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LANDESAMT FÜR UMWELT, LANDWIRTSCHAFT UND GEOLOGIE | Freistaat SACHSEN

# Haptoglobin in milk – immunologic biomarker for monitoring health status by on-farm analysis


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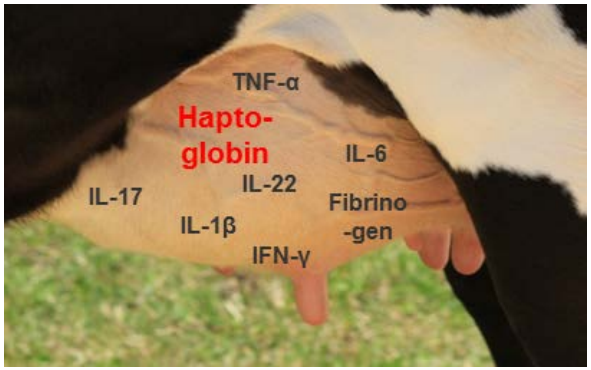
# Introduction

- **Acute Phase Response (APR) plays a central role** in the action of the non-specific innate immune system (systemic response)
- Is triggered when an animal is **subjected to challenges**, such as infection, inflammation, trauma or stress




- **Haptoglobin (Hp)** is a major Acute Phase Proteins (APP) - one of the most sensitive bovine APP → *low concentration in normal animals + rapid increase during inflammation + rapid decrease with the resolution of the disease*
- Hp acts in plasma as a **scavenger molecule** and has **antioxidant activities**
- Hp in plasma is **clinically** a useful parameter for measuring the occurrence and severity of inflammatory responses
- Several studies showed: **Hp is secreted in bovine milk** during clinical mastitis
- **Different hypothetical pathways** for the presence of bovine Hp in milk (exported to the mammary gland or extrahepatic production)

# Objectives

- **Investigate relations between Hp in milk and systemic inflammatory reactions of the body**
  - Based on a new on-farm technique to measure Hp in milk (ELISA)
  - How I have to collect the samples
  - How stable is the test after repeated measures
  - What is the sensitivity and the specificity of the test as an early indicator
  - Which thresholds and expected values could be assumed during the lactation

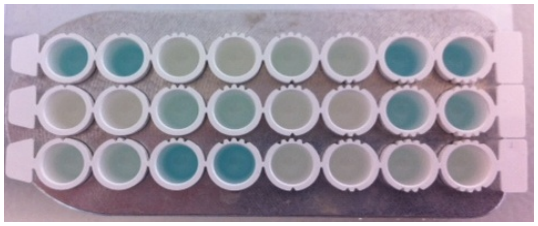
- **Can Hp in milk with the new on-farm test be used …**
  - … as a early indicator of systemic inflammatory reactions of the body?
  - … as a biomarker to monitor the health status of dairy cows?

# Materials and Methods



## On-farm test system for Hp in milk

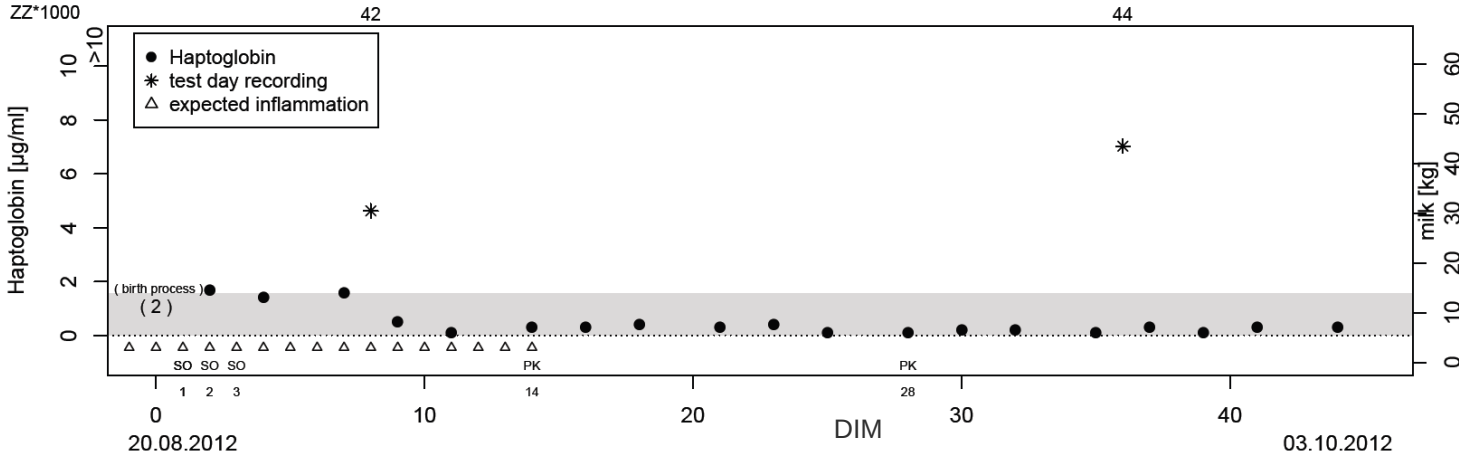
- On farm device eProCheck 2.0 was used
  *(test version of FrimTec GmbH [www.**frimtec**.de](http://www.frimtec.de))*
- Sandwich-ELISA
- Only 50 µl of milk samples must be pipetted in prepared testwells
- 22 samples and 2 standards per analysis
- 60 – 80 minutes per cycle
- Color reaction is analyzed – values in µg/ml in a range of 0 to about 30 with one decimal place

