Light Scattering Particle Immunoassays in Lab-on-a-Chip for Infectious Agents in Livestock Barns

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Mission statement

- Quarantine and inspection
- Sanitary control for livestock products
- Animal disease control
- Research and development (in veterinary science)
- Animal medicine
- Animal protection
- Veterinary diagnostics
Veterinary diagnostic tool that is:

- Early
- Point-of-care
- Ultra-low detection limit
- Automated

To detect infectious diseases in livestock and poultry:

- Bovine Viral Diarrhea (BVD) - cattle
- Porcine Reproductive and Respiratory Syndrome (PRRS) - swine
- Salmonella - poultry
- Avian influenza (AI) - poultry
• BVDV & PRRSV

BVDV: one of the most significant viral infections in cattle
- Affects both dairy and beef cattle
- Infected cattle are more susceptible to many respiratory and intestinal pathogens

PRRSV: positive-strand RNA virus
- 50-65 nm (enveloped virus); 25-35 nm (nucleocapsid core)
- Abortions, stillbirths, weak piglets
- Respiratory diseases
- Secondary infections in adult pigs
BVDV Virus

- Neurological defects in calves
- Infertility, abortion
- Nasal discharge
- Genital ulcers
- Oral ulcers
- Erosions
- GI ulcers
- Immunotolerance vs Immunosuppression
- BRD (immunosuppