EAAP 2018
69th Annual Meeting of the European Federation of Animal Science
Dubrovnik, Croatia, 27th to 31st August 2018

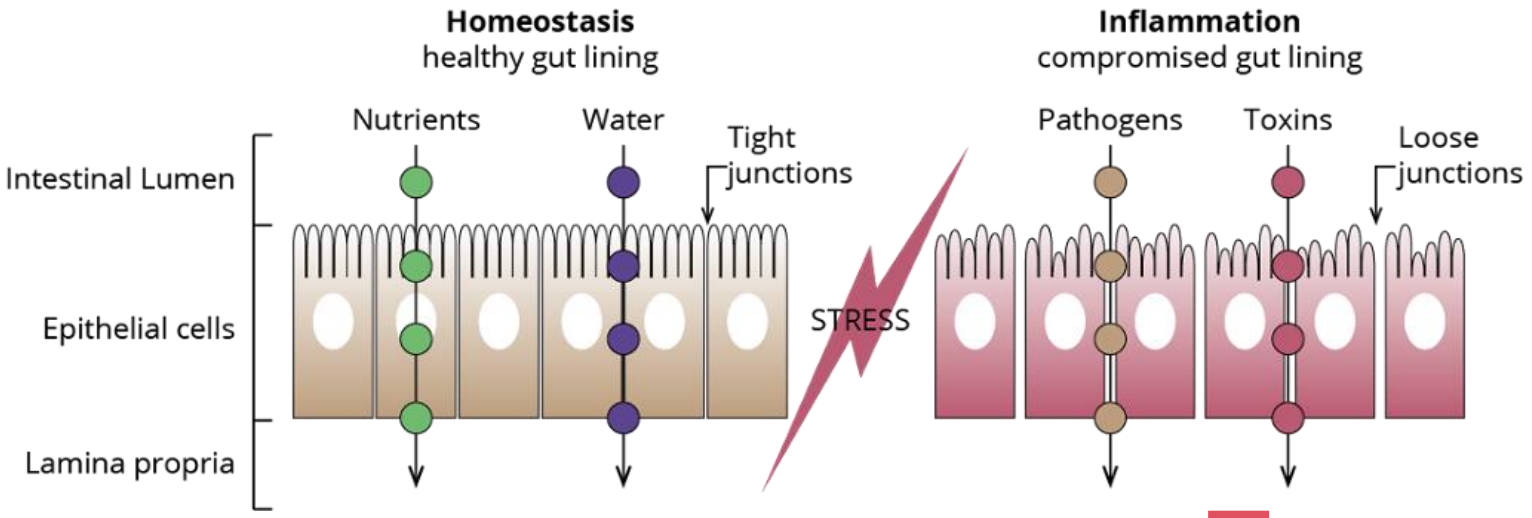
Development of a new ELISA test for Pancreatitis Associated Protein detection in pig

Mariani E.[1,2], Savoini G[1]. and Niewold T.[2]

[1] Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Milan, Italy
[2] Nutrition and Health Unit, Department of Biosystems, Faculty of Bioscience Engineering, KU Leuven, Heverlee, Belgium.

Background: Intestinal Inflammation

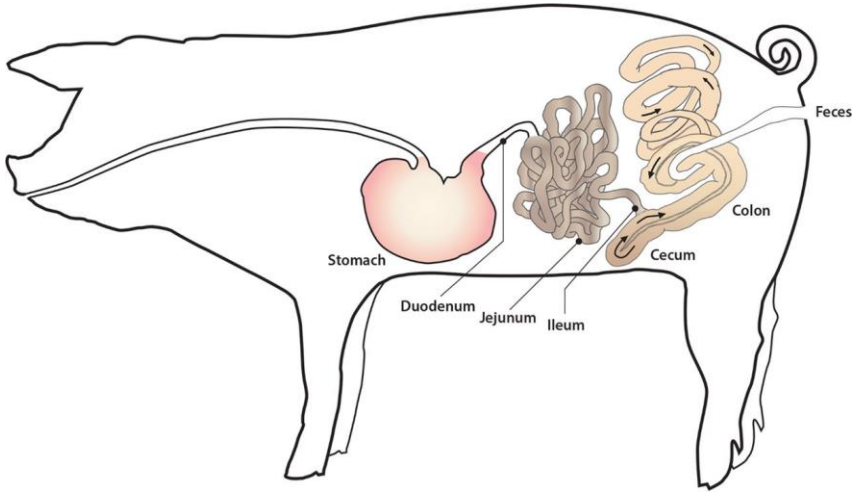
Gastro intestinal tract is a site of interaction between pig and the environment