# Materials and Methods

## Test design

- Investigations under **field conditions**
  - In a dairy cattle farm with 1350 cows / 2 x 40 side by side milking parlour
  - Average milk yield  9.500 kg / a
- 30.07.2012 – 05.10.2012 / 100 cows / 2nd day p.p. up to the 44 d p.p. in an 2 or 3 - day –rhythm (Mo – We – Fr)
  - Composite sample (mix from all quarters)
  - Documentation of veterinary diagnoses and treatments
  - Test-day records, calving ease



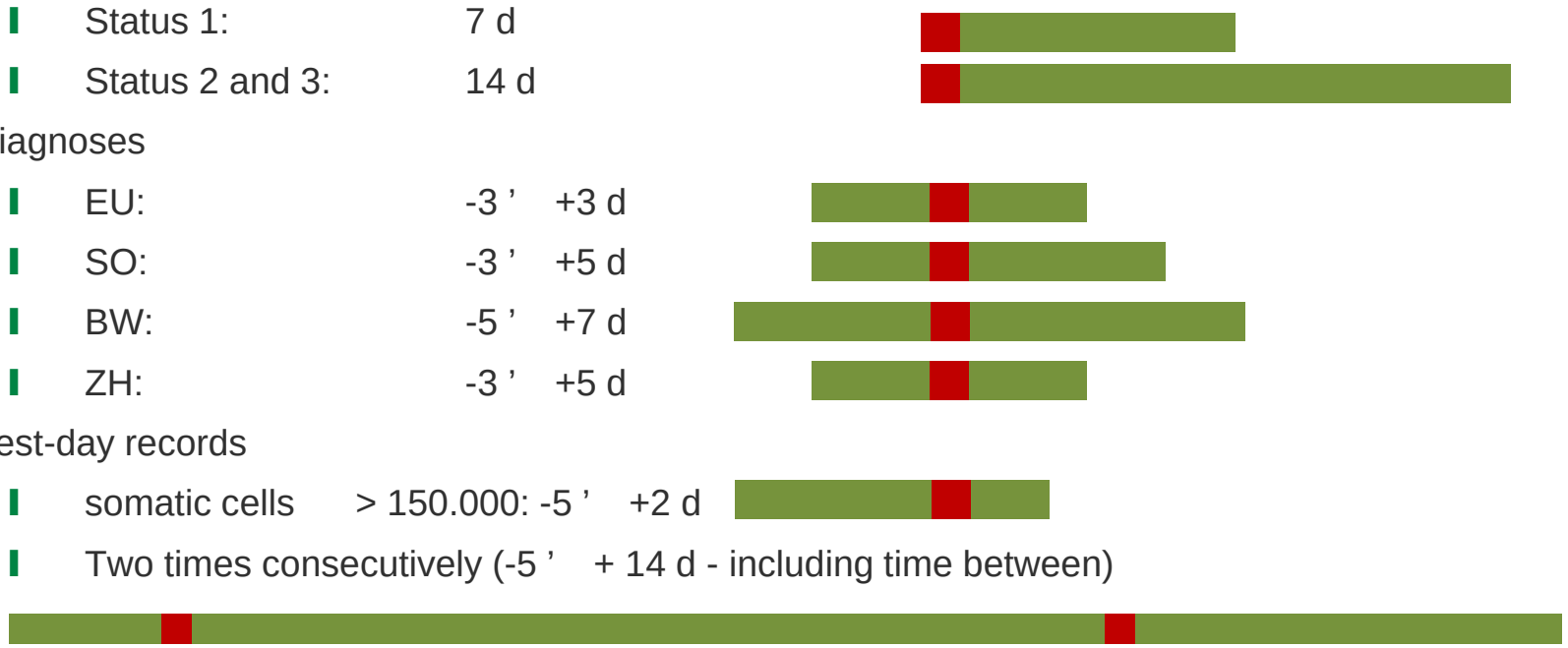
# Materials and Methods



## Expected inflammatory reaction as a reference

We assume that the cow has a inflammatory reaction **around a incidence detected**:

- Calving ease / dystocia (KV)
  - Status 1: 7 d
  - Status 2 and 3: 14 d
- Diagnoses
  - EU: -3 ’ +3 d
  - SO: -3 ’ +5 d
  - BW: -5 ’ +7 d
  - ZH: -3 ’ +5 d
- Test-day records
  - somatic cells > 150.000: -5 ’ +2 d
  - Two times consecutively (-5 ’ + 14 d - including time between)

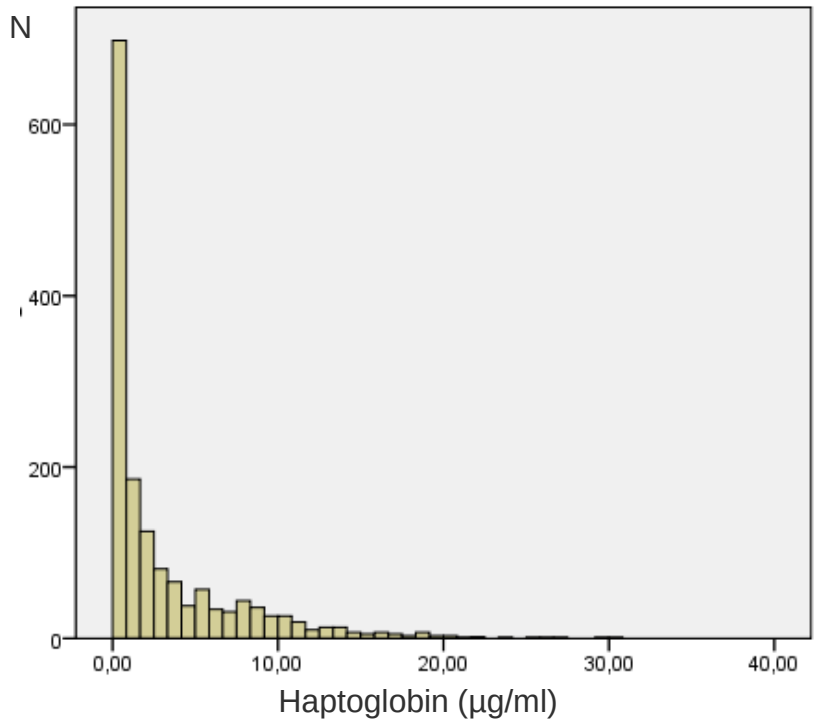


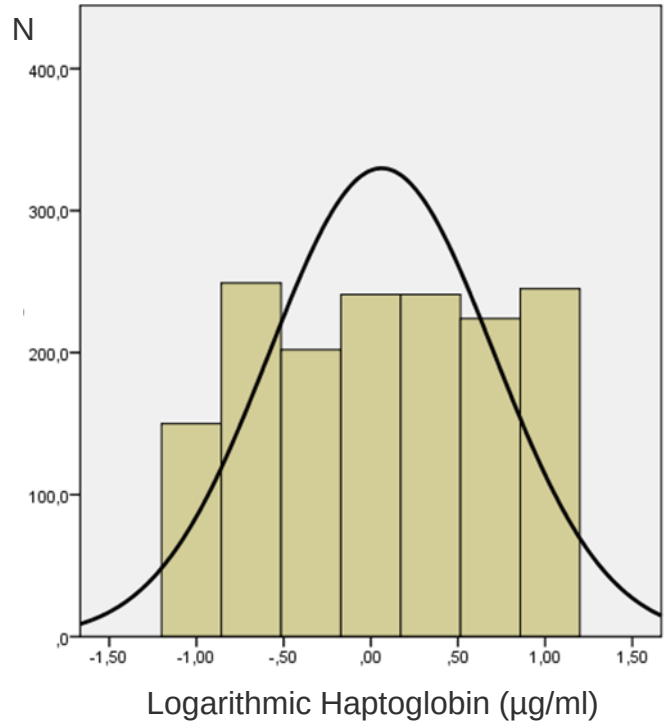
EU = udder diseases / mastitis
KV = dystocia
SO = fever, digestive and respiratory diseases
BW = lameness and hoof disorders
ZH = puerperal diseases

