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# Comparison of Lateral Flow to Other Pregnancy Determination Methods in Order to Determine Accuracy of Pregnancy Status in Beef Cattle Pre and Postpartum

Adalaide C. Kline

Julie Walker

Taylor N. Andrews

Saulo Menegatti Zoca

Kaitlin M. Epperson

*See next page for additional authors*

## Authors

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## Comparison of lateral flow to other pregnancy determination methods in order to determine accuracy of pregnancy status in beef cattle pre and postpartum

*Adalaide C. Kline, Julie A. Walker, Taylor N. Andrews, Saulo Menegatti Zoca, Kaitlin M. Epperson, Lacey K. Quail, Jaclyn N. Ketchum, Jerica J. J. Rich, Jim R. Rhoades, George A. Perry*

### Objective

The objective of this study was to compare the accuracy of multiple pregnancy-associated glycoproteins (PAGs) tests to transrectal ultrasonography, and to determine how many days postpartum (dpp) is necessary for the clearance of PAGs from the previous pregnancy to avoid false positives when utilizing the lateral flow test.

### Study Description

Blood samples were collected from six different Bos taurus herds between day 27 and 285 of gestation (heifers n = 1,205 and cows n = 1,539). Blood samples to determine PAG clearance interval were collected weekly postpartum for up to 12 weeks (heifers n = 418 and cows n = 657). Serum was tested using the lateral flow test and were read by two technicians who were blind to pregnancy status. Level of agreement between the tests were determined by Pearson's correlation coefficients and Kappa scores. The MIXED procedure of SAS was used to evaluate the effect of dpp and age (heifers or cows) on postpartum test results. There was a positive correlation between transrectal ultrasonography and the lateral flow test ($r^2 = 0.71$; $P < 0.01$), and agreement between the two tests was very good (Kappa = 0.84). Of the animals that were diagnosed nonpregnant by transrectal ultrasonography, 5.61% were called pregnant by the lateral flow test. Of the animals diagnosed pregnant by transrectal ultrasonography, 2.00% were called not pregnant by the lateral flow test. Thus, a 92.38% agreement occurred between the two methods. For postpartum samples, there was no effect ($P = 0.21$) of age, but there was an effect of dpp ($P < 0.01$) and a tendency for a dpp by age interaction ($P = 0.06$). All animals were still considered pregnant from the previous pregnancy through 35 dpp (100 ± 2.58%). The percentage of females receiving a false positive test result further decreased with time postpartum, by 77 dpp there were 13.72 ± 3.16% of the females positive for pregnancy and at 84 dpp there were 4.11 ± 4.39% positive for pregnancy detection.

### Take Home Points

There is very good agreement between transrectal ultrasonography and the lateral flow PAG test, but if the test is used at less than 40 dpp the likelihood of a false positive result is extremely likely. Thus, the test should be utilized at least 42 dpp to prevent obtaining false positive results.

### Introduction

Within a beef operation, there are reproductive management strategies that can be implemented to increase the success of the breeding season: insemination of all cows towards the beginning of breeding season,

detection of all nonpregnant cows as early as possible, and rebreed nonpregnant females as soon as possible (Bó et al., 2016). In the United States, within large (≥ 200 cows) and small operations (≤ 50 cows) there are 21.9% and 69.6%, respectively, who do not use any type of reproductive technology, which include artificial insemination, breeding soundness exam, pregnancy detection, and body condition scoring to name a few (USDA, 2017). Time and labor were the most cited reasons for not implementing reproductive technologies within an operation (USDA, 2017). In order to increase the percentage of operations that utilize a pregnancy detection method and improve in their reproductive management strategies, the method needs to be accurate and easy to use.

Annual cost (feed, fuel, and labor) to maintain a female ranges from $380 to $900 per head (USDA, 2009; Gray et al., 2012), so without early pregnancy detection there could be monetary loss and missed opportunities for management decisions regarding particular females that are not pregnant. Transrectal ultrasonography has become the gold standard for pregnancy detection, as it can accurately determine pregnancy status as early as d 26 post-insemination (Curran et al., 1986). There are, however, high incidences of pregnancy losses that occur after d 25 of pregnancy (Kastelic et al., 1991; Perry and Cushman, 2016), so determination later in gestation is beneficial to confirm pregnancy is maintained. Even though transrectal ultrasonography can determine pregnancy early in gestation it requires specialized training, is physically demanding, and is costly, which can deter the use of this method (Kastelic et al., 1991; Perry and Cushman, 2016).

