The Detection of Pregnancy Associated Glycoproteins (PAGs) in Routine Milk Recording Samples as an Indicator of Pregnancy in Dairy Cattle

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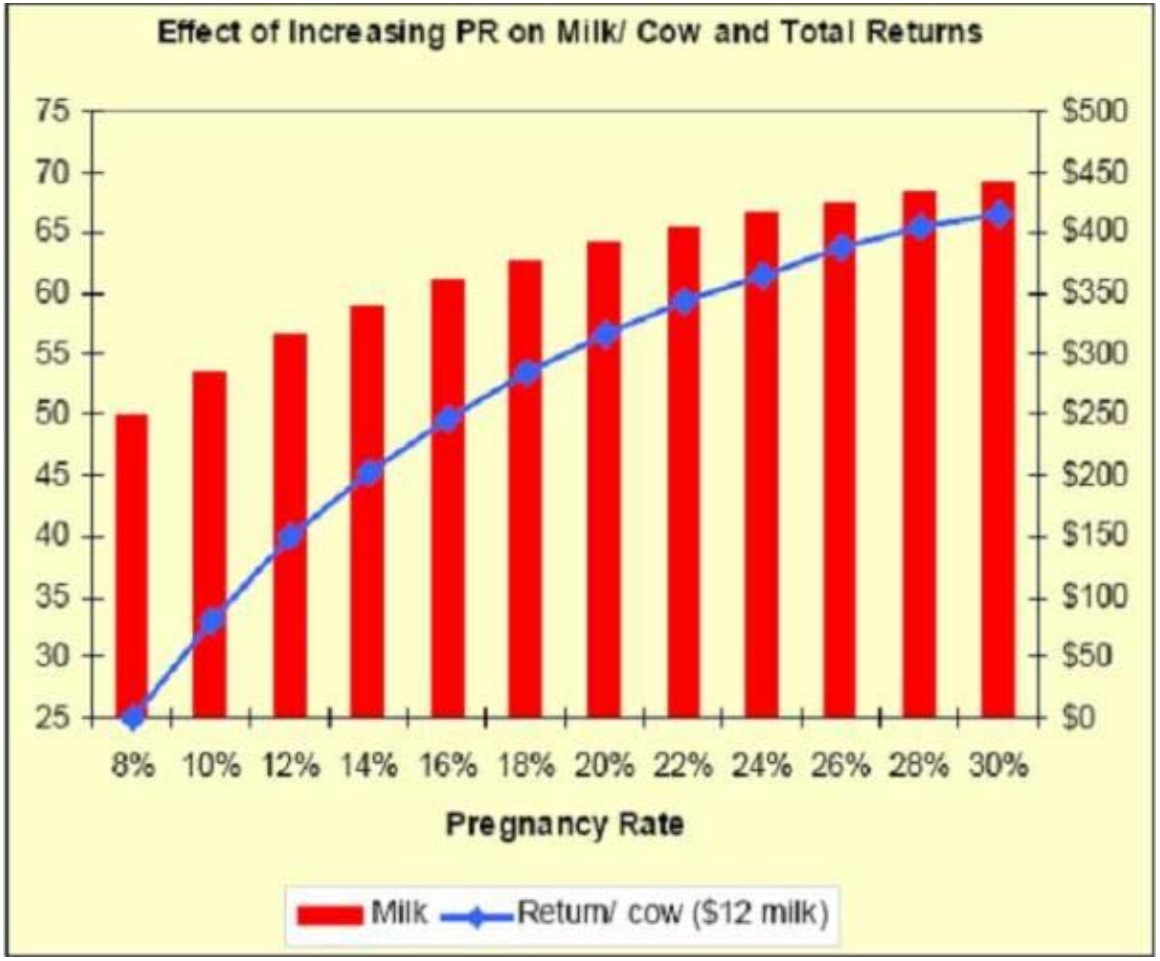
The Importance of Reproductive Efficiency in Dairy Herds



http://www.extension.org/pages/11007/pregnant-vs-open:-getting-cows-pregnant-and-the-money-it-makes