- Lower appetite
- Muscle catabolism
- Pathogens
- Disease/Pathology

(Niewold et al.,2015)

Background: Biomarkers



The larger part of GI-tract is accessible only throughout invasive techniques

Endoscopic technique

Mucosal Biopsies

Surgery

Non invasive biomarkers

- MPO
- IFABP
- PAP/Reg3

Aim of the study

The aim of this study was to develop a new Sandwich ELISA test for the detection and quantification of Pancreatitis Associated Protein (PAP/Reg3γ) in pig

Materials and Methods: Developing phase

- Sandwich ELISA protocol
  - Polyclonal rabbit antibody
  - Pure peptide
  - Biotinylated detection antibody
  - Streptavidin HRP-conjugated
  - ABTS

- VICTOR3™ multilabel plate reader, for absorbance measurement (after 90 minutes of dark incubation)

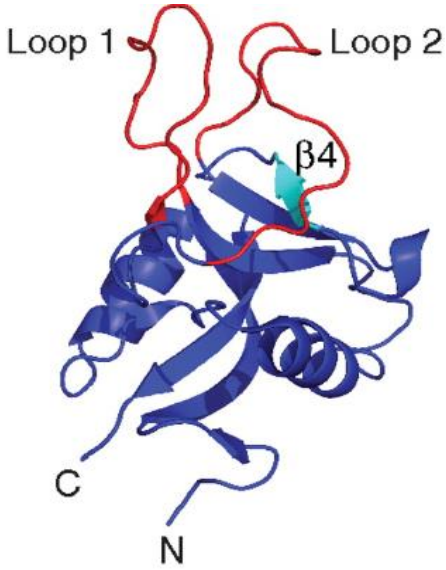
Materials and Methods: Developing phase

Pure peptide: most immunogenic part of native protein sequence (Soler et al., 2015):