Determining presence of pregnancy-associated glycoproteins is another pregnancy detection method that has increased in popularity because it is easy to perform and is highly accurate (95 to 99%) with only a 1 to 5% false positive rate (Pohler et al., 2016) compared to transrectal ultrasonography. Pregnancy-associated glycoproteins increase in concentration throughout gestation, peak at parturition, and then decrease postpartum (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005). Previous studies (Zoli et al., 1992; Kiracofe et al., 1993) have reported that PAGs have a long half-life in circulation; thus, determining the clearance of PAGs is essential to increasing the accuracy of the test by decreasing false positive rate due to PAG concentrations from the previous pregnancy. Previously, a downside to PAG tests have included the time and/or equipment needed to conduct the assay, which can impact the use or acceptance of this method. A new method of pregnancy determination using PAGs has become commercially available (IDEXX Alertys OnFarm Pregnancy Test), which is a chute side test with results within 20 minutes. Therefore, the objective of this study was to determine if the IDEXX Alertys OnFarm Pregnancy Test is comparable to other pregnancy detection methods in accuracy, and to determine when concentrations have cleared post calving to prevent false positive results.

## Experimental Procedures

### *Experimental Design*

Blood samples from six different Bos taurus herds in the state of South Dakota were utilized in this study. Pregnancy detection was performed by transrectal ultrasonography between 30- and 75-days post-insemination in all animals.

### *Blood Sampling*

Blood samples [(heifers n = 1,205 and cows n = 1,539) and postpartum n = 1,066] were collected over a three-year period (2018, 2019, and 2020). Postpartum samples were collected from one group of animals (heifers n = 48 and cows n = 66) and were collected once a week for up to 12 weeks post calving (range of first and last sample was 1-7 to 84-91 days postpartum; dpp). All blood samples were collected from either the tail or jugular vein into 10-mL Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and stored at room temperature (20 °C) for approximately two hours until centrifuged. Samples were centrifuged at 2,000 x g for 30 min for serum collection and stored at -20 °C until testing.

### *Ultrasonography*

All animals were evaluated for pregnancy by transrectal ultrasonography by a trained technician with an Ibex EVO ultrasound and linear probe on d 28 following their first insemination. Pregnancy diagnosis was based on the visualization of a fetus or absence of one. A final pregnancy diagnosis occurred between 31 and 80 d following the end of the breeding season to determine if embryonic loss occurred.

### *IDEXX Alertys OnFarm Pregnancy Test (lateral flow)*

Samples were tested using commercially available blood pregnancy test, IDEXX Alertys OnFarm Pregnancy Test (IDEXX, Westbrook, ME) according to the manufacturer's directions. Briefly, 150 µL of serum or plasma was pipetted into the well of the lateral flow test followed by 25 µL of chase buffer. After waiting 20 min the tests were read by the same two technicians that were blind to ultrasonography pregnancy status. Interpretation of IDEXX Alertys OnFarm Pregnancy Test with one line visible indicates the female was not pregnant, while two visible lines means the female was pregnant at time of blood sample (Figure 1).

### *IDEXX Alertys Rapid Visual Pregnancy Test (RVPT)*

Whole blood samples were collected on d 28 and tested using the commercially available blood pregnancy test, IDEXX Alertys Rapid Visual Pregnancy Test (IDEXX, Westbrook, ME) according to the manufacturer's directions. Briefly, positive/negative controls, and whole blood samples were pipetted into coated plates, and plates were washed and treated with reagents according to the manufacturer's instructions. Visual evaluation of the plates based on a numerical scale, established by color intensity were made upon completion of the procedure by one technician. Color intensity evaluation and description was according to Northrop et al. (2019). The scoring system included a yes/no assignment and numerical value from 0 - 3 based on color in comparison to the controls.

### *IDEXX Alertys Ruminant Pregnancy test (RPT)*

Samples were tested using the commercially available blood pregnancy test, IDEXX Alertys Ruminant Pregnancy Test (IDEXX, Westbrook, ME) according to the manufacturer's directions. Briefly, controls and blood samples were pipetted in duplicate into wells of the coated plates, and plates were washed and treated with reagents according to the manufacturer's instructions. The results from the RPT were analyzed on a Molecular Devices SpectraMax 190 microtiter plate reader (San Jose, California).