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Pregnancy Diagnosis

- Rectal Palapation/Ultrasound
  - Advantages
    - Accurate, real-time, pregnancy plus….
  - Disadvantages
    - Labor and technically intensive, disruptive to pregnancy
- Blood Chemical Assays
  - Progesterone / ECF/ estrone sulphate
  - Pregnancy Associated Glycoproteins
    - Advantages
      - Accurate, simple
    - Disadvantages
      - Labor intensive
- Milk Chemical Assays
  - Progesterone / estrone sulphate

IDEXX Milk Pregnancy Test

A New Opportunity for Laboratory-Based Pregnancy Testing

- **Accurate** determination of pregnancy status - High levels of sensitivity and specificity, from 60 days after calving. Day of detection for commercial kit to be confirmed
- **Trusted,** timely results - Obtain results in less than 3.5 hours using proven IDEXX ELISA technology
- **Expanded** testing options - Test for pregnancy from routine DHI milk samples
- **Improved** reproductive performance - Through earlier identification and rebreeding of open cows, shorter calving intervals and increased milk production

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IDEXX Milk Pregnancy Test

Kit Overview

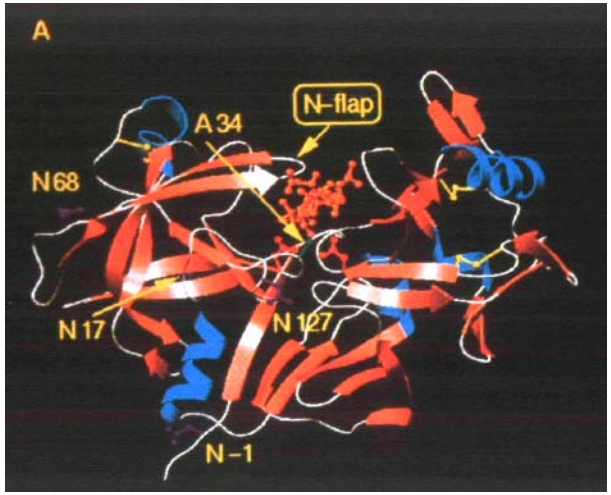
- ELISA strip well format
- DHI whole milk samples
- ~3.5 hours to results
- Ready to use reagents
- Standard laboratory equipment
- Detects pregnancy associated glycoproteins (PAGs)

IDEXX Milk Pregnancy Test

Kit Overview

Pregnancy Associated Glycoproteins (PAGs)

- Target antigen for the IDEXX Milk Pregnancy ELISA
- Placenta-specific expression
  - Expressed in maternal & embryonic regions of placenta
- Subgroup of Aspartic Protease family
  - 22+ bovine transcribed genes identified
- Temporally expressed
  - Variable gene expression at different stages of pregnancy