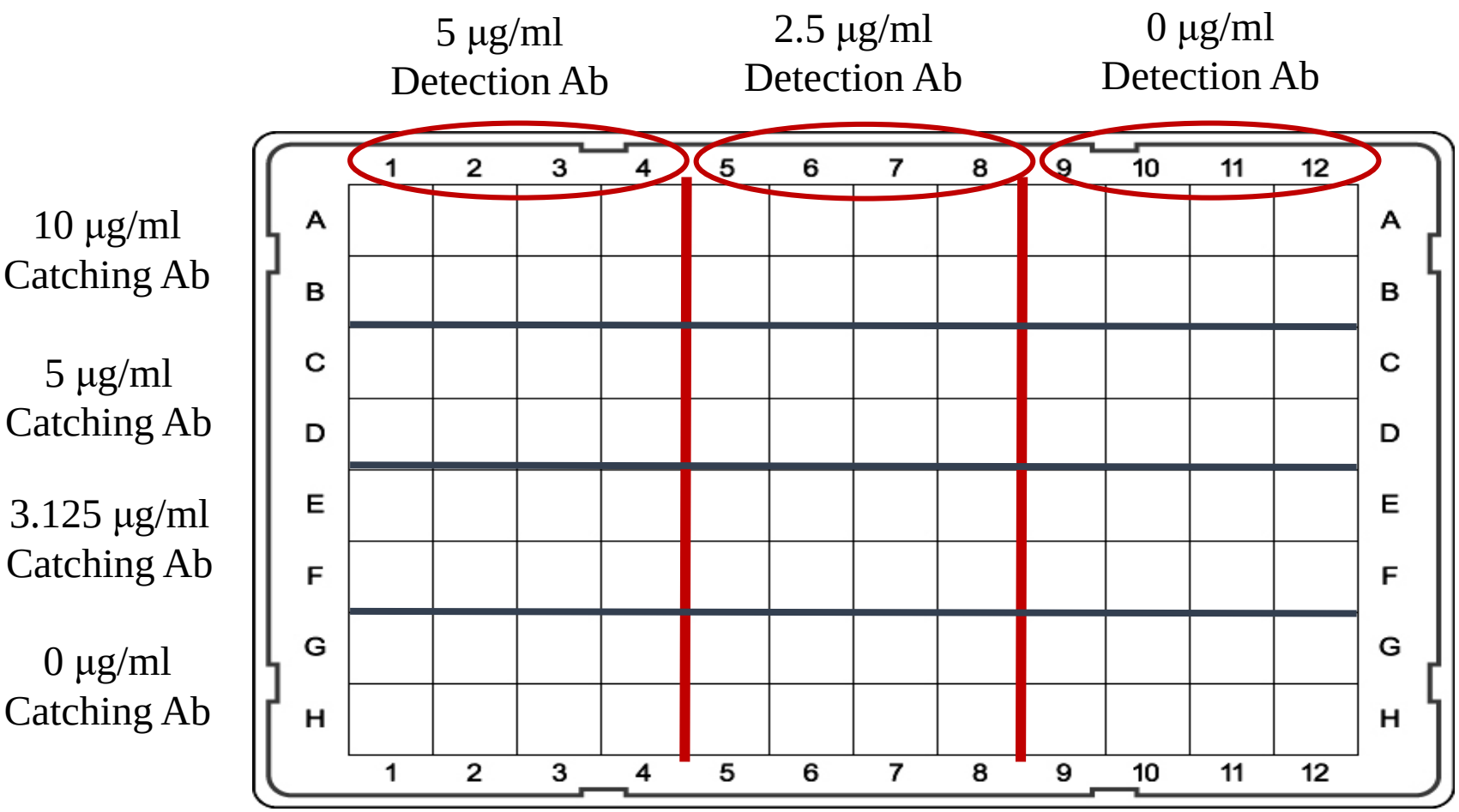
MMLPSMSLPSLSWMLLSCLMLLSQVQGE DSPADTPSARISCPKGSMAYASY CYALFITPK **60**

TWMGADMACQKRPSGHLASVLSGAEASFVSSLIKNNLNALSDVWIGLHDPTEGLEPNAGG **120**

WEWSSSDVLNYVAWERNPSTSSYPGYCGSLSRNTGYLKWRDYNCYVNLPYVCKFKG **176**



Materials and Methods: Developing phase



Materials and Methods: Validation phase

- Two fecal samples with known MPO activity values
  - Low: 78.85 mill Units/mL
  - High: 350.09 mill Units/mL
- Extraction in PBS (1g of feces + 1mL PBS → double centrifugation)