### *Statistical Analysis*

Samples were analyzed as a correlation coefficient using the CORR procedure of SAS (9.4) to assess the agreement between tests: ultrasound, RVPT, RPT and lateral flow in the model. Since all variables had a significant correlation between tests further analysis was made using the FREQ procedure in SAS evaluating the frequency between each one comparatively to each other. The Kappa scoring scale is as follows: 0.80 - 1.00 = Very good, 0.60 - 0.80 = Good, 0.40 - 0.60 = Moderate, 0.20 - 0.40 = Fair, and < 0.20 = Poor. Statistical significance was considered at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$ for analysis.

## Results and Discussion

Agreement based on Kappa scores was very good amongst all tests in the study (Table 1). Additionally, there was a positive correlation among all tests (Table 2).

Of the 1,096 animals that were diagnosed nonpregnant by transrectal ultrasonography, 5.61% were diagnosed pregnant by the lateral flow test. Of the 1,648 animals diagnosed pregnant by transrectal ultrasonography, 2.00% were diagnosed nonpregnant by the lateral flow test. Thus, an 92.38% agreement occurred between the two methods (Table 3). In comparison the RPVT had a 90.73% agreement and the RPT had a 92.61% agreement with transrectal ultrasonography (Table 3).

Comparisons were also made between the lateral flow test and the two other IDEXX blood tests to determine accuracies amongst the tests. Of the 816 animals that were diagnosed nonpregnant by the RVPT, 3.56% were called pregnant by the lateral flow test, and the 644 animals diagnosed as pregnant by RPVT, 1.37% were called nonpregnant by the lateral flow test. Thus, a 95.07% agreement occurred between RVPT and lateral flow (Table 3). Lastly, the 724 animals that were diagnosed as nonpregnant by RPT, 1.31% were called pregnant by lateral flow test. Of the 1,636 animals diagnosed as pregnant by RPT, 2.46% were called nonpregnant by the lateral flow test. Thus, 96.22% agreement occurred between RPT and lateral flow test (Table 3).

For postpartum samples utilizing the lateral flow test, there was not a difference ($P = 0.21$) in age, but there was an effect of dpp ($P < 0.01$) and a tendency for a dpp by age interaction ($P = 0.06$). All animals regardless of age were still considered pregnant from the previous pregnancy through 35 dpp (100 ± 2.58%), whereas by 42 dpp, 98.16 ± 2.55% were considered pregnant (Figure 2.A). The percentage of females receiving a false positive test result further decreased with time postpartum. By 77 dpp, there were 13.72 ± 3.16% of the females positive for pregnancy, and at 84 dpp, there were 4.11 ± 4.39% positive for pregnancy (Figure 2.A). The detection of false positives rapidly decreased from 42 to 70 dpp then slowly decreased from 70 to 84 dpp (Figure 2.A). There was a tendency for an age by dpp interaction ($P = 0.06$; Figure 2.B). Between 63 to 77 dpp there was a greater decrease in false positives among heifers compared to cows (at 77 dpp 5.12 ± 4.26% and 22.31 ± 4.67%; respectively). At 84 dpp 4.66 ± 6.03% of cows and 3.56 ± 6.38% of heifers were still considered pregnant (Figure 2).

## Implications

The utilization of the IDEXX Alertys OnFarm Pregnancy Test, lateral flow, is a competitive alternative in pregnancy detection compared to the gold standard transrectal ultrasonography with a 92.38% agreement comparison in postpartum females. Of the three IDEXX Laboratories tests available, the lateral flow test is the most user-friendly method. Additionally, it provides the tools necessary for performing the test included in the kit once the blood has been collected. With the exception of transrectal ultrasonography where the pregnancy detection results are immediate, the time required for results from the lateral flow compared to the other two PAG pregnancy tests is shorter and does not require any specialized training or equipment. Caution should be used with the utilization of the lateral flow test postpartum in either heifers or cows until at least 70 dpp due to the remaining residual PAGs. Thus, allowing 30 dpp for uterine involution to occur before breeding a female then testing females for pregnancy status should be performed at least an additional 40 dpp (or d 40 of gestation) to decrease the amount of false positive results.