Guruprasad, K et al. 1996 Protein Engineering. 9:849

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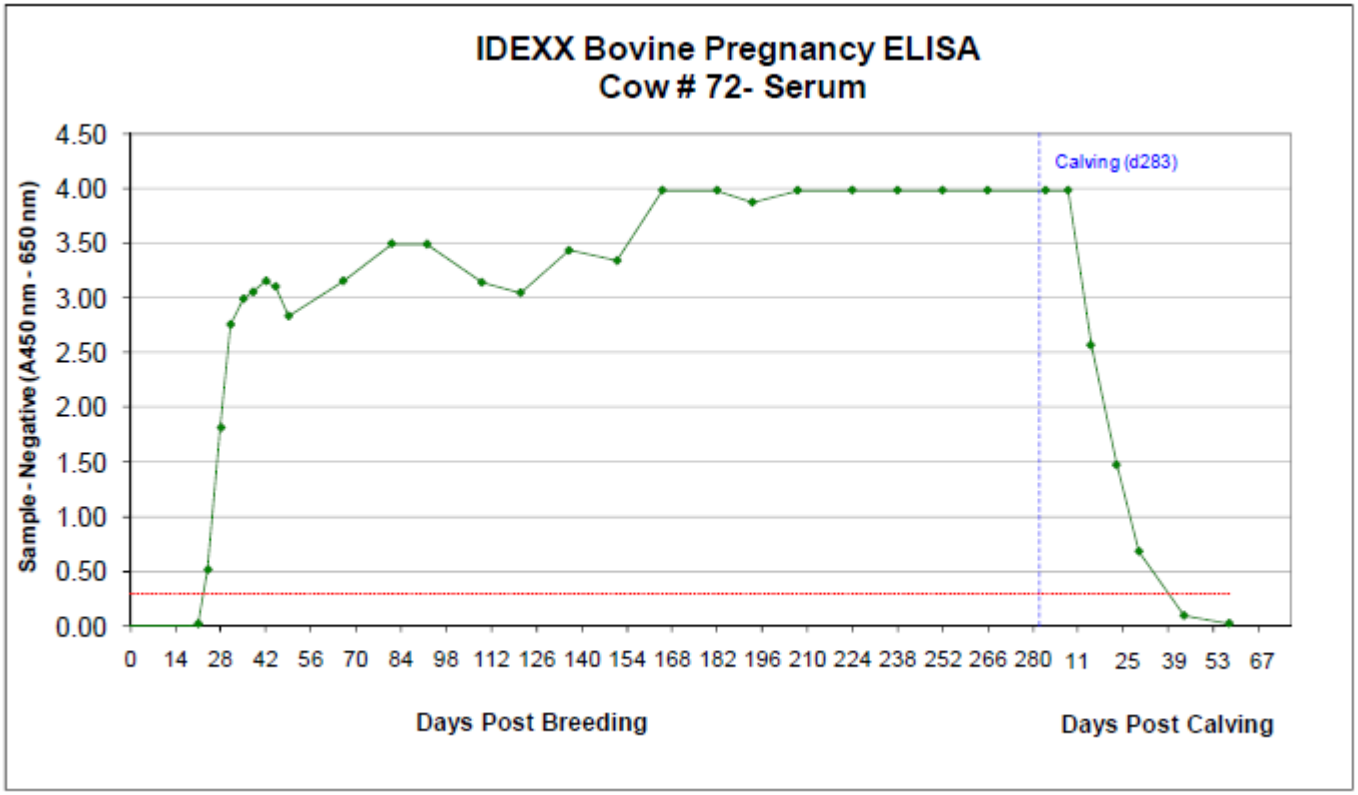
PAG Levels in Serum

PAGs are present throughout pregnancy



PAGs present throughout pregnancy. Early detection (from 28 days post AI).
PAGs decline post calving: test returns to negative from 60 days post calving

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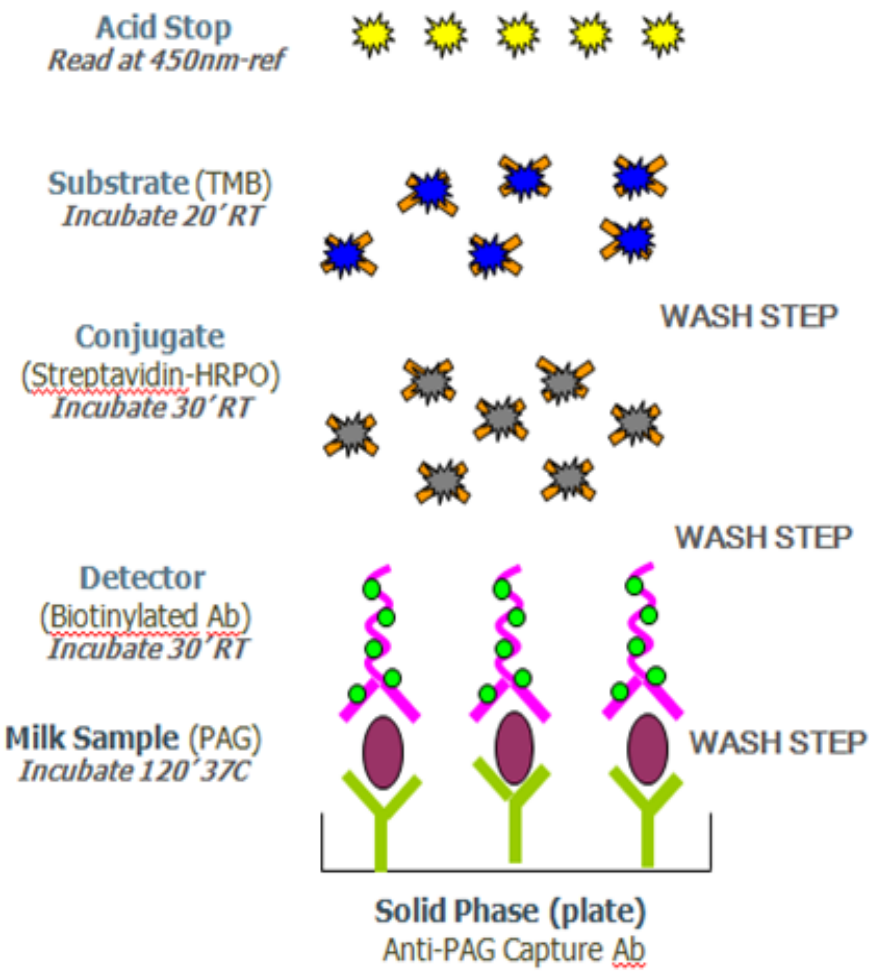
ELISA Protocol