→Dilution factor of extracts: 1:10 – 1:50 – 1:100

Hypothesis: High MPO = High Reg3γ

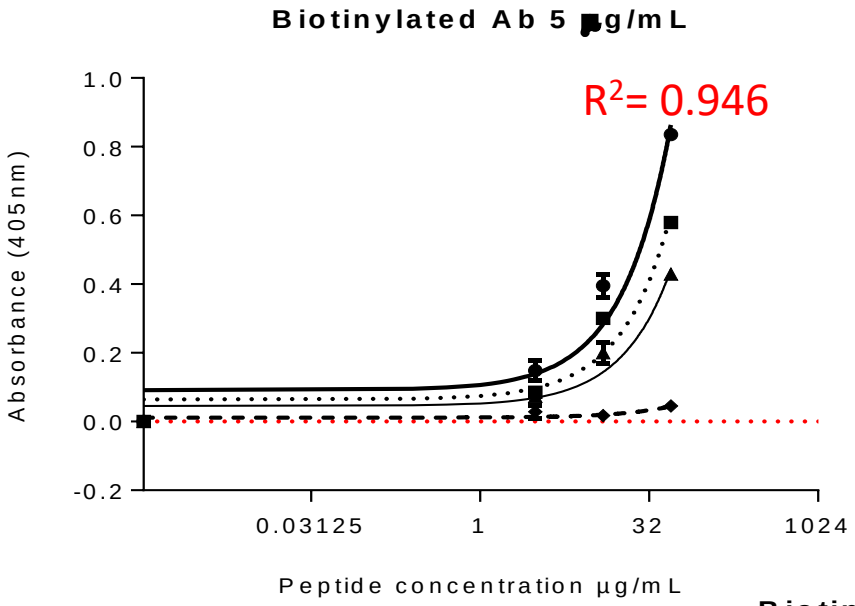
Materials and Methods: Stability test

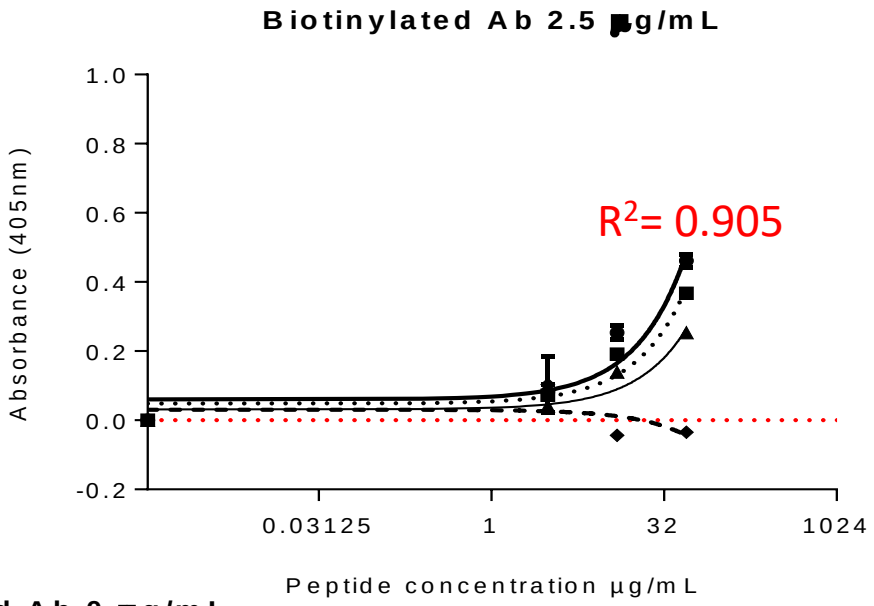
- With and without Protease Inhibitor cocktail (PIC)
- Three different conditions:
  - Cold room: +4° C
  - Room Temperature (RT)
  - Water bath: +37° C
- Time sampling: +4h, +8h, +24h
- Control: immediately frozen supernatant (+/- PIC)

