Pregnancy & Disease Detection from Milk Samples
A Global Overview - ICAR Chile 2016 - 25 October, 11.20-11.40
Agenda

• The Milk Sample
• Diagnostic Solutions
• IDEXX Milk Pregnancy Test:
  - What have we learned since the launch?
• Global Overview of Milk Pregnancy Testing Services
  - Europe
  - North America
  - Australia
  - Latin America
The Milk Sample

- Cattle, Sheep, Goat, Buffalo, ...
- Native, skimmed, conserved
- Multiple information:
  - Milk quality parameters
  - Mastitis, SCC
  - Disease: antibodies, antigen, DNA, RNA
  - Pregnancy-associated glycoproteins
  - Progesterone
  - More?
- Easy to collect sample
- Not so easy to store...getting sour easily
- Conserving milk helps stabilize the sample for use in the lab
- Tends to build flocks upon multiple freeze/thaw cycles
Diagnostic Solutions: Adding Diagnostic Value

• More results from every milk sample
• Milk is a simple, accurate medium for regular disease testing
  - Bulk tank, individual milk
• Simplified collection and logistics
• Offer veterinarians and producers greater value with every result
• Antibody disease testing:
  - BVDV (bulk tank and individual)
  - IBR (bulk tank and individual)
  - EBL (BLV) (bulk tank and individual)
  - Brucellosis (bulk tank and individual)
  - Johne’s (individual)
  - Fasciolosis (bulk tank)
  - Q Fever (individual)
• PCR:
  - RealPCR BVDV RNA Test
  - RealPCR MAP DNA Test (in validation)
IDEXX Milk Pregnancy Test

- From 28 days post breeding (cows and goats)
- Coming soon:
  - Water Buffalo: from 30 days post breeding
  - Sheep: from 60 days post breeding
- ELISA test designed for large scale testing in DHI laboratories
IDEXX Milk Pregnancy Test – Lab-Based Milk Test

Value Proposition

• The IDEXX Milk Pregnancy Test helps labs, veterinarians and producers detect pregnant and open cows accurately as early as 28 days post breeding and throughout gestation with the first milk-based laboratory test in a cost-effective and time-efficient manner which allows re-breeding of open cows to improve reproductive efficiency.

• Dairy producer benefits
  - Shorter calving intervals, improved calving rates, increased milk production
  - Save $3-5 per day open
  - Easy to collect routine samples
  - Less animal handling, cow comfort benefit

• Veterinarian benefits
  - Optimize on-farm time for investigating open cows and providing more value added services
  - Systematically capture pregnancy data to improve long-term reproductive management

• Laboratory benefits
  - New value-added service addresses top producer need using existing milk samples
  - Easy-to-run, familiar and trusted, high-throughput ELISA platform
IDEXX Milk Pregnancy Test Performance

• Early and simple:
  - From 28 days post breeding and throughout gestation
  - Test for pregnancy from routine DHI milk samples
  - Bovine and caprine milk
    - Whole or skim
    - Fresh or preserved

  - Performance (bovine milk):

  **Sensitivity** = 98.7% (LCL: 98%)*
  **Specificity** = 94.4% (LCL: 92%)*
  Rechecks: 3% of total tested (2% pregnant and 1% open)

*See IDEXX Milk Pregnancy Test validation report for complete performance data. Excluding IDEXX Milk Pregnancy Test recheck results.
IDEXX Milk Pregnancy Test Worldwide

- Mainly introduced to DHIs that service different dairy production systems
- Adoption of pregnancy diagnosis service in milk
  - Logistics: use routine samples from existing flow to lab and also use individual samples anytime
  - Install new testing parameter besides milk quality, mastitis PCR testing
    - IDEXX recommends instruments (shaker-incubator) and supports with all facets of installation (many DHIs are new to ELISA testing)
  - Recommended: participate in regular proficiency tests
  - Reporting: website (login), e-mail, SMS, fax and letter rare
  - Invoicing: routine, mostly on monthly basis for DHI members
  - Promotion:
    - Advertisements, producer events
    - Leverage regular presence of DHI technicians on farm
    - Examples of successful collaboration between DHI, breeding associations and genetic companies
  - Technical challenges
    - Carry over
    - The dip
    - Decline of PAGs after early embryonic death (EED) and abortion
    - Optional recheck zone
Carry Over Contamination

• There is a technical risk of carry-over contamination* for the IDEXX Milk Pregnancy Test
  - Carryover of <1% does not present a significant risk for false positive or recheck sample
  - 2.5-5% carryover may increase # of recheck results but low risk of false positives
  - >10% carryover could significantly increase number of recheck and false positive results

• However, in reality, we see very few ‘field-based’ issues of carryover contamination
  - >2000 samples tested from routine DHI collections with very few false positive samples that could have been attributed to carryover contamination
  - Specificity in field trials and validation testing exceptionally high (>97%)

• In order to mitigate the risk of carryover contamination, IDEXX has the following recommendations included in the test protocol:

  Sample Quality and Handling
  - Care should be taken to minimize the likelihood of milk carryover from cow to cow during sample collection, particularly when using samples collected for routine herd recording.