Assay Format:

- Sandwich ELISA
- Biotin & SA-HRPO detection
- Color development = pregnant

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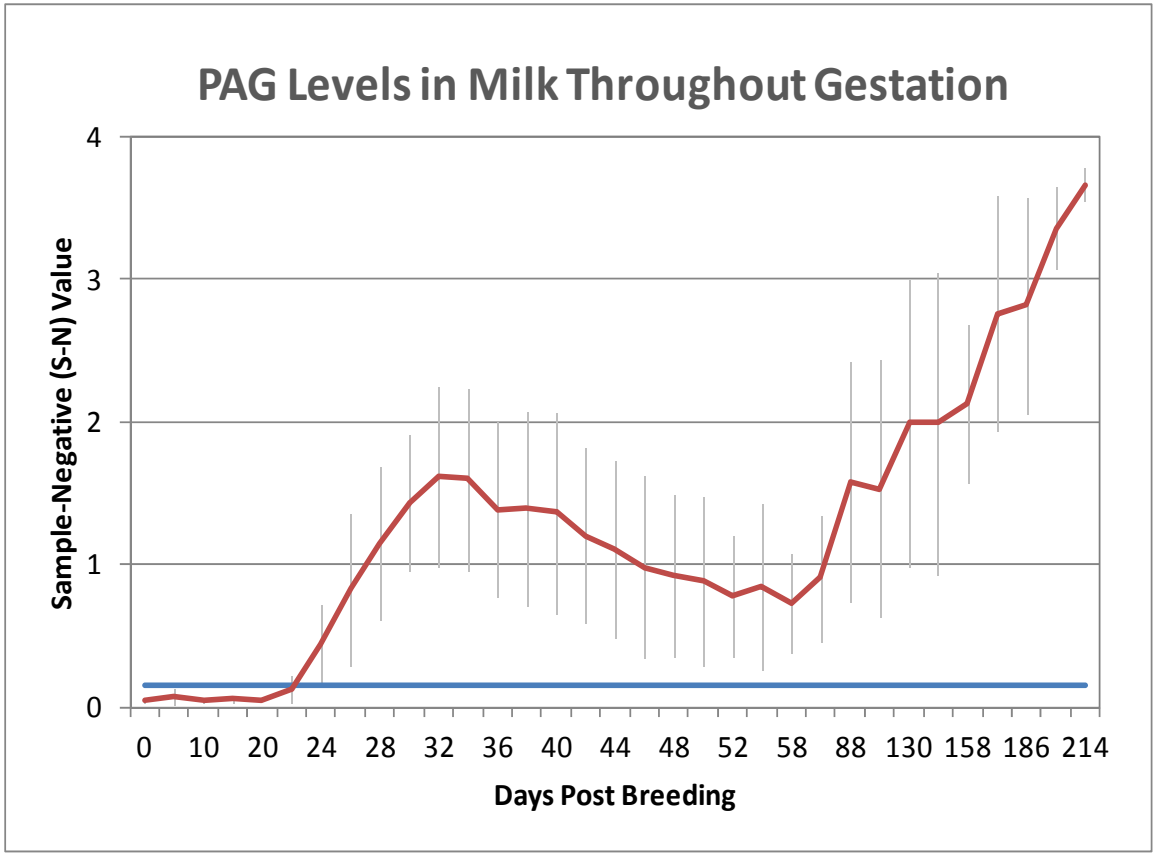
Temporal Study

