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Novel diagnostic assay (based on glucometer) for bovine infectious diseases

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Background

- Current disease control strategies in cattle industry are based on improving animal health management and reducing the risk of disease introduction by animal additions
- These control strategies rely on efficient disease detection methods.
- Current diagnostic methods are lab based and expensive
- Therefore there is an urgent need for new diagnostic tests that can be performed on farm

Objectives

Develop a split enzyme able to detect the exposure to specific infectious pathogens in a timely manner that will be compatible with pen-side diagnostic testing.

Aims

1) Design a diagnostic test for detection of various markers of exposure to infectious diseases (antibodies) or infectious pathogens themselves based on the use of a common glucometer.

2) Apply the diagnostic test to detect: antibodies (e.g Bovine Leukemia Virus (BLV)) and whole bacterial pathogens (e.g. *Staphylococcus aureus (SA)*);

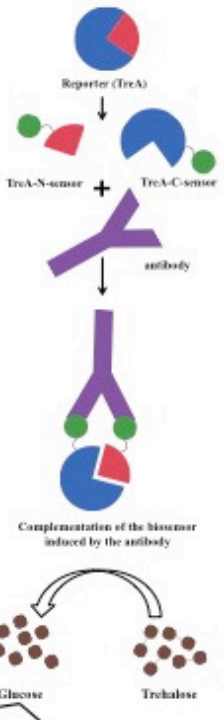
Biosensor Principle

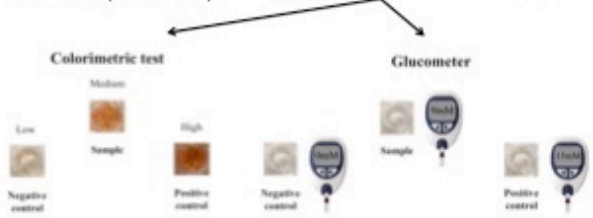
Step 1: *The choice of the reporter/ signal*. This detection assay is based on *E. coli* enzyme trehalase (TreA) that produces glucose from trehalose and therefore enables the use of plain glucometer.

Step 2: *Design of detection assay*. TreA is split in 2 non-functional fragments (N-terminus and C-terminus) fused with peptides (e.g. epitopes) or whole proteins (e.g antigens) that act as sensors for disease related analyte (e.g. antibodies, bacterial cells).

Step 3: *Signal detection*. Interaction between sensors and analytes specific to the sensors leads to the complementation and subsequent reporter activation which then produces glucose from trehalose.

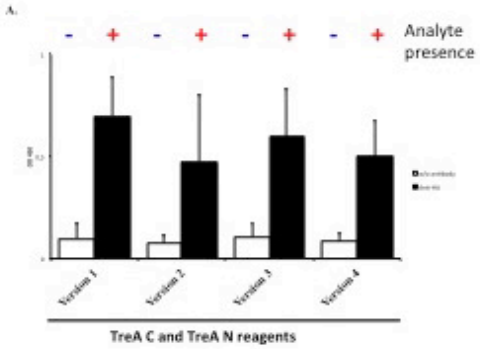
Step 4: *Measurement of the ouput signal*. Signal is then detected in two ways: by a glucometer (mM of glucose) or by a colorimetric assay including Glucose oxidase (GOx), Horseradish peroxidase (HRP) and O-dianisidine (=substrate).





Results

Detection of antibodies (e.g. detection of exposure to viral diseases like BLV, influenza or bacterial diseases like Johne's disease)



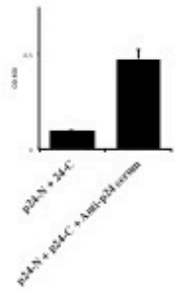


Fig. 1.Detection of antiviral antibodies by TreA split detection assay. A) TreA reagents C and N fragments carrying small viral epitope (HA) were incubated with anti-HA monoclonal antibodies or without. A) TreA C and N fragment caring viral antigenic protein (p24) as a sensor incubated with non purified anti-p24 serum or without.
Signal was detected with colorimetric Gox-HRP assay.

Detection of total antibodies (e.g. detection of IgG content in colostrum)

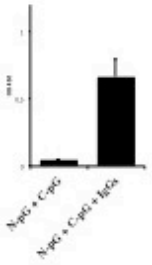
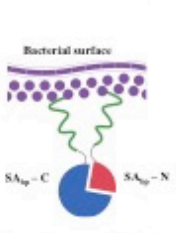


Fig. 2. Detection of total antibodies by TreA split detection assay. TreA C and N fragments carrying protein G (recognizes total IgG) were incubated with purified bovine IgG or without.
Signal was measured with colorimetric Gox-HRP assay

Detection of whole pathogens (e.g. detection of bacteria like *Staphyloccocus aureus* – mastitis pathogen)



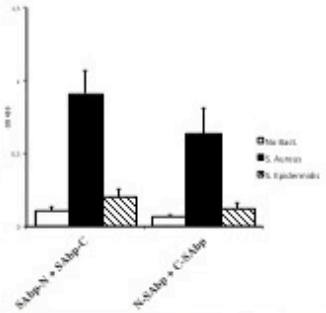


Fig. 3. Detection of whole bacterial pathogens by TreA split detection assay. TreA C and N fragments carrying *S. aureus* binding peptide (SAbp) were incubated with *S. aureus*, *S. epidermidis* or without.
Signal was determined with colorimetric Gox-HRP assay

Relevance

The outcome of the proposed project will be an easy-to-use diagnostic test for several infectious diseases in the cattle industry, that will facilitate and encourage more frequent monitoring of individual animals and entire herds and will be used in decision making strategies to control and manage these diseases.

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