0.642	0.613	0.624	0.622	0.649	0.487	0.623	0.632	0.053	8.41%	
B	0.690	0.691	0.667	0.656	0.652	0.643	0.612	0.606	0.620	0.631	0.628	0.644	0.645	0.028	4.31%	
C	0.675	0.695	0.702	0.638	0.670	0.624	0.645	0.612	0.619	0.624	0.576	0.591	0.639	0.040	6.19%	
D	0.693	0.667	0.669	0.620	0.652	0.627	0.623	0.627	0.614	0.634	0.593	0.637	0.638	0.028	4.34%	
E	0.706	0.673	0.656	0.627	0.624	0.628	0.616	0.639	0.625	0.646	0.590	0.620	0.638	0.030	4.71%	
F	0.687	0.679	0.650	0.612	0.627	0.631	0.614	0.629	0.632	0.647	0.622	0.614	0.637	0.025	3.86%	
G	0.700	0.670	0.660	0.653	0.627	0.626	0.622	0.618	0.624	0.623	0.607	0.615	0.637	0.028	4.34%	
H	0.707	0.719	0.676	0.682	0.654	0.645	0.628	0.647	0.623	0.647	0.612	0.601	0.653	0.037	5.59%	
Columns																
Mean	0.692	0.688	0.664	0.643	0.644	0.633	0.622	0.625	0.622	0.638	0.589	0.618	Overall	0.640	0.034	5.28%
S.D.	0.012	0.019	0.020	0.023	0.016	0.009	0.011	0.014	0.005	0.011	0.045	0.017				
CV	1.70%	2.79%	3.01%	3.58%	2.55%	1.36%	1.77%	2.16%	0.84%	1.71%	7.60%	2.81%				

II. Repeatability

B. Interlot Variability

Purpose: To assess the interlot variability of the IDEXX Milk Pregnancy Test.

Procedure: A set of pregnant and open bovine samples was tested in duplicate across three lots of the IDEXX Milk Pregnancy Test, according to the standard assay protocol and interpretation.

Results/Conclusions: Mean test results for the three lots of the IDEXX Milk Pregnancy Test are listed in figure 2. Samples from cows that had been identified as open or pregnant based on ultrasound/palpation were correctly identified as pregnant or open by all three test lots. Two postcalving (fresh) samples yielded results within the recheck zone for each of the three lots. The three lots demonstrated good repeatability for the bovine samples evaluated.

Figure 2. Repeatability

Sample ID	Cow Status	Kit KAV-232		Kit Z901		Kit Z911	
		S-N	Result	S-N	Result	S-N	Result
1	Open	-0.01	-	-0.01	-	0.00	-
2	Open	-0.02	-	-0.01	-	-0.01	-
3	Open	-0.03	-	-0.02	-	-0.01	-
4	Open	-0.01	-	0.00	-	0.00	-
5	Open	-0.01	-	0.01	-	0.01	-
6	Open	0.01	-	-0.01	-	-0.01	-
7	Open	0.00	-	0.00	-	0.00	-
9	Fresh	0.21	Rchk	0.19	Rchk	0.19	Rchk
10	Fresh	0.24	Rchk	0.19	Rchk	0.20	Rchk
8	Pregnant	0.33	+	0.26	+	0.32	+
11	Pregnant	0.93	+	0.85	+	0.91	+
12	Pregnant	1.05	+	0.86	+	0.86	+
13	Pregnant	1.03	+	0.88	+	0.88	+
14	Pregnant	1.09	+	0.87	+	0.86	+
15	Pregnant	0.80	+	0.61	+	0.66	+
16	Pregnant	2.75	+	2.32	+	2.23	+
17	Pregnant	1.52	+	1.33	+	1.21	+
18	Pregnant	1.61	+	1.43	+	1.36	+
19	Pregnant	1.48	+	1.20	+	1.22	+
20	Pregnant	1.34	+	1.05	+	1.08	+