# Materials and Methods

## Haptoglobin in milk

- After data preparation **1552 values** were usable
- 0 – 30 (mean 3.1, median 1.1, s 4.3) - extreme skew distribution
- Logarithmic transformation (mean 0.06, median 0.04, s 0.64)

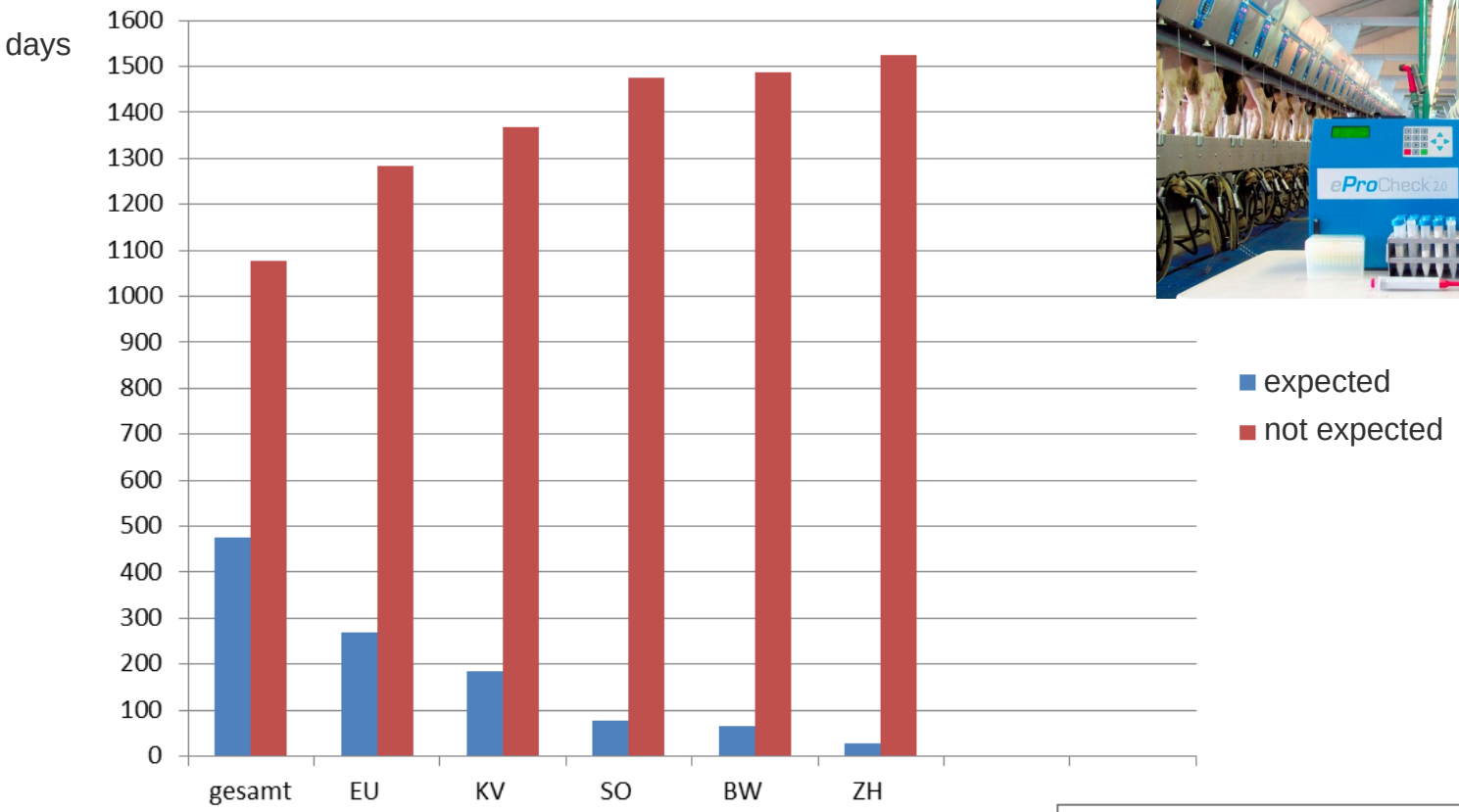


# Materials and Methods



## Days with an expected inflammatory reaction





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# Results

## Results of some preliminary investigations

- **What samples** should be used?
  - Starting milk (a sample immediately after udder cleaning and forestripping) or collection milk (like milk recording): u = 0,85 → both is possible
  - Quarter sample (sample from each quarter) or composite sample:
    - Hp content shows a high variation between quarters
    - All quarters must be includes in a composite sample
- How large is the **repeatability** of the Hp analysis of comparable samples for starting milk, collection milk and the composite sample
  - u = 0,96 - 0.99
  - Test shows a high repeatability