*Data from laboratory –based dilution studies, not field samples
The Dip

- Pregnant cows show rapid increase of PAGs up to day 32 post breeding. Levels decline above cut-off and increase again to day 70 post breeding creating a dip.
- Similar findings in published study (Ricci et al.)
- Conclusion: there is a dip between 32 and 70 days post breeding resulting in lower PAG levels. However, test performance remains at high levels.
Milk PAG Levels in Danish Milk recorded Cows 2013-2016
294,584 positive results in Holsteins, Jersey, Red Nordic, and Cross breed

Milk PAG Levels (S-N) and Days Post Breeding

Cut-off (S-N) 0.100-0.250

Decline of PAGs after EED or Abortion

- PAGs are produced by trophoblast cells in the placenta. If placenta loses functionality due to early embryonic death (EED)/abortion PAGs will not be produced anymore and concentration of PAGs in blood and milk will decline over time.

<table>
<thead>
<tr>
<th>Time of loss</th>
<th>Estimated (1) duration of PAG decline in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-60 days of pregnancy (EED)</td>
<td>Up to 10 or more days</td>
</tr>
<tr>
<td>&gt; 60 days of pregnancy (abortion)</td>
<td>Up to 60 days</td>
</tr>
</tbody>
</table>

(1) no exact data available yet. IDEXX is collecting data to learn more about it.
Decline of PAGs after EED or Abortion

- Study at University of Hannover, Germany with IDEXX Bovine Pregnancy Test:
Optional Recheck Zone

- To summarize overall sensitivity and specificity for the IDEXX Milk Pregnancy Test on bovine milk samples using the optional method for interpretation of results for cows over 45 days postbreeding. With this method, cows more than 45 days postbreeding are considered pregnant if the S-N is greater than 0.10 (there is no recheck zone).

<table>
<thead>
<tr>
<th>Days Bred</th>
<th>Standard Interpretation</th>
<th>Optional Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cows</td>
<td>Se</td>
</tr>
<tr>
<td>28–34</td>
<td>524</td>
<td>99%</td>
</tr>
<tr>
<td>35–45</td>
<td>132</td>
<td>98%</td>
</tr>
<tr>
<td>46–55</td>
<td>125</td>
<td>97%</td>
</tr>
<tr>
<td>56–65</td>
<td>167</td>
<td>96%</td>
</tr>
<tr>
<td>66–75</td>
<td>62</td>
<td>98%</td>
</tr>
<tr>
<td>76–85</td>
<td>34</td>
<td>97%</td>
</tr>
<tr>
<td>86–95</td>
<td>54</td>
<td>100%</td>
</tr>
<tr>
<td>96–105</td>
<td>66</td>
<td>100%</td>
</tr>
<tr>
<td>&gt;105</td>
<td>411</td>
<td>100%</td>
</tr>
<tr>
<td>Overall</td>
<td>1,839</td>
<td>99%</td>
</tr>
</tbody>
</table>

*See IDEXX Milk Pregnancy Test validation report for complete performance data. Excluding IDEXX Milk Pregnancy Test recheck results.
Recommended Times to Assess Pregnancy Status
With IDEXX Milk Pregnancy Test

• P1: 28-35 days in gestation (post AI)
  - The opportunity to find non-pregnant (open) cows
  - Estrous Synchronization programs allow these cows to re-enter the program quicker

• P2: 45-70 days in gestation
  - Peak period of Early Embryonic Death (EED)
  - Best practice management programs

• P3: 90-110 days of gestation
  - Early Embryonic Death (EED) peak is now past
  - Cow approaching break-even phase of lactation curve

• P4: 200-230 days of gestation (dry-off)
  - Although uncommon, pregnancy loss can occur between 100-230 days
  - Important decision point for culling after finishing lactation
Frequency of Positive Pregnancy Checks
Danish milk recorded cows, between day 25 and 254 postbreeding

Number of positive results

Global Overview of Milk Pregnancy Testing Services

• Germany
  - Milchprüfring Bayern e.V. Routine DHI (https://www.mpr-bayern.de/eng)
    ‣ One of the largest independent milk testing laboratories for automated analyses of payment and DHI samples worldwide.
    ‣ 275,000 pregnancy tests since launch two years ago
    ‣ Routine DHI samples and individual samples for members and also non-members
    ‣ Shortly available: mpr-App «mpr-mobil» for online reception of results.

