DEVELOPMENT OF AN ELISA FOR DETECTION OF BOVINE PREGNANCY

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Introduction: Accurate and timely detection of pregnancy in dairy cows is an essential component of today's reproductive management programs. It enables a faster rebreeding and shortens the calving interval to maximize milk production and revenue for the farm.

Objective: IDEXX Labs, Inc. is developing an ELISA for the accurate detection of pregnancy as early as 28 days post breeding.

Materials & methods: The bovine pregnancy ELISA detects the early pregnancy-associated glycoproteins (PAGs) in bovine serum or plasma. An anti-PAG antibody is coated onto the solid phase to bind PAGs in sample. Second anti-PAG antibody, coupled with biotin is used as the detection reagent along with streptavidin-horseradish peroxidase (SA-HRP). TMB substrate is used as a colorimetric indicator and the reaction is stopped with an acid stop solution. Read at 450nm, wells with color development above the assay threshold are considered positive, indicating a pregnant animal. Wells with little or no color development indicate non-pregnant animals.

An evaluation of bovine pregnancy ELISA has been conducted with serum and plasma samples from about 50 bull bred Holstein heifers. Blood samples were taken every 3 days post breeding for the first 45 days of pregnancy and then every 2 weeks until calving. After calving, samples were taken from the cows once weekly for 10 weeks. Transrectal ultrasound was performed between days 27 and 35 post breeding to confirm pregnancy, and once weekly through 60 days of pregnancy. Fifteen control animals weren’t bred, and were bled every three days for 30 days.

Results: Specificity results were 100%; none of the cows unsuccessfully bred were considered pregnant by the ELISA. Samples from confirmed pregnant cows were considered positive by ELISA at least 95% of the time by day 27 after breeding. After 30 days post breeding, 100% of the cows pregnant by ultrasound indicated positive results on the ELISA.

Conclusion: Initial evaluations of this test indicate that the test is an useful adjunct tool to existing reproductive programs. It reliably distinguishes between pregnant and open animals 27-30 days post breeding, and during pregnancy. Veterinarians could use the test during regular herd checks to evaluate individual animals when it is too soon after breeding to palpate accurately. Also veterinarians may also recommend the test for use when farmers do not have regular access to a veterinarian or an experienced palpator.

Keywords: Pregnancy test, PAG, Day 27 to 30