Alertys OnFarm Pregnancy Test

I. Introduction

The Alertys* OnFarm Pregnancy Test is a lateral flow test for the detection of pregnancy-associated glycoproteins (PAGs) in bovine, caprine, and ovine whole blood, serum, or plasma as a marker for pregnancy. As the sample and buffer are applied to the test strip, pregnancy-associated glycoproteins (PAGs) flow across bioactive regions of the test and complex with labeled anti-PAG antibodies. The labeled PAG-antibodies and PAG complexes are then captured by a reagent at the Test Line position in the read window. The presence of a pink or red line of any intensity at the Test Line position indicates a pregnant result. A pink or red line of any intensity at the Control Line position confirms a valid test.

Table 1. Alertys OnFarm Pregnancy Test product information

Part number	Number of units
98-26441-00	25

II. 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.

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 nonpregnant (open) animals that test open in the assay; nonpregnant animals that test pregnant in the assay are considered to have false-positive results.

III. Diagnostic sensitivity

Purpose: To evaluate the sensitivity of the Alertys OnFarm Pregnancy Test at different time points after breeding.

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 Alertys OnFarm Pregnancy Test according to the standard assay protocol.

Results/conclusions: The results of this study are shown in Tables 3.1, 3.2, 3.3, and 3.4. The Alertys OnFarm Pregnancy Test detected confirmed-pregnant animals with 98.3%–100% sensitivity, depending on days postbreeding and sample type.

Table 3.1. PAG detectability at Day 28 pregnant cow in the whole-blood samples

Days pregnant	28
Total # of cows	18
Sensitivity (%)	100

Table 3.2. Sensitivity at different gestation periods in the whole-blood samples

Days pregnant	28–50	51–100	101–150	151–200	≥201	All
Total # of cows	66	58	56	56	54	290
Sensitivity (%)	100	99.2	99.1	100	100	99.7

Table 3.3. PAG detectability at Day 28 pregnant cow in the plasma samples

Days pregnant	28
Total # of cows	18
Sensitivity (%)	100

Table 3.4. Sensitivity at different gestation periods in Serum/plasma samples

Days pregnant	28–50	51–100	101–150	151–200	≥201	All
Total # of cows	60	60	60	60	60	300
Sensitivity (%)	99.2	98.3	100	100	100	99.5

IV. Diagnostic specificity

Purpose: To assess the detectability of declining PAG levels after calving. 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 70 days earlier. Cows in this study were not rebred and were therefore considered open. Each sample was tested using the Alertys OnFarm Pregnancy Test according to the standard protocol.

Results/conclusions: Study results are shown in Tables 4.1 and 4.2. Specificity ranged from 94.5%–98.1%, depending on sample type, on the Alertys OnFarm Pregnancy Test in cows more than 70 days after calving.

Table 4.1. Whole-blood specificity

Days postcalving	≥70
Total # of cows	80
Specificity (%)	98.1

Table 4.2. Serum/plasma specificity

Days postcalving	≥70
Total # of cows	100
Specificity (%)	94.5%

V. Overall sensitivity and specificity

Purpose: To summarize the overall sensitivity and specificity of the Alertys OnFarm 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 after breeding. Samples used to assess specificity were obtained 70 or more days after calving. Each sample was tested using the Alertys OnFarm Pregnancy Test according to the standard assay protocol.

Results/conclusions: Table 5 represents the overall sensitivity and specificity for all samples tested. The Alertys OnFarm Pregnancy Test accurately detected pregnant animals with a sensitivity of 99.7% and 99.5% for whole blood and EDTA plasma/serum samples, respectively, for animals greater than 28 days post-breeding. The specificity for whole blood and EDTA plasma/serum samples was 98.1% and 94.5%, respectively, for animals greater than 70 days after calving.

Table 5. Overall sensitivity and specificity

Sample type	Sensitivity (28+ DPB)	Specificity (70+ DPC)
Whole blood	99.7%	98.1%
Serum/Plasma	99.5%	94.5%

VI. Caprine samples (Specificity and pregnancy detection post-breeding)

Purpose: To summarize performance for the Alertys OnFarm Pregnancy Test when testing Caprine samples post-breeding.