III. Sensitivity—Bovine Milk Samples

A. Diagnostic Sensitivity—Temporal

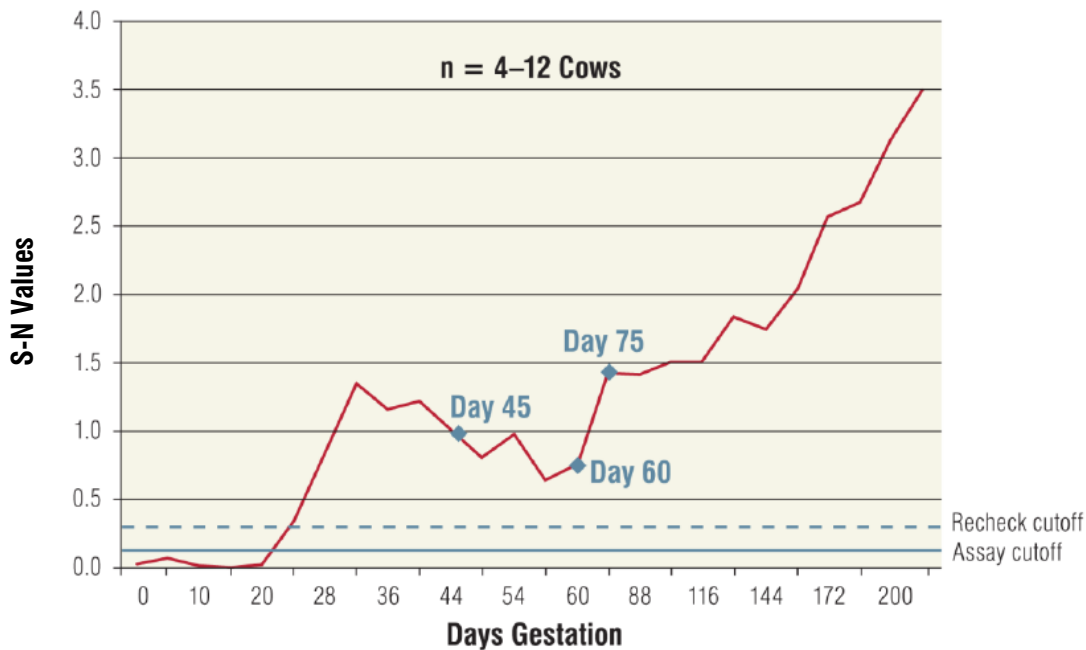
Purpose: To evaluate the IDEXX Milk Pregnancy Test using bovine milk samples collected from cows throughout gestation.

Procedure: Bovine milk samples were collected from cows from a Beijing, China, dairy, starting at day of breeding and continuing at different time points throughout the pregnancy. A total of 4–12 samples were tested for each time point. Samples were tested and results interpreted according to the standard assay protocol.

Results/

Conclusions: The average PAG levels in samples collected at the different time points are represented in figure 3. There was a rapid increase in early PAG levels, with pregnancy consistently detected by day 28. PAG levels declined slightly in midpregnancy around day 60, returning to high levels prior to calving.

Figure 3: Temporal Response of PAGs



III. Sensitivity—Bovine Milk Samples

B. Diagnostic Sensitivity—Throughout Gestation

Purpose: To evaluate the sensitivity of the IDEXX Milk Pregnancy Test on bovine milk samples at different time points postbreeding, corresponding to time points of typical pregnancy testing.

Procedure: Bovine milk samples were collected from cows in multiple regions of the United States and Europe. Cows that were confirmed pregnant by ultrasound or palpation were included in this study, and results were analyzed by the number of days postbreeding at which the milk sample was collected. Each sample was tested and results interpreted according to the standard assay protocol.

Results/Conclusions: The results of this study are shown in figure 4. The IDEXX Milk Pregnancy Test detected confirmed pregnant cows with sensitivity between 96% and 100%, depending on stage of gestation. Recheck results were not included in the calculations for sensitivity.

Figure 4: Diagnostic sensitivity throughout gestation

Pregnant Cows		IDEXX Milk Pregnancy Test (Bovine Samples)				Performance		
		Total	Pregnant	Recheck	Open	Sensitivity*	LCL	Recheck
Days After Breeding	28–34	256	252	2	2	99%	97%	1%
	35–45	65	63	1	1	98%	91%	2%
	46–55	124	94	15	3	97%	91%	12%
	56–65	153	123	25	5	96%	91%	16%
	66–75	53	51	1	1	98%	89%	2%
	76–85	32	31	0	1	97%	83%	0%
	86–95	49	48	1	0	100%	91%	2%
	96–105	63	63	0	0	100%	93%	0%
>105	398	396	0	2	100%	98%	0%	

*Sensitivity excludes IDEXX Milk Pregnancy Test recheck results.

