IDEXX Rapid Visual Pregnancy Test

Validation Data Report

The IDEXX Rapid Visual Pregnancy Test is an enzyme-linked immunosorbent assay (ELISA) designed to detect the early presence of pregnancy-associated glycoproteins (PAGs) in bovine serum, EDTA plasma, and whole blood as a marker for pregnancy. A microtiter plate format has been configured by coating an anti-PAG antibody onto the plate. The test sample and a PAG specific antibody (detector solution) are co-incubated in the coated wells. Unbound antibody is washed away and a horseradish peroxidase conjugate is added to the wells. 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. Color development greater than the negative control observed in a test sample well indicates the presence of PAGs and is interpreted as a positive pregnancy result.

I. Glossary of terms

The following definitions have been taken from the Glossary of Terms section of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (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.

Reproducibility—Ability of a test method to provide consistent results when applied to aliquots of the same sample tested by the same method in different laboratories.

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. Operator variability

Purpose: To assess the interoperator variability of the IDEXX Rapid Visual Pregnancy Test.

Procedure: A panel of four samples was tested by independent operators according to the standard test protocol.

Results/ Conclusions: Visual test result interpretations are shown for each operator in table 1. Samples previously identified as open or pregnant based on ultrasound or palpation were correctly identified as open or pregnant by all operators using the IDEXX Rapid Visual Pregnancy Test. These results indicate good test method repeatability of the IDEXX Rapid Visual Pregnancy Test.

Table 1. Interoperator variability of the IDEXX Rapid Visual Pregnancy Test

	Operator	A	B	C
Sample	Status	Visual result	Visual result	Visual result
1	Pregnant	Pregnant	Pregnant	Pregnant
2	Pregnant	Pregnant	Pregnant	Pregnant
3	Pregnant	Pregnant	Pregnant	Pregnant
4	Open	Open	Open	Open

B. Lot-to-lot variability

Purpose: To assess the variability between multiple lots of the IDEXX Rapid Visual Pregnancy Test.

Procedure: A set of two open and four pregnant samples was tested by the same operator on multiple lots of the IDEXX Rapid Visual Pregnancy Test.

Results/ Conclusions: Test results for two lots of the IDEXX Rapid Visual Pregnancy Test are indicated in table 2. Samples previously identified as open or pregnant based on ultrasound or palpation were correctly identified as open or pregnant on all test lots of the IDEXX Rapid Visual Pregnancy Test. These results demonstrate good test repeatability across lots of the IDEXX Rapid Visual Pregnancy Test.

Table 2. Lot-to-lot variability of the IDEXX Rapid Visual Pregnancy Test

	Lot	Lot A	Lot B
Sample	Status	Visual result	Visual result
1	Open	Open	Open
2	Pregnant	Pregnant	Pregnant
3	Pregnant	Pregnant	Pregnant
4	Pregnant	Pregnant	Pregnant
5	Pregnant	Pregnant	Pregnant
6	Open	Open	Open

III. Reproducibility

Purpose: To assess the variation of the IDEXX Rapid Visual Pregnancy Test conducted at multiple test locations.

Procedure: The sample panel, consisting of four open and eight pregnant samples, was tested at three different sites. All samples were tested using the same lot of the IDEXX Rapid Visual Pregnancy Test.

Results/ Conclusions: Test results for the three test locations are indicated in table 3. Samples previously identified as open or pregnant based on ultrasound or palpation were correctly identified as open or pregnant at all three locations using the IDEXX Rapid Visual Pregnancy Test. These results demonstrate good test reproducibility of the IDEXX Rapid Visual Pregnancy Test.

Table 3. Reproducibility of the IDEXX Rapid Visual Pregnancy Test

		Site A	Site B	Site C
Sample ID	**Status**	**Visual result**	**Visual result**	**Visual result**
1	Pregnant	Pregnant	Pregnant	Pregnant
2	Open	Open	Open	Open
3	Pregnant	Pregnant	Pregnant	Pregnant
4	Open	Open	Open	Open
5	Pregnant	Pregnant	Pregnant	Pregnant
6	Pregnant	Pregnant	Pregnant	Pregnant
7	Pregnant	Pregnant	Pregnant	Pregnant
8	Pregnant	Pregnant	Pregnant	Pregnant
9	Open	Open	Open	Open
10	Pregnant	Pregnant	Pregnant	Pregnant
11	Pregnant	Pregnant	Pregnant	Pregnant
12	Open	Open	Open	Open

III. Diagnostic sensitivity

Purpose: To evaluate the sensitivity of the IDEXX Rapid Visual Pregnancy Test at different time points postbreeding.

Procedure: Serum, EDTA plasma, and whole blood samples were collected from cows at different points during pregnancy. Animals were categorized as pregnant based on ultrasound or palpation. Each sample was then tested using the IDEXX Rapid Visual Pregnancy Test according to the standard assay protocol.

Results/ Conclusions: The results of this study are shown in tables 4a, 4b, and 4c. The IDEXX Rapid Visual Pregnancy Test detected confirmed-pregnant animals with 98.4%–99.1% sensitivity, depending on sample type.