- Animal Health Bavaria
  ‣ www.tiergesundheit.bayern.de
  ‣ Do not slaughter high pregnant cows!
  ‣ Test options recommended:
    - PD by vet or technician
    - PAG test using blood or milk

Dead unborn calf in abattoir, “0.6% of high pregnant cows have been slaughtered in Bavaria in first half of 2016.” Information leaflet tiergesundheit.bayern, Sept 2016
Global Overview of Milk Pregnancy Testing Services

• Switzerland
  - The only Swiss DHI lab, Suisselab offers pregnancy testing services (www.fertalys.ch) together with breeding associations and swissgenetics (Swiss semen company), results via e-mail or SMS
  - Easy contact options: fertalys@suisselab.ch
  - Online order options for sample kits
  - Currently establishing program to promote 2nd sample from same cow (confirmation)

Fertalys: Result via text message (SMS) to farmer

Fertalys: advertisement
Global Overview of Milk Pregnancy Testing Services

• Denmark
  - RYK - Eurofins Steins, www.vfl.dk/ryk
    ‣ Great experience with samples from robot units
Global Overview of Milk Pregnancy Testing Services

- France
  - Clasel, French DHI lab
    - Gestadetect, a reliable, simple pregnancy indicator

- United Kingdom
  - National Milk Recording (NMR)
    - [https://www.nmr.co.uk/breeding/pregnancy-testing](https://www.nmr.co.uk/breeding/pregnancy-testing)
    - Why use?
      - It’s accurate, flexible, easy, cost-effective, pays a check, pays a dry-off check
  - The Cattle Information Service – CIS
    - [http://www.thecis.co.uk/theCIS/CIS_PregCheck.aspx](http://www.thecis.co.uk/theCIS/CIS_PregCheck.aspx)
    - Managing the reproductive cycle on your farm
Global Overview of Milk Pregnancy Testing Services

• Antelbio USA, a division of NorthStar Cooperative
  - http://www.northstarcooperative.com/pregnancy/
  - Collaborated to validate the first milk pregnancy test

• USA, Dairy One
  - http://dairyone.com/analytical-services/pregnancy-testing/
  - Dairy One Ithaca Lab, routine samples and individual samples (free shipping), sample transportation system, FAQs,
  - BOTH OPTIONS *Now Offering free “RECHECK’s”*

• The Dairy Authority, Colorado, USA
  - A vet clinic, offering Milk Preg Test 24 hrs TAT
Global Overview of Milk Pregnancy Testing Services

• Heart of America DHIA
  - [http://www.hoadhia.com/lab.html](http://www.hoadhia.com/lab.html)
  - ELISA submission form for Pregnancy and Johne’s

• Lancaster DHIA
  - [http://www.lancasterdhia.com/](http://www.lancasterdhia.com/)
  - “Let us help you find open cows”
  - Routine and individual samples, “call your local DHI technician“
    offer a mailing box, members and non-members

• Texas DHIA
  - [http://www.texasdhia.com/SERVICES.html](http://www.texasdhia.com/SERVICES.html)
  - Our milk pregnancy test is 96% accurate and the results are collected within 6 hours of receiving the samples.
  - This is the industry leading test for reducing invasiveness and unnecessary stress on the animal.
  - Milk pregnancy testing eliminates the need for additional animal sorting, restraining, and sampling. It also allows for better time management between the producer and veterinarian.
Global Overview of Milk Pregnancy Testing Services

• Canada
  - Valacta (www.valacta.com) DHI, Quebec:
    • Gestalab, now available every day (since 3rd October 2016)
      - With sampling kits
      - In collaboration with CIAQ (www.ciaq.com, a semen company)
  - CanWest DHI (www.canwestdhi.com)
    • Accurate, easy & cost-effective
    • Use as 1st check, Recheck and dry-off check

• Australia
  - IDEXX Pregnancy Test, 28 Day Milk Pregnancy Testing
Global Overview of Milk Pregnancy Testing Services

• Brazil
  - Clinica do Leite
    ‣ Promoting P-CHECK

  - APCBRH, Parana
    http://www.apcbrh.com.br/
Global Overview of Milk Pregnancy Testing Services

• Chile
  - Cooprinsem (http://cooprinsem.cl/home/)
What Customers Are Saying

• “Our producer clients are really seeing the benefits of getting a pregnancy test milk sample in the parlor without the extra efforts of having to lock up or sort cows. The process is efficient and eliminates potential delays in breeding programs, which saves valuable time.”
  Bruce W. Hoffman, DVM President, Animal Profiling International, Inc., USA

• “Testing milk samples for pregnancy during our DHI test is a lot easier, and it's on my schedule every month.”
  Mark Bontekoe, Touchdown Dairy, LLC, USA