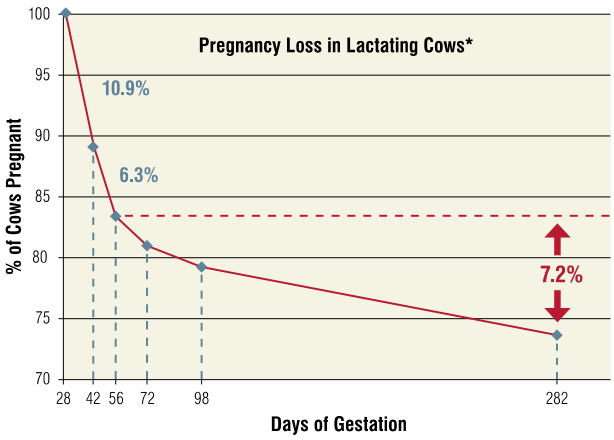
IDEXX Milk Pregnancy Test##

Pregnant? Confirm it with Milk

Complements Traditional Methods of Early Pregnancy Detection

Accurate determination of pregnancy status—High levels of sensitivity and specificity from day 28 post-breeding and throughout gestation

- **Trusted,** timely results—Obtain results in less than 4 lab hours using proven IDEXX ELISA technology
- **Expanded** testing options—Test for pregnancy from routine DHI milk samples
- **Improved** reproductive performance—Earlier identification and rebreeding of open cows results in shorter calving intervals and increased milk production
- Detects **pregnancy associated glycoproteins (PAGs)**
  - **Advantages**
    - Serum and milk (convenient use of milk samples already collected)
    - Pregnancy specific
    - Accurate, simple, safe
  - **Disadvantages**
    - Residual PAGs after embryo loss



- Total pregnancy loss (day 28 to calving) of 24.4%
- 7.2% of pregnancy loss occurs day 56 to calving
- Pregnancy confirmation throughout gestation aids timely identification of open cows

Value Proposition

$4.60 per cow

Improve dairy cattle reproductive performance by identifying open cows using noninvasive milk samples in a cost-effective and time-efficient manner

Dairy producer benefits

- Convenient use of samples already collected for DHIA testing
- Less animal handling/welfare benefit
- Shorter calving intervals/increased milk production

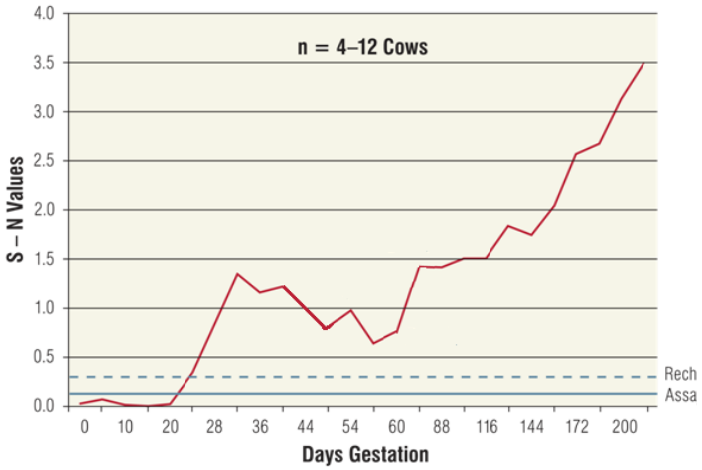
Veterinarian benefits

- Additional information to improve reproductive management
- Timely diagnosis of open cows for veterinary investigation
- Increased time availability for proactive, preventative veterinary medicine

PAG Levels in Milk

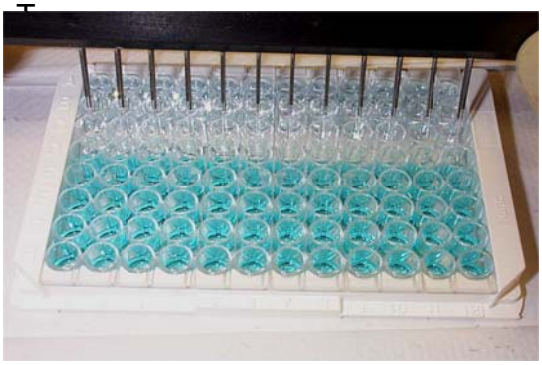
Pregnancy Associated Glycoproteins **(PAGs)** are detectable early in pregnancy and throughout gestation

- Variability in PAG levels detected from different cows*
- Very strong signal in late gestation through calving
- Values differ by each animal and by stage of gestation.
- Studies have shown that PAG levels “dip” between days 46-74 during pregnancy, as shown below.



et antigen for the IDEXX Milk Pregnancy Test

- Placenta-specific expression
  - Expressed in maternal and embryonic regions of the placenta
- Subgroup of aspartic protease family
  - 22+ bovine transcribed genes identified
- Temporally expressed
  - Variable gene expression at different stages of pregnancy



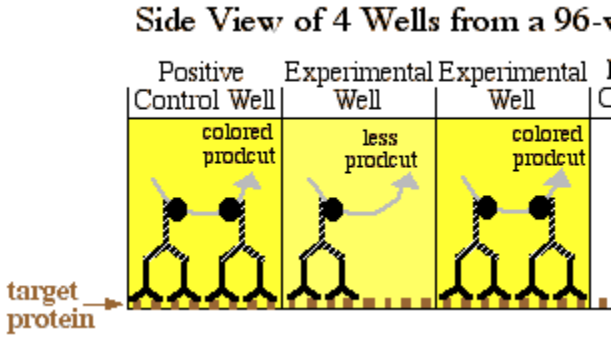
Minnesota DHIA and IDEXX encourage you to work closely with your veterinarian to develop a reproductive management program that is appropriate for your operation

Consequences of Poor Dairy Herd Fertility

- Loss of milk production
- Enforced culling
- Reduced calf sales
- Loss of valuable genetics
- Additional AI costs
- Extra veterinary treatment costs
- Associated welfare issues
- Linkage with other problems

ELISA (Enzyme-Linked ImmunoSorbant Assay)

- The purpose of an ELISA is to determine if a particular protein is present in a sample and if so, how much. There are two main variations on this method: you can determine how much antibody is in a sample, or you can determine how much protein is bound by an antibody. The distinction is whether you are trying to quantify an antibody or some other protein. In this example, we will use an ELISA to determine how much of a particular antibody is present in an individuals blood.
- ELISAs are performed in 96-well plates which permits high throughput results. The bottom of each well is coated with a protein to which will bind the antibody you want to measure. Whole blood is allowed to clot and the cells are centrifuged out to obtain the clear serum with antibodies (called primary antibodies). The serum is incubated in a well, and each well contains a different serum (see figure below). A positive control serum and a negative control serum would be included among the 96 samples being tested.



- After some time, the serum is removed and weakly adherent antibodies are washed off with a series of buffer rinses. To detect the bound antibodies, a secondary antibody is added to each well. The secondary antibody would bind to all human antibodies and is typically produced in a rodent. Attached to the secondary antibody is an enzyme such as peroxidase or alkaline phosphatase. These enzymes can metabolize colorless substrates (sometimes called chromagens) into colored products. After an incubation period, the secondary antibody solution is removed and loosely adherent ones are washed off as before. The final step is the addition the enzyme substrate and the production of colored product in wells with secondary antibodies bound.
- When the enzyme reaction is complete, the entire plate is placed into a plate reader and the optical density (i.e. the amount of colored product) is determined for each well. The amount of color produced is proportional to the amount of primary antibody bound to the proteins on the bottom of the wells. The University of Arizona has an excellent QuickTime animation and quiz if you want further information.