PAG Levels Throughout Pregnancy

- High degree of variability in PAG levels detected from different cows
- PAGs are detectable early in pregnancy and throughout gestation
- Assay response declines slightly between day 40 through day 90
- Very strong signal in late gestation through calving



5-13 cows sampled at each time point; average response and standard deviation shown on graph

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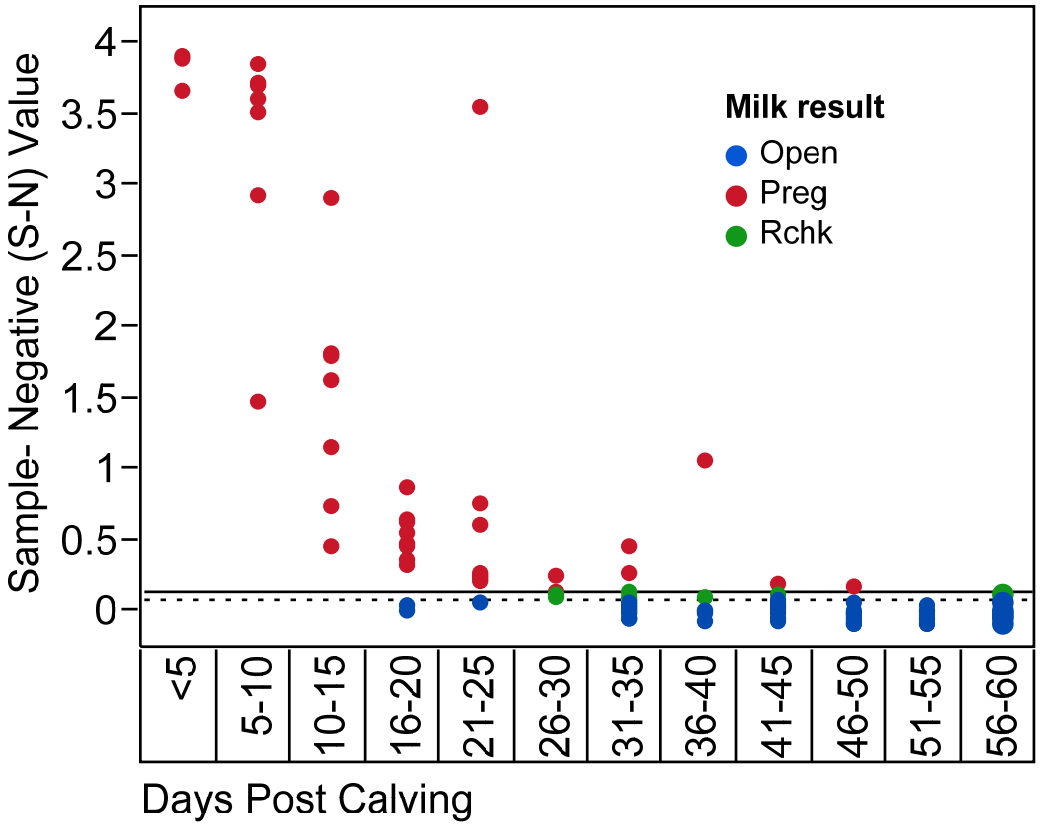
PAG Levels Post-Calving

134 cows sampled after calving, and prior to next breeding;
Assay response for individual cows is shown on the graph



- PAG levels decline rapidly after calving
- Specificity of **100%** by **60 days** (8.5 weeks) after calving
- No interference when testing for the next pregnancy