# Results

## Test of influences on Hp in milk

$$y = \mu + ln + lw + diag + animal + e$$

| | | |
|---|---|---|
| y | - | lg of daily Hp in composite milk in µg/ml |
| ln | - | lactation number |
| lw | - | lactation week |
| diag | - | complex of diagnoses |

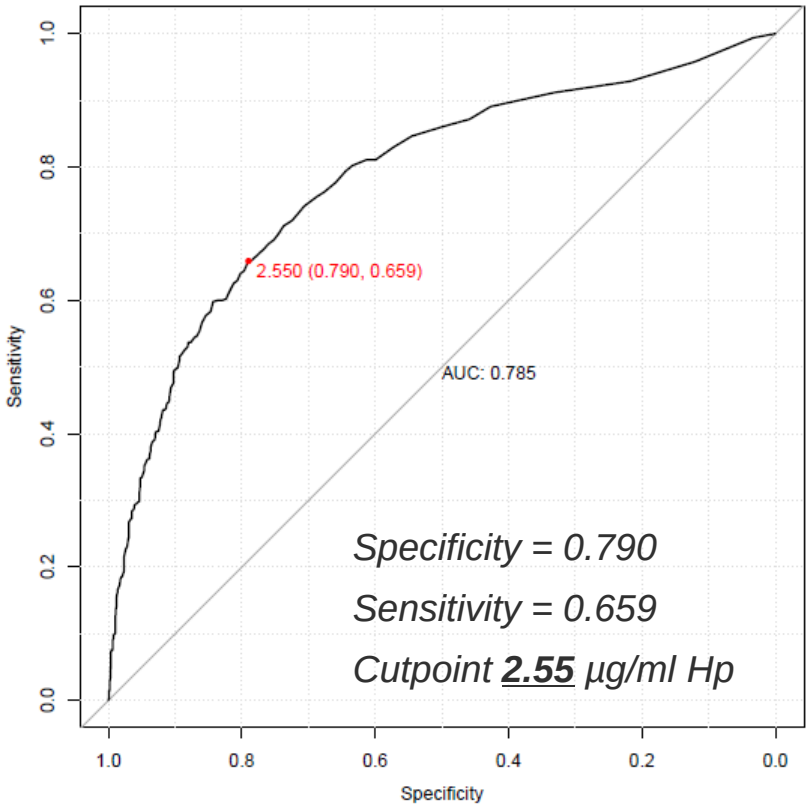
- **Lactation number** has no significant influence
- **Lactation week** has significant influence – first two weeks show higher Hp-concentration
- **Diagnoses**
  - Alle Erkrankungen 0,000***
  - EU 0,000***
  - SO 0,001***
  - ZH 0,029*
  - BW 0,056

EU = udder diseases / mastitis
SO = fever, digestive and respiratory diseases
ZH = puerperal diseases
BW = lameness and hoof disorders