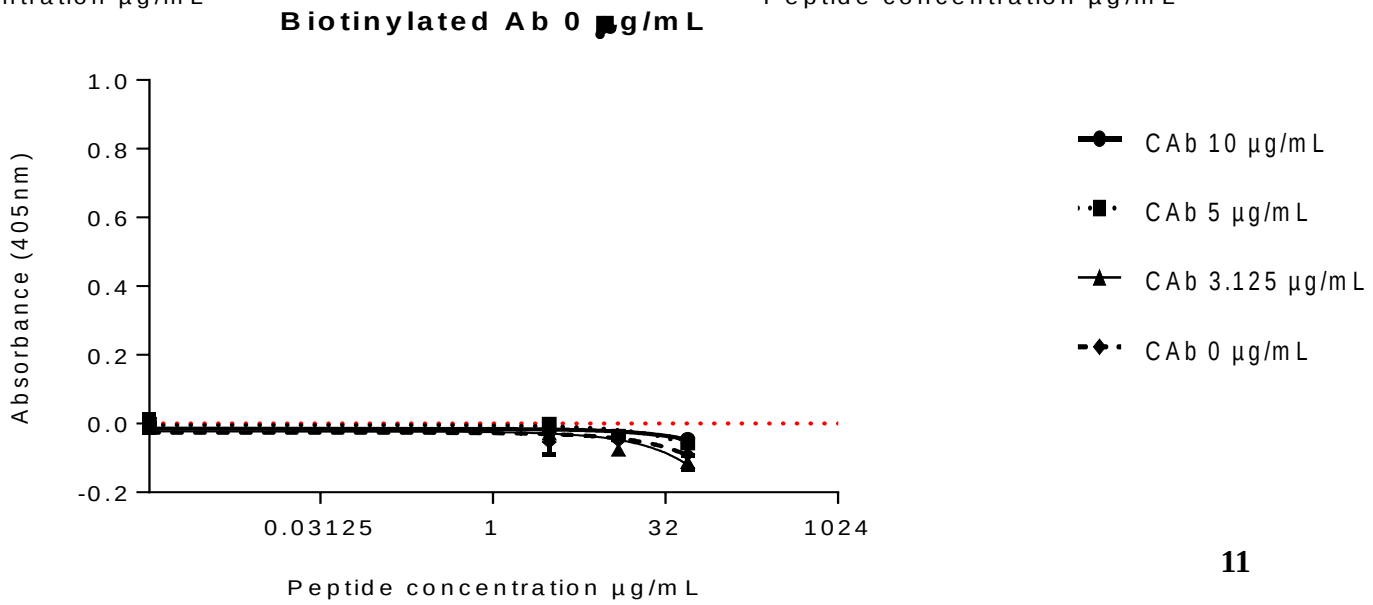
Materials and Methods: Statistical Analysis

- Linear regression and Interpolation of standard curve was performed with GraphPad Prism® v.7.04 software.
- Results from stability test were analysed with a GLM and a MIXED procedure using SAS 9.4.