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IDEXX Milk Pregnancy Test

Results – Herd A

New England Dairy : 192 cows sampled

All cows > 60 DIM and not bred or >40 days post insemination

IDEXX Milk ELISA

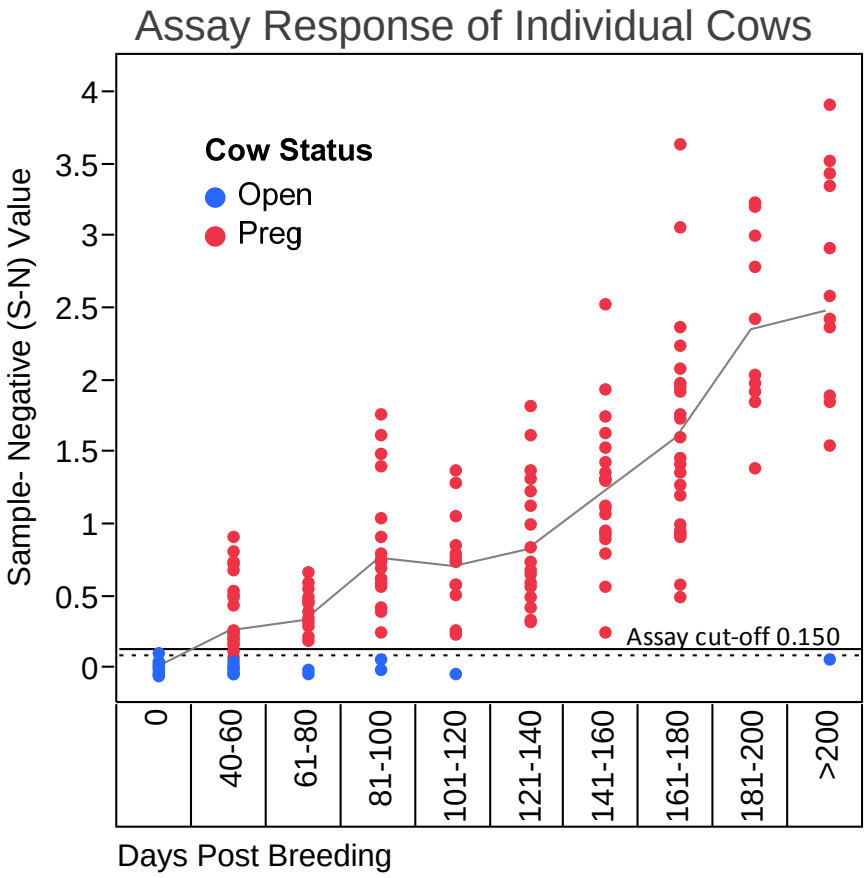
	Ultrasound or Palpation: Pregnant	Ultrasound or Palpation: Open
Pregnant	155	0
Recheck	*2*	*1*
Open	1	33

Total of 192 Cows Tested

Sensitivity* = 99.4% (95% CI: 96.0-100%)

Specificity* = 100% (95% CI: 87.3-100%)

**Recheck results excluded*



IDEXX Milk Pregnancy Test

Results – Herd B

Midwestern Dairy: 120 cows sampled

All cows > 60 DIM and not bred or >40 days post insemination

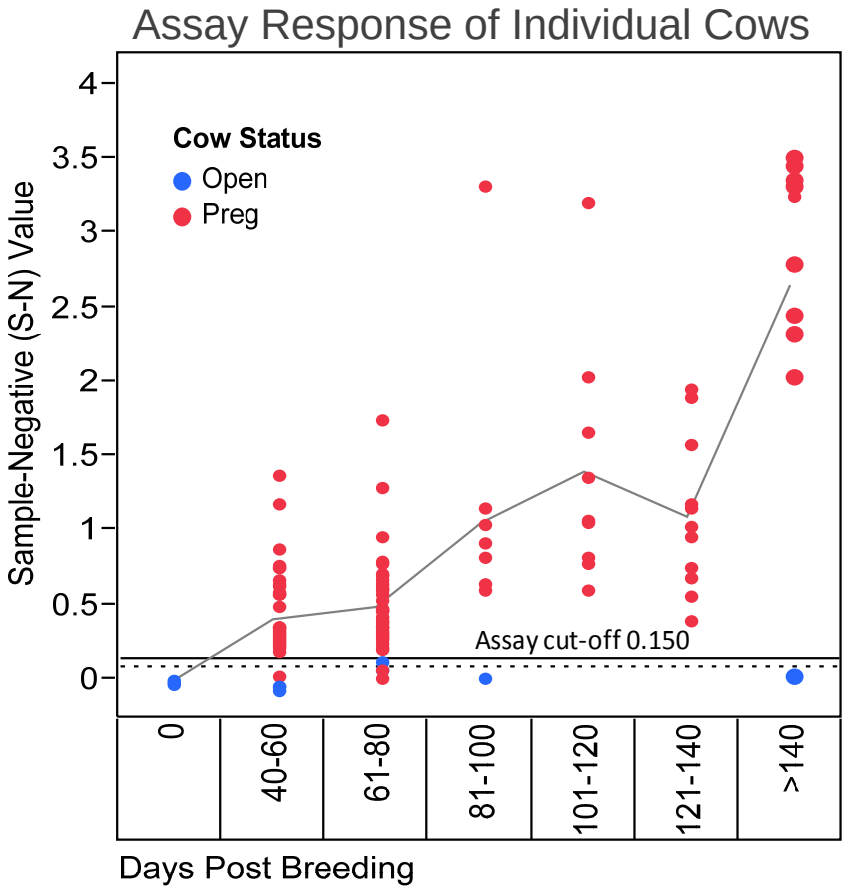
IDEXX Milk ELISA	Ultrasound or Palpation: Pregnant	Ultrasound or Palpation: Open
Pregnant	104	0
Recheck	*0*	*1*
Open	3	12