IV. Specificity—Bovine Milk Samples

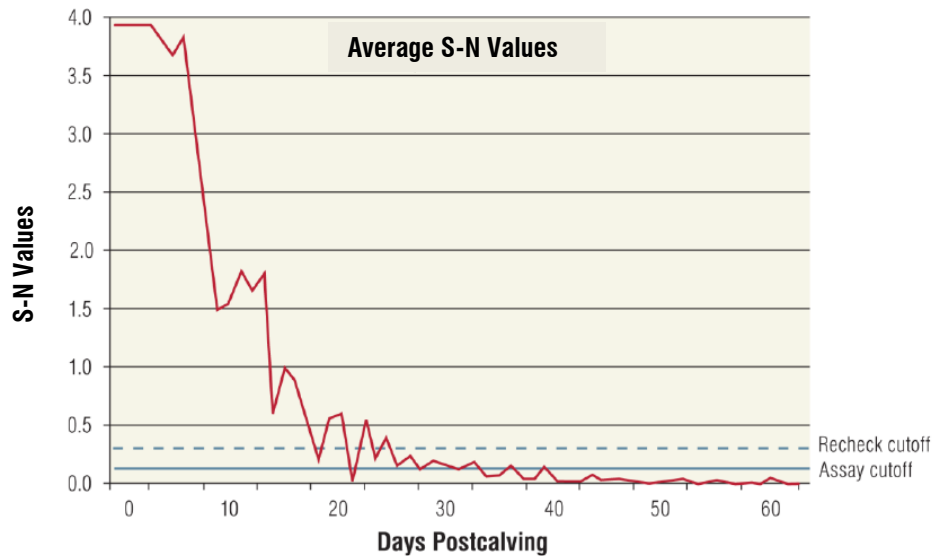
A. Diagnostic Specificity—Postcalving

Purpose: To evaluate declining PAG levels as detected by the IDEXX Milk Pregnancy Test. Cows typically have very high PAG levels at the time of calving. It is important that PAGs from the first pregnancy have declined and are not detected when the cow is tested to confirm the next pregnancy.

Procedure: Milk samples were collected from cows in multiple regions of the United States and Europe. A total of 345 cows that had calved up to 60 days prior to sampling was included in this study, and each sample was tested according to the standard assay protocol. Results were analyzed by the number of days postcalving at which the milk sample was collected.

Results/Conclusions: The results of this study are shown in figure 5. A total of 1–23 samples was tested for each time point, and the average S-N value was plotted. PAG levels declined quickly postcalving, and all cows tested negative by 60 days postcalving.

Figure 5. Temporal decline of PAGs after calving



IV. Specificity—Bovine Milk Samples

B. Diagnostic Specificity—Throughout Gestation

Purpose: To evaluate the specificity of the IDEXX Milk Pregnancy Test on bovine milk samples at different time points postbreeding.

Procedure: Bovine milk samples were collected from cows in multiple regions of the United States and Europe. Cows that were confirmed open by ultrasound or palpation were included in this study, and results were analyzed by the number of days postbreeding at which the milk sample was collected. Each sample was tested and results were interpreted according to the standard assay protocol.

Results/

Conclusions: The results of this study are shown in figure 6. The IDEXX Milk Pregnancy Test detected confirmed open cows with specificity of 90% to 100%, depending on stage of gestation. Specificity in early pregnancy was somewhat compromised by the detection of PAGs for several days following the loss of a pregnancy, which occurs with greater frequency in early weeks of gestation than later in pregnancy. Recheck results were not included in the calculations for specificity.

Figure 6: Diagnostic specificity throughout gestation

Open Cows		IDEXX Milk Pregnancy Test (Bovine Samples)				Performance		
		Total	Pregnant	Recheck	Open	Specificity*	LCL	Recheck
Days After Breeding	Not Bred	264	3	4	257	99%		2%
	28–34	268	26	11	231	90%	86%	4%
	35–45	67	6	2	59	91%	86%	3%
	46–55	13	0	1	12	100%	71%	8%
	56–65	14	0	0	14	100%	74%	0%
	66–75	9	0	1	8	100%	62%	11%
	76–85	2	0	0	2	100%	29%	0%
	86–95	5	0	0	5	100%	51%	0%
	96–105	3	0	0	3	100%	38%	0%
	>105	13	1	1	11	92%	62%	8%

*Specificity excludes IDEXX Milk Pregnancy Test recheck results.

V. Overall Sensitivity and Specificity—Bovine Milk Samples

Purpose: To summarize overall sensitivity and specificity for the IDEXX Milk Pregnancy Test on bovine milk samples.

Procedure: Milk samples were collected from cows in multiple regions of the United States and Europe. Cows were confirmed pregnant or open by ultrasound or palpation. A total of 1,839 samples from cows that were 28 or more days postbreeding and/or 60 or more days postcalving was included in this study. Each sample was tested according to the standard assay protocol.