Table 4a. Diagnostic sensitivity of the IDEXX Rapid Visual Pregnancy Test— whole blood

Whole blood			
Days postbreeding	**Total cows tested**	**Total visually pregnant**	**Sensitivity**
28–34	75	73	97.3%
35–45	19	19	100.0%
46–55	25	25	100.0%
56–65	33	33	100.0%
66–75	15	15	100.0%
76–85	7	7	100.0%
86–95	3	3	100.0%
96–105	21	21	100.0%
>105	22	22	100.0%
Total	220	218	99.1%

Table 4b. Diagnostic sensitivity of the IDEXX Rapid Visual Pregnancy Test— EDTA plasma

EDTA plasma			
Days postbreeding	**Total cows tested**	**Total visually pregnant**	**Sensitivity**
28–34	65	63	96.9%
35–45	15	15	100.0%
46–55	16	15	93.8%
56–65	37	37	100.0%
66–75	23	23	100.0%
76–85	13	13	100.0%
86–95	6	6	100.0%
96–105	20	20	100.0%
>105	40	40	100.0%
Total	235	232	98.7%

Table 4c. Diagnostic sensitivity of the IDEXX Rapid Visual Pregnancy Test— serum

Serum			
Days postbreeding	**Total cows tested**	**Total visually pregnant**	**Sensitivity**
28–34	147	146	99.3%
35–45	12	12	100.0%
46–55	16	15	93.8%
56–65	30	30	100.0%
66–75	15	14	93.3%
76–85	14	14	100.0%
86–95	5	5	100.0%
96–105	20	20	100.0%
>105	54	52	96.3%
Total	313	308	98.4%

IV. Diagnostic specificity

Purpose: To assess the detectability of declining PAG levels postcalving. 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 next tested to confirm subsequent pregnancy.

Procedure: Serum, EDTA plasma, and whole blood samples were collected from cows that had calved more than 60 days earlier. Cows in this study were not rebred and were therefore considered open. Each sample was tested using the IDEXX Rapid Visual Pregnancy Test according to the standard protocol.

Results/ Conclusions: Study results are shown in tables 5a, 5b, and 5c. Specificity ranged from 95.0%–98.9%, depending on sample type, on the IDEXX Rapid Visual Pregnancy Test in cows more than 60 days postcalving.

Table 5a. Diagnostic specificity of the IDEXX Rapid Visual Pregnancy Test—whole blood

Whole blood			
Days postcalving	**Total cows tested**	**Total visually open**	**Specificity**
>60	91	88	96.7%
>70	15	12	80.0%
>80	5	5	100.0%
>90	4	4	100.0%
>100	5	5	100.0%
Total >60	120	114	95.0%

Table 5b. Diagnostic specificity of the IDEXX Rapid Visual Pregnancy Test—EDTA plasma

EDTA plasma			
Days postcalving	**Total cows tested**	**Total visually open**	**Specificity**
>60	58	58	100.0%
>70	16	16	100.0%
>80	6	5	83.3%
>90	3	3	100.0%
>100	4	4	100.0%
Total >60	87	86	98.9%

Table 5c. Diagnostic specificity of the IDEXX Rapid Visual Pregnancy Test—serum

Serum			
Days postcalving	**Total cows tested**	**Total visually open**	**Specificity**
>60	46	44	95.7%
>70	6	6	100.0%
>80	2	2	100.0%
>90	0	0	NA
>100	3	3	100.0%
Total > 60	57	55	96.5%

V. Overall sensitivity and specificity

Purpose: To summarize the overall sensitivity and specificity of the IDEXX Rapid Visual Pregnancy Test.

Procedure: Serum, EDTA plasma, and whole blood samples were collected from cows at different points during pregnancy. Animals were confirmed as pregnant or open based on ultrasound or palpation test results. Samples used to assess sensitivity were obtained 28 or more days postbreeding. Samples used to assess specificity were obtained 60 or more days postcalving. Each sample was tested using the IDEXX Rapid Visual Pregnancy Test according to the standard assay protocol.

Results/ Conclusions: Tables 6a and 6b represent overall sensitivity and specificity, respectively, by sample type for all samples tested. The IDEXX Rapid Visual Pregnancy Test accurately detected pregnant animals with sensitivity results of 99.1%, 98.7% and 98.4% on whole blood, EDTA plasma, and serum samples, respectively, for animals greater than 28 days postbreeding. The IDEXX Rapid Visual Pregnancy Test's specificity results on whole blood, EDTA plasma, and serum were 95.0%, 98.9%, and 96.5%, respectively, for animals tested more than 60 days postcalving.

Table 6a. Sensitivity of IDEXX Rapid Visual Pregnancy Test—all sample matrices

Diagnostic sensitivity			
Sample type	Total cows tested	Total visually pregnant	Sensitivity
Whole blood	220	218	99.1%
Plasma	235	232	98.7%
Serum	313	308	98.4%

Table 6b. Specificity of IDEXX Rapid Visual Pregnancy Test—all sample matrices

Diagnostic specificity			
Sample type	**Total cows tested**	**Total visually open**	**Specificity**
Whole blood	120	114	95.0%
Plasma	87	86	98.9%
Serum	57	55	96.5%

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