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## Tables

**Table 1.** Agreement between Transrectal Ultrasonography (Ultrasound), IDEXX Alertys Rapid Visual (RVPT), IDEXX Alertys Ruminant Pregnancy test (RPT), and IDEXX Alertys OnFarm Pregnancy Test (Lateral Flow) to determine accuracy of pregnancy detection. Values depicted above the diagonal line are the Kappa scores, while values below the diagonal are the overall agreement of the tests based on the Kappa score. The Kappa scoring scale is: 0.80 - 1.00 = Very good, 0.60 - 0.80 = Good, 0.40 - 0.60 = Moderate, 0.20 - 0.40 = Fair, and < 0.20 = Poor.

| Test | Ultrasound | RVPT | RPT | Lateral Flow |
|---|---|---|---|---|
| **Ultrasound** | | 0.8108 | 0.8344 | 0.8388 |
| **RVPT** | very good | | 0.9472 | 0.9005 |
| **RPT** | very good | very good | | 0.9122 |
| **Lateral Flow** | very good | very good | very good | |

**Table 2.** Correlation between Transrectal Ultrasonography (Ultrasound), IDEXX Alertys Rapid Visual (RVPT), IDEXX Alertys Ruminant Pregnancy test (RPT), and IDEXX Alertys OnFarm Pregnancy Test (Lateral Flow) to determine accuracy of pregnancy detection. Values depicted above the diagonal line is the correlation coefficient of the tests, while values below the diagonal are the P-values of all the tests. A positive correlation and significant difference were found amongst the tests in comparison to each other.

| Test | Ultrasound | RVPT | RPT | Lateral Flow |
|---|---|---|---|---|
| **Ultrasound** | | 0.81311 | 0.83856 | 0.84126 |
| **RVPT** | < 0.0001 | | 0.94739 | 0.90138 |
| **RPT** | < 0.0001 | < 0.0001 | | 0.91257 |
| **Lateral Flow** | < 0.0001 | < 0.0001 | < 0.0001 | |

**Table 3.** Agreement between pregnancy detection tests to determine accuracy amongst tests.

| Test[1] | Agreement, % | False Positive[2], % | False Negative[3], % | Samples, n |
|---|---|---|---|---|
| **Ultrasound[4]:Lateral Flow[5]** | 92.38 | 5.61 | 2.00 | 2,744 |
| **Ultrasound[4]:RVPT[6]** | 90.73 | 6.46 | 2.80 | 1,533 |
| **Ultraound[4]:RPT[7]** | 92.61 | 5.91 | 1.48 | 2,436 |
| **RVPT[6]:Lateral Flow[5]** | 95.07 | 3.56 | 1.37 | 1,460 |
| **RPT[7]:Lateral Flow[5]** | 96.22 | 1.31 | 2.46 | 2,360 |
| **RPVT[6]:RPT[7]** | 97.36 | 1.80 | 0.83 | 1,443 |

[1]Comparison between tests first:second
[2] False Positive = a result that shows a female is pregnant when she is actually non-pregnant
[3]False Negative = a result that shows a female is non-pregnant when she is actually pregnant
[4]Ultrasound = transrectal ultrasonography
[5]Lateral Flow = IDEXX Alertys OnFarm Pregnancy Test
[6]RVPT = IDEXX Alertys Rapid Visual Test
[7]RPT = IDEXX Alertys Ruminant Pregnancy Test

## Figures


**Figure 1.** Alertys Ruminant OnFarm Pregnancy Test (Lateral Flow). Test on the left is a test result for a pregnant female, while the test on the right is a test result for a nonpregnant female. There should always be a control line visible indicated by the “C”, unless the test is defective, which in that case rerun that sample on a new test. If a line is visible on the test line indicated by a “T” then the female is pregnant, while no visible line then the female is nonpregnant.