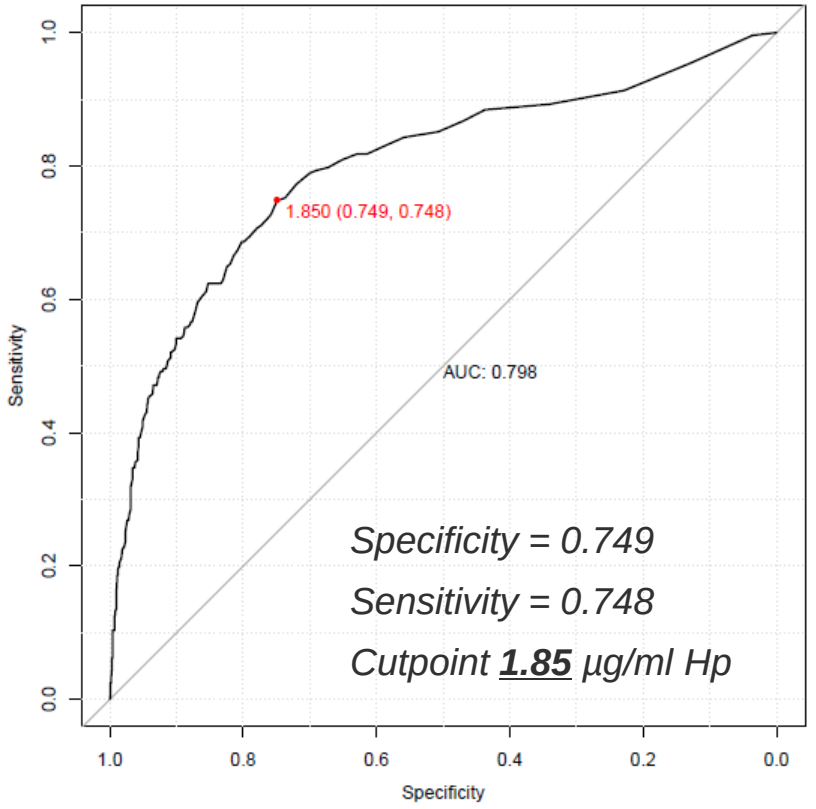
# Results

## Receiver Operating Characteristic (ROC)

All expected inflammatory reactions




All expected inflammatory reactions DIM > 14

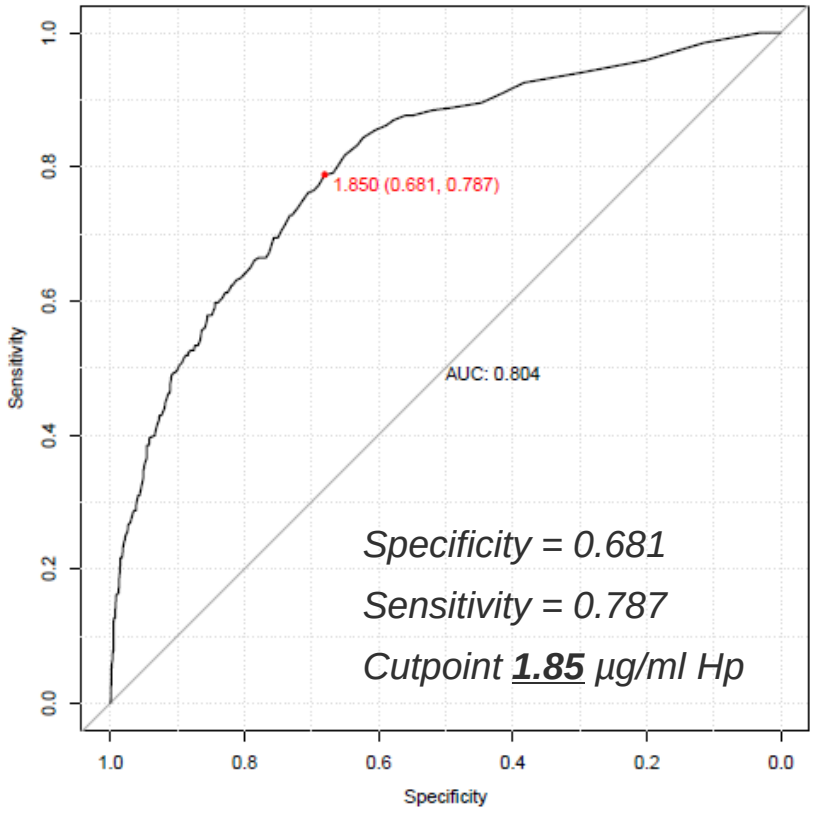


# Results



## Receiver Operating Characteristic (ROC)

Expected inflammatory reactions
from udder diseases

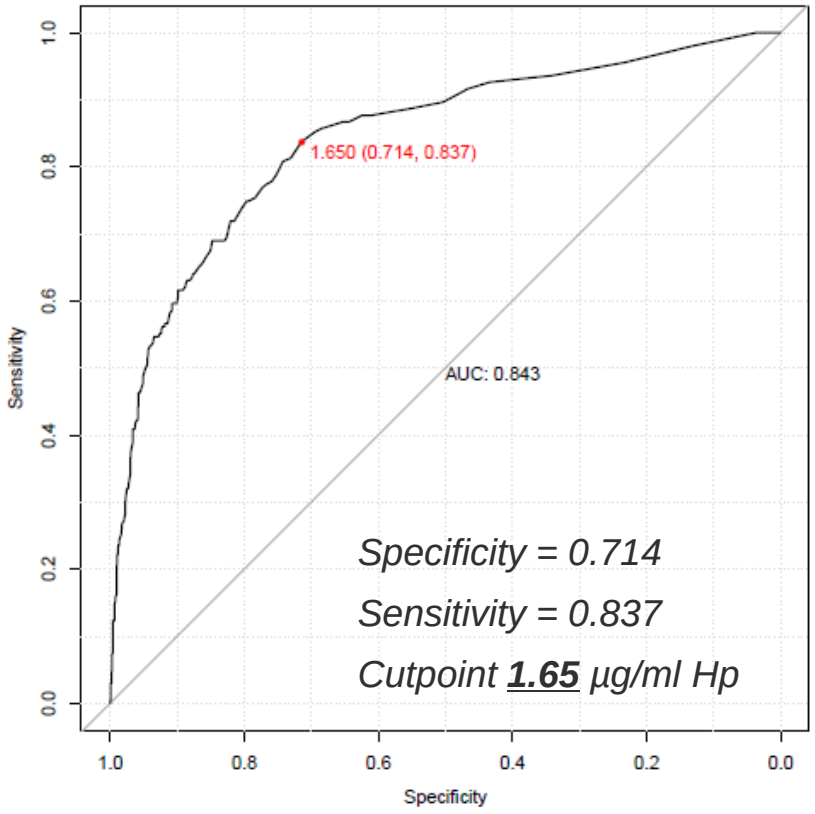



*Specificity = 0.681*

*Sensitivity = 0.787*

*Cutpoint **1.85** µg/ml Hp*

Expected inflammatory reactions
from udder diseases DIM > 14



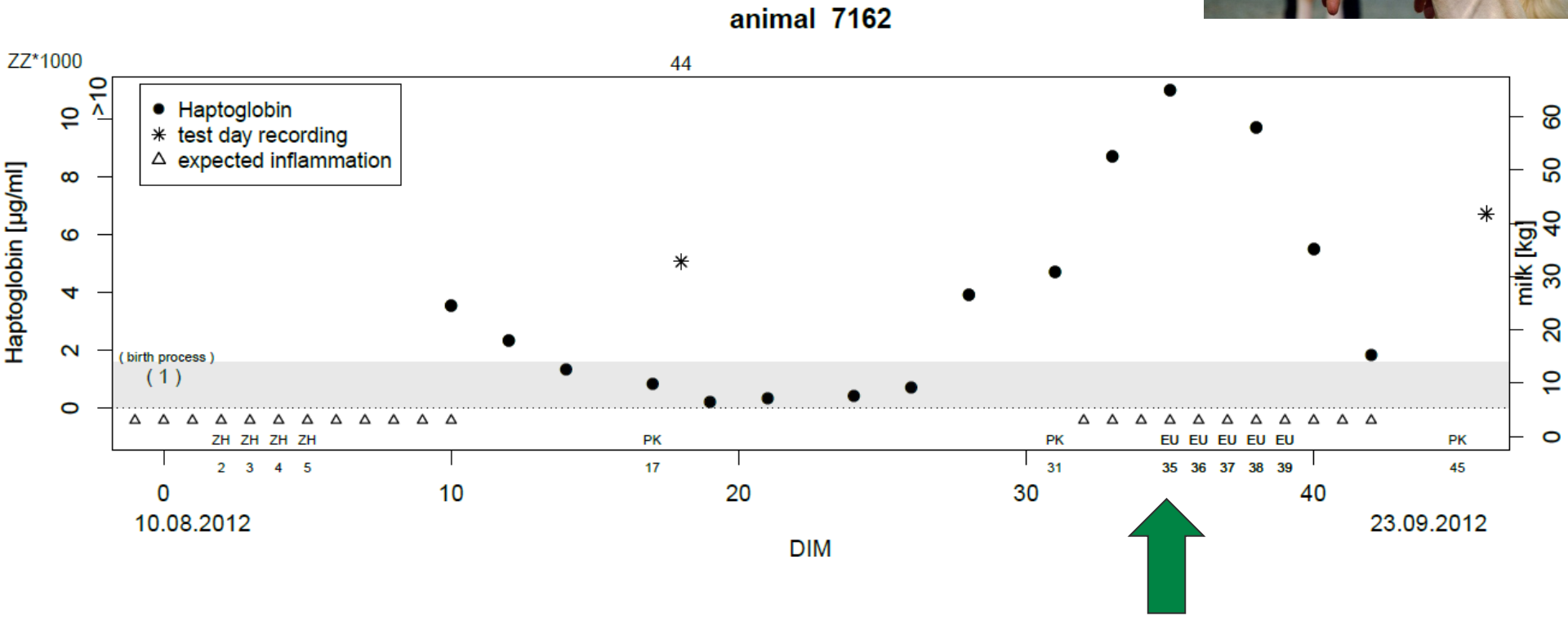

*Specificity = 0.714*

*Sensitivity = 0.837*

*Cutpoint **1.65** µg/ml Hp*

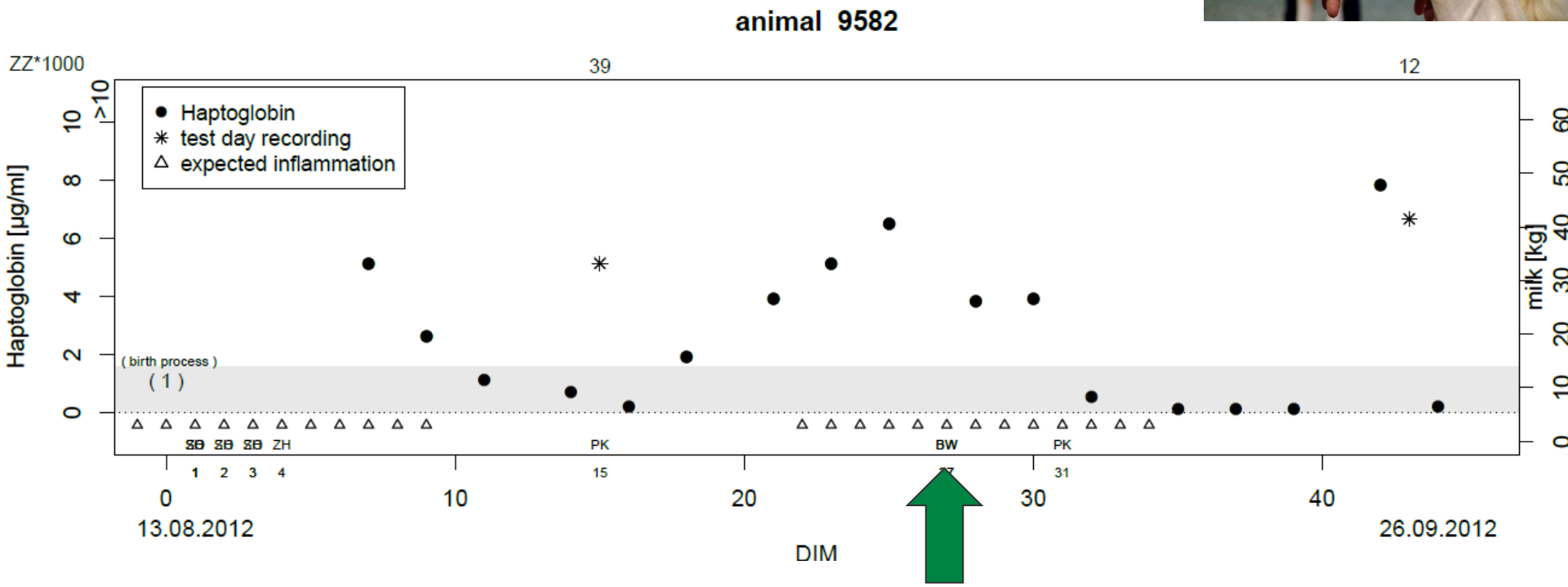
# Results

## Example for mastitis




# Results

## Example for hoof disorders




# Conclusions

- Hp assay in milk **can be performed on-farm** with eProCheck 2.0 device using a Sandwich-ELISA (but costs and effort must be have in mind)
- Test system yields a **high repeatability**
- healthy cows had **undetectable** levels of Hp | increased levels **varied markedly**
- Beside the diseases only the **lactation week** shows a significant influence on Hp-concentration (especially during the first two weeks of lactation)
- **Sensitivity and specificity** is about 0.75, **Hp-threshold** about 1.85
  - from this value a inflammatory reactions seems to proceed
- It seems that Hp in milk can be used as an **early indicator** for systemic inflammatory reactions of the body– not only for mastitis
  - for different disease complexes
  - up to 3-5 day before first visible symptoms
- The test should be used mainly in **sensitive situations**: Start of lactation, dry cow treatment, before first insemination
- could be useful in **diagnosis**, **prognosis** and in **monitoring** response to therapy
- If the test system is able to **monitor the health status of dairy herd (**general health screening) needs further investigation

# Acknowledgements

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