Results: Developing phase





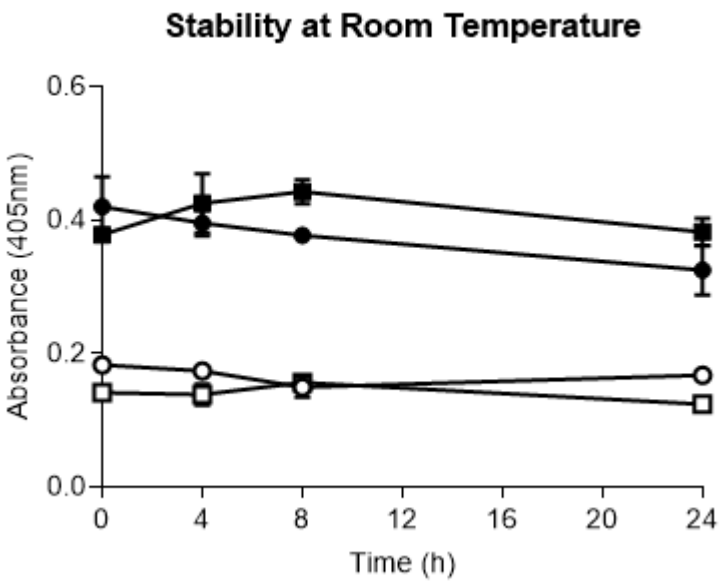


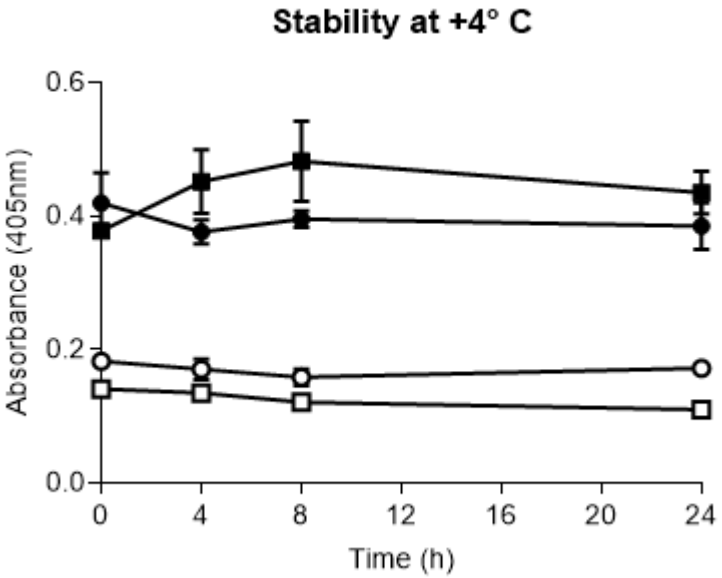
Results: Validation phase

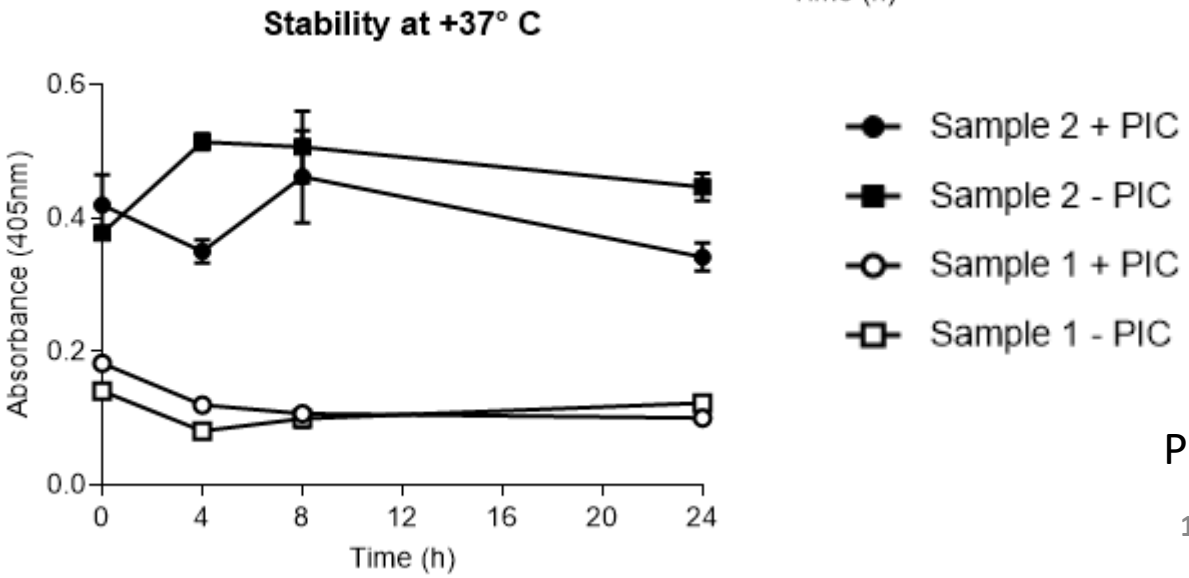
Sample	MPO Activity (millU/mL)	Dilution Factor	Corrected average absorbance value (405nm)	Final Reg3γ concentration (μg/mL)
1	78.85	10	0.169	129.552
		50	0.083	296.907
		100	0.028	177.474
2	350.09	10	0.505	507.032
		50	0.157	599.053
		100	0.079	562.570

Table 1: Average absorbance values (n=2) and Reg3γ concentrations (μg/mL of peptide equivalent) of samples with known MPO activity values.

Results: Stability Test







P>0.05

Conclusions

- The set up protocol works and has an high specificity for the peptide's sequence
- The sandwich ELISA test developed is able to detect porcine Reg3γ in complex matrices as faeces.
- Correlation between MPO and PAP/Reg3γ (??)
- Immunoreactivity stable at different temperatures

PAP/Reg3γ could be a promising biomarker!

Thank you for the kind attention