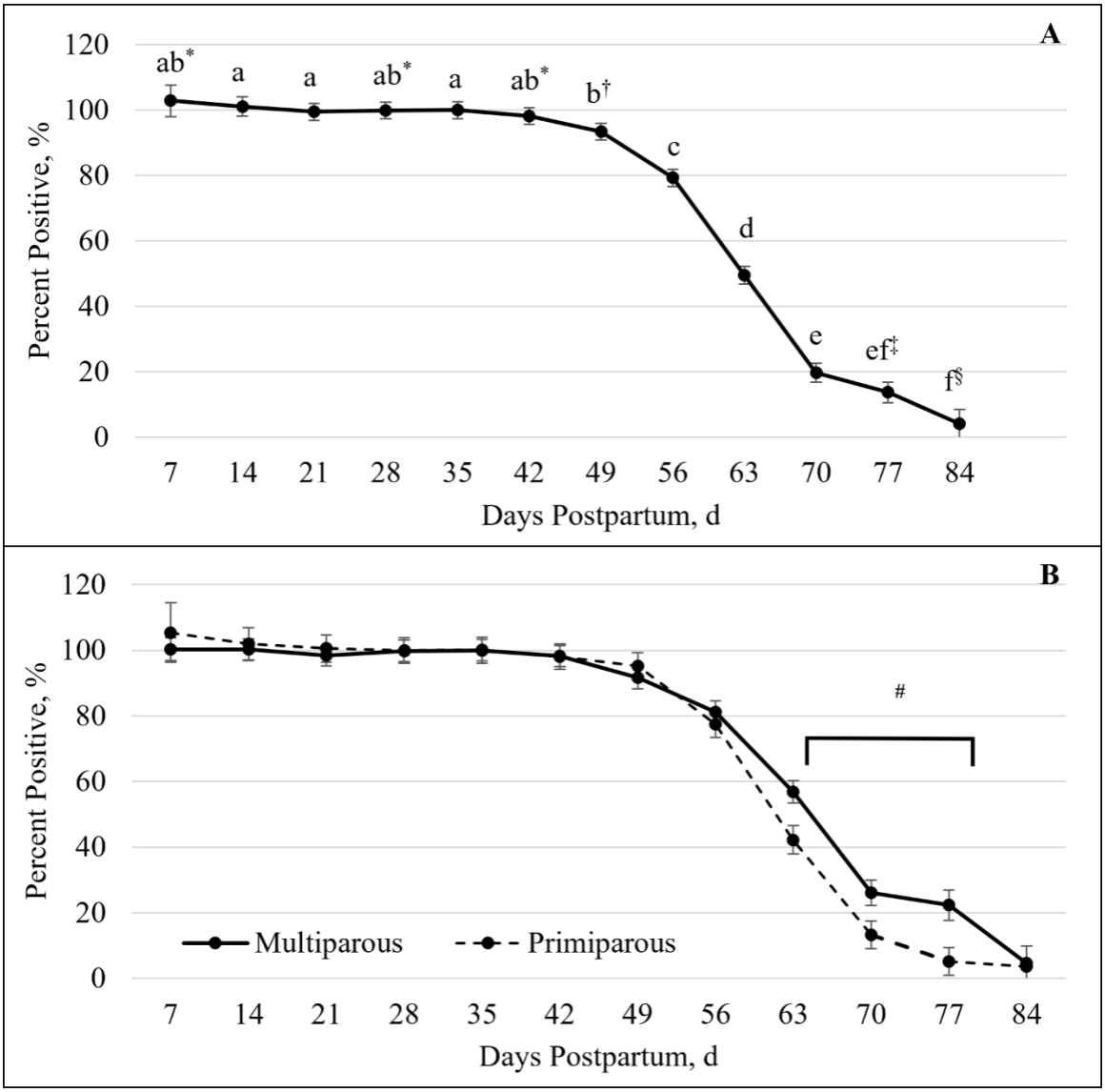


**Figure 2.** False positive percentage of days postpartum (A) and age (B) on clearance utilizing the lateral flow test. Postpartum samples were analyzed by the IDEXX Alertys OnFarm Pregnancy Test, lateral flow, to determine an accurate timeframe to test pregnant females without getting a false positive test from the residual pregnancy-associated glycoproteins (PAGs) from the previous pregnancy. There was a significant effect of dpp ($P < 0.01$; A). All animals were still considered pregnant from the previous pregnancy on 35 dpp (100%; A). Days postpartum by age tended to be different ($P = 0.06$; B).

a-f values not sharing the same superscripts differed $P < 0.05$

*† values not sharing the same superscripts differed $P \leq 0.08$

‡§ values not sharing the same superscripts differed $P \leq 0.07$

# values between cows and heifers between 63 to 77 dpp differed $P < 0.05$