Total of 120 Cows Tested

Sensitivity* = 97.2% (95% CI: 91.6-99.4%)

Specificity* = 100% (95% CI: 71.3-100%)

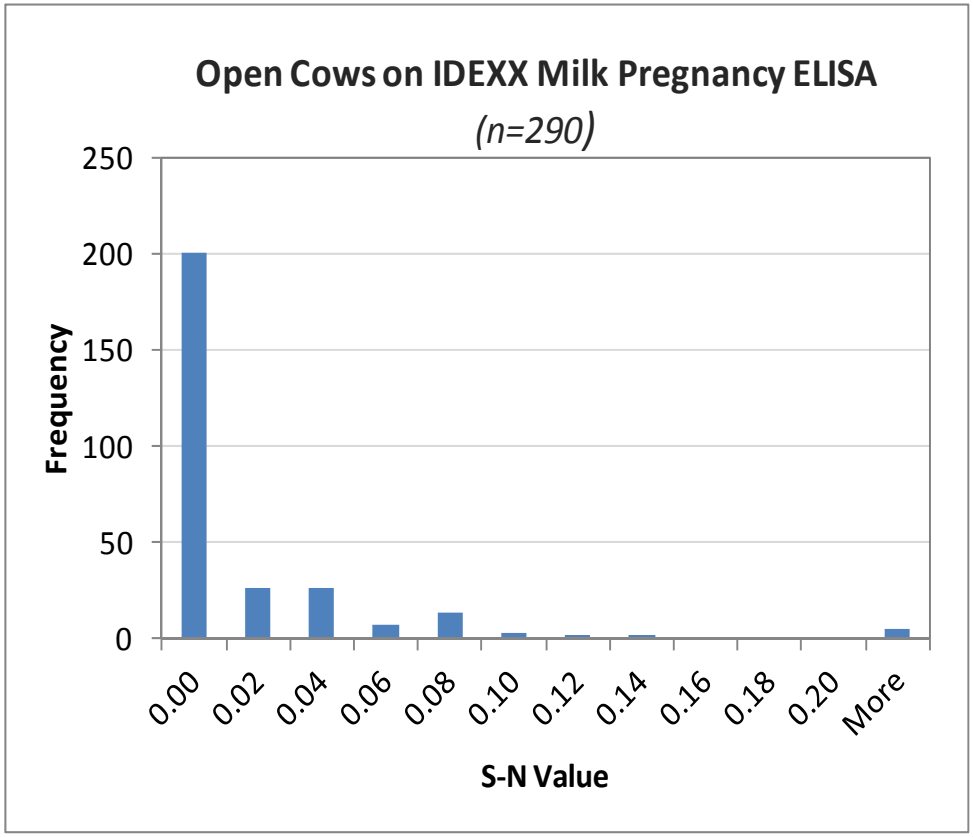
**Recheck results excluded*



IDEXX Milk Pregnancy Test

Detection of Open Cows

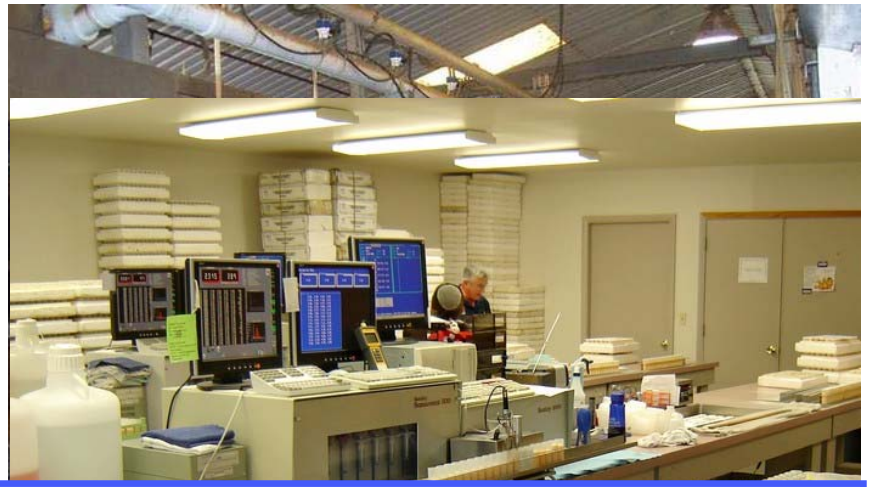
- 290 cows from 5 geographically diverse herds
- All cows > 60 DIM and not bred or >40 days post insemination
- Bred cows confirmed open by ultrasound or palpation



- Overall specificity = 96.7% (95% CI: 94.9-98.9%)
- Fewer open samples available in late gestation, but specificity is very good (100% after day 90)
- Specificity is lower prior to day 40 of pregnancy, likely due to high rates of embryonic death and residual PAGs in the cow's system

Integrating Milk Pregnancy Testing into Routine Herd Recording Programmes

- Field
  - Versatile Application
    - Early detection and confirmations
    - Preselected by days since bred
- Laboratory
  - Simple ELISA platform
  - Uses existing samples
- Data Processing Centers
  - Rapid turnaround
  - Reproduction reports
  - Hot sheet



025 - VET - COW PREG CK, POST PARTUM, OPEN, NOT BRED, & FRESH

UNIV OF IL DAIRY HERD - 33090003
Ref:06-17

GRP	Barn Name	Reason on Report	Days In Milk	Svc Heat Int	No Br	C D E	Bred Heat Date	Days Sinc Br-H	Most Recent Health Code Current Lactation MMDDYY	Code	T	Remarks	BCS Mst Rct
3	DARLENE	PREGCK	332	31	7		5-09	40	041304	HRMX	Y	POSTSYNCH GNRH	