Results/

Conclusions: As seen in figure 7, the IDEXX Milk Pregnancy Test had 98.7% sensitivity and 94.4% specificity for this population, when compared to ultrasound or palpation. Recheck results were not included in the calculations for sensitivity or specificity and comprise 3% of the total population tested. For this population, 2% of the cows interpreted as rechecks were actually pregnant and 1% were open when examined by ultrasound or palpation. These results demonstrate good overall sensitivity and specificity for the IDEXX Milk Pregnancy Test.

Figure 7. Overall performance of the IDEXX Milk Pregnancy Test on bovine milk samples

		Ultrasound/Palpation	
		Pregnant	Open
IDEXX Milk Pregnancy Test	Pregnant	1,122	36
	Recheck	44	20
	Open	15	602

Sensitivity: 98.7% (LCL 98%)*

Specificity: 94.4% (LCL 92%)*

Rechecks: 3% of total tested (2% pregnant and 1% open)

*Excluding IDEXX Milk Pregnancy Test recheck results

VI. Overall Performance of the IDEXX Milk Pregnancy Test with Optional Recheck—Bovine Milk Samples

Purpose: To summarize overall sensitivity and specificity for the IDEXX Milk Pregnancy Test on bovine milk samples using the optional method for interpretation of results for cows over 45 days postbreeding. With this method, cows more than 45 days postbreeding are considered pregnant if the S-N is greater than 0.10 (there is no recheck zone). Results for cows fewer than 45 days postbreeding are interpreted with the standard S-N recheck zone of 0.10–0.25, and S-N >0.25 is considered pregnant.

Procedure: Milk samples were collected from cows in multiple regions of the United States and Europe. Cows were confirmed pregnant or open by ultrasound or palpation. A total of 1,839 samples from cows that were 28 or more days postbreeding and/or 60 or more days postcalving was included in this study. Each sample was tested according to the standard assay protocol and interpreted with either the standard or optional recheck criteria.

Results/Conclusions: As seen in figure 8 the IDEXX Milk Pregnancy Test had 98.6% sensitivity and 93.2% specificity for this population, when compared to ultrasound or palpation. Recheck results were not included in the calculations for sensitivity or specificity and comprise 3% of the total population tested. For this population, 2% of the cows interpreted as rechecks were actually pregnant and 1% were open when examined by ultrasound or palpation. These results demonstrate good overall sensitivity and specificity for the IDEXX Milk Pregnancy Test when analyzing bovine milk samples.

Figure 8. Overall performance of the IDEXX Milk Pregnancy Test on milk samples with cutoff at 0.1 and no recheck zone for cows >45 days postbreeding

	Standard Interpretation				Optional Interpretation		
	(0.10–0.25 Current Recheck)				(0.10 Cutoff and No Recheck)		
	Performance				Performance		
Days Bred	Cows	Se	Sp	% Recheck	Cows	Se	Sp
28–34	524	99%	90%	2%	n/a	n/a	n/a
35–45	132	98%	91%	2%	n/a	n/a	n/a
46–55	125	97%	100%	13%	125	97%	92%
56–65	167	96%	100%	15%	167	97%	100%
66–75	62	98%	100%	3%	62	98%	89%
76–85	34	97%	100%	0%	34	97%	100%
86–95	54	100%	100%	2%	54	100%	100%
96–105	66	100%	100%	0%	66	100%	100%
>105	411	100%	92%	0%	411	99%	85%
Overall	1,839	99%	94%	3%	1839	99%	93%

VII. Overall Sensitivity and Specificity—Caprine Milk Samples

Purpose: To summarize overall sensitivity and specificity for the IDEXX Milk Pregnancy Test when used with caprine (goat) milk samples.

Procedure: 160 goats were selected from a dairy goat herd in the U.S. All goats had been bred between the months of September and January. Goats were evaluated for pregnancy by IDEXX Milk Pregnancy Test and by ultrasound on January 24 and were retested by both methods on March 7 and again on March 22. Each sample was tested and results were interpreted according to the standard IDEXX Milk Pregnancy Test protocol.

Results/

Conclusions: As seen in figure 9, the IDEXX Milk Pregnancy Test had 94.9% sensitivity and 100% specificity for the goat population, when compared to ultrasound. Recheck results were not included in the calculations for sensitivity or specificity and comprise 2.6% of the total population tested. These results demonstrate good overall sensitivity and specificity for the IDEXX Milk Pregnancy Test when analyzing goat milk samples.

Figure 9. Overall performance of the IDEXX Milk Pregnancy Test on caprine (goat) milk samples

		Ultrasound/Palpation	
		Pregnant	Open
IDEXX Milk Pregnancy Test	Pregnant	56	0
	Recheck	4	0
	Open	3	92