Procedure: Nineteen (18) Boer does were synchronized and bred to billy goats. Markings on does were observed to note breeding dates. Serum, plasma and whole blood was collected prior to breeding, then on days 20, 28 and 70; ultrasound was performed on days 45 and 70 post-breeding. Samples were tested on the Alertys OnFarm Pregnancy Test according to the standard test protocol.

Results/conclusions: As shown in Table 6, the Alertys OnFarm Pregnancy Test detected pregnancy as early as day 20 post-breeding. Day 28 sensitivity (vs day 45 ultrasound) was 100% for all three sample types (11 pregnant animals). Similar results were obtained on day 70 where 10 animals determined as pregnant by ultrasound were also pregnant by Alertys OnFarm. One animal (#18) was pregnant by ultrasound although visually different than other animals. This animal was determined as open, on Alertys OnFarm, on all 3 sample types. Two animals (#7069, #8038) aborted by day 70.

As shown in Table 7, when samples collected prior to breeding were tested, specificities for whole blood, serum and plasma were all 100% (18/18).

These results demonstrate excellent performance of the Alertys OnFarm Pregnancy Test when testing Caprine samples post-breeding.

Table 6. Sensitivity vs Ultrasound

	Day 20 Post-breeding			Day 28 Post-breeding			Ultrasound	Day 70 Post-breeding			
Goat ID	**WB**	**Serum**	**Plasma**	**WB**	**Serum**	**Plasma**	**45 day**	**Ultrasound**	**WB**	**Serum**	**Plasma**
Goat 4	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open
Goat 5	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 6	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open
Goat 8	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 18	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	Open	Open	Open
Goat 105	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 0480	Pregnant	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 0607	Open	Open	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 0630	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 0633	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open
Goat 1203	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open
Goat 7069	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	Open	Open	Open	Open
Goat 8020	Open	Open	Open	Open	Open	Open	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 8038	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	Open	Open	Open	Open
Goat 8045	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 9156	NS	NS	NS	Open	Open	Open	Open	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**
Goat 1190	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open
Goat X009	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**	**Pregnant**

Table 7. Specificity prior to breeding

	Pre-bleed		
Goat ID	**WB**	**Serum**	**Plasma**
Goat 4	Open	Open	Open
Goat 5	Open	Open	Open
Goat 6	Open	Open	Open
Goat 8	Open	Open	Open
Goat 18	Open	Open	Open
Goat 105	Open	Open	Open
Goat 0480	Open	Open	Open
Goat 0607	Open	Open	Open
Goat 0630	Open	Open	Open
Goat 0633	Open	Open	Open
Goat 1203	Open	Open	Open
Goat 7069	Open	Open	Open
Goat 8020	Open	Open	Open
Goat 8038	Open	Open	Open
Goat 8045	Open	Open	Open
Goat 9156	Open	Open	Open
Goat 1190	Open	Open	Open
Goat X009	Open	Open	Open
Specificity	**100%**	**100%**	**100%**

VII. Ovine serum samples (Specificity and pregnancy detection post-breeding)

Purpose: To summarize performance for the Alertys OnFarm Pregnancy Test when testing Ovine samples post-breeding.

Procedure: Thirty (30) Rambouillet ewes were synchronized and bred to rattle-painted rams for 2 estrous cycles. The date of breeding was recorded as determined by visualization of rattle marking on ewes. Serum, plasma and whole blood was collected prior to breeding and then at days 20, 35 and 70 post-breeding; ultrasound was performed on days 35 and 70 post-breeding. Samples were tested on the Alertys OnFarm Pregnancy Test according to the standard test protocol.

Results/conclusions: As shown in Table 8, the Alertys OnFarm Pregnancy Test detected pregnancy as early as day 20 post-breeding. All 10 animals determined as pregnant by ultrasound on day 35 were pregnant by Alertys OnFarm, on all three sample types (100% sensitivity). Twenty-eight animals were determined as pregnant by ultrasound on day 70; at day 70, all animals were pregnant by Alertys OnFarm when testing serum or plasma (100% sensitivity). One animal was determined as open when using whole blood (96.4% sensitivity). One animal (#845) was pregnant on day 35 but aborted by day 70; one animal (#109) never conceived.

As shown in Table 9, when samples collected prior to breeding were tested, specificities for whole blood, serum and plasma were 96.7%, 93.3% and 96.7% respectively.