3	LASSY	PREGCK	168	22	3		5-07	42	042004	HRMX	Y	POSTSYNCH GNRH	
3	LEOTA	PREGCK	358	42	7	R	5-06	43	060804	PALP	N	PRH EED?	
3	EDNA	RECK-P	203	56	2	P	3-25	85	042704	PALP	N	PRH	
3	MILLY	PREGCK	297	42	6	R	5-06	43	060804	HRMX	Y	OVSYNCH GNRH1	
3	BEAUTY	PREGCK	430	49	7	R	5-06	43	060804	PALP	N	PRH	
3	CANDEE	PREGCK	108		1	R	5-06	43	060804	PALP	N	PRH	
3	DARLENE	RECK-P	269	42	4	P	4-01	78	050404	PALP	N	PRH	3.5
3	7453	RECK-P	188	42	2	P	3-25	85	042704	PALP	N	PLH	
3	SUZANN	RECK-P	155		1	P	3-18	92	042704	PALP	N	PRH	
3	CHEERFU	PREGCK	187	42	3	R	5-06	43	060804	PALP	N	PRH	
3	PRECISI	RECK-P	204	49	2	P	3-18	92	042004	PALP	N	PLH	
3	SUZANNA	RECK-P	145		1	P	3-25	85	050404	PALP	N	PLH	
3	LASSIE	PREGCK	201	20	4		5-09	40	041304	HRMX	Y	OVSYNCH GNRH1	

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Conclusions

- PAGs can be detected by the IDEXX Milk Pregnancy Test throughout gestation
- The IDEXX Milk Pregnancy Test offers a expanded testing options for herd recording laboratories
- Herd recording samples for pregnancy diagnosis - hassle-free and cost-effect reproductive management in dairy herds
- Application of a milk-based pregnancy test coupled with management data from herd recording could help to improve reproductive efficiency in dairy herds

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For more information please contact...

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