These results demonstrate excellent performance of the Alertys OnFarm Pregnancy Test when testing Ovine samples post-breeding.

Table 8. Sensitivity vs Ultrasound

	Day 20 Post-breeding			Day 35 Post-breeding				Day 70 Post-breeding			
Sheep ID	**WB**	**Serum**	**Plasma**	**Ultrasound**	**WB**	**Serum**	**Plasma**	**Ultrasound**	**WB**	**Serum**	**Plasma**
Sheep 009	NS	Open	Open	Inconclusive	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 014	Open	Open	Open	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 019	Open	Open	Open	Open	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 048	Open	**Preg +**	**Preg +**	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 101	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 102	Open	Open	Open	Inconclusive	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 105	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 109	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open	Open
Sheep 111	Open	Open	Open	Open	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 140	Open	Open	Open	Inconclusive	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 544	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	Open	**Preg +**	**Preg +**
Sheep 551	Open	**Preg +**	**Preg +**	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 601	Open	**Preg +**	**Preg +**	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 640	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 646	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 655	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 656	NS	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 660	Open	**Preg +**	**Preg +**	Inconclusive	NS	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 723	Open	Open	Open	Inconclusive	**Preg +**	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 730	Open	Open	Open	Open	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 753	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 755	Open	Open	Open	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 758	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 801	Open	Open	Open	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 831	Open	Open	Open	Inconclusive	Open	Open	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 845	Open	**Preg +**	**Preg +**	Inconclusive	**Preg +**	**Preg +**	**Preg +**	Inconclusive	Open	Open	Open
Sheep 907	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 914	**Preg +**	**Preg +**	**Preg +**	Inconclusive	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 937	Open	Open	Open	**Open**	Open	**Preg +**	Open	**Preg +**	**Preg +**	**Preg +**	**Preg +**
Sheep 940	Open	Open	Open	Inconclusive	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**	**Preg +**

Preg + = Pregnant

Table 9. Specificity prior to breeding

	Pre-bleed		
Sheep ID	**WB**	**Serum**	**Plasma**
Sheep 009	Open	Open	Open
Sheep 014	Open	Open	Open
Sheep 019	Open	**Pregnant**	Open
Sheep 048	Open	Open	Open
Sheep 101	Open	Open	Open
Sheep 102	Open	Open	Open
Sheep 105	Open	Open	Open
Sheep 109	Open	Open	Open
Sheep 111	Open	Open	Open
Sheep 140	Open	Open	Open
Sheep 544	Open	Open	Open
Sheep 551	Open	Open	Open
Sheep 601	Open	Open	Open
Sheep 640	**Pregnant**	**Pregnant**	**Pregnant**
Sheep 646	Open	Open	Open
Sheep 655	Open	Open	Open
Sheep 656	Open	Open	Open
Sheep 660	Open	Open	Open
Sheep 723	Open	Open	Open
Sheep 730	Open	Open	Open
Sheep 753	Open	Open	Open
Sheep 755	Open	Open	Open
Sheep 758	Open	Open	Open
Sheep 801	Open	Open	Open
Sheep 831	Open	Open	Open
Sheep 845	Open	Open	Open
Sheep 907	Open	Open	Open
Sheep 914	Open	Open	Open
Sheep 937	Open	Open	Open
Sheep 940	Open	Open	Open
Specificity	**96.7%**	**93.3%**	**96.7%**

VIII. Lot-to-lot reproducibly

Purpose: To assess the reproducibility between multiple lots of the Alertys OnFarm Pregnancy Test.

Procedure: A set of two open (D and E) and two pregnant (B and C) panels were tested on three lots of the Alertys OnFarm Pregnancy Test.

Results/conclusions: Test results for three lots of the Alertys OnFarm Pregnancy Test are indicated in Table 10. Using known pregnant and open samples, the test results demonstrate good test reproducibility across three lots of the Alertys OnFarm Pregnancy Test.

Table 10. Performance of Alertys OnFarm Pregnancy Test on three device lots.

Panels	Status	Lot A	Lot B	Lot C
B	Pregnant	Pregnant	Pregnant	Pregnant
C	Pregnant	Pregnant	Pregnant	Pregnant
D	Open	Open	Open	Open
E	Open	Open	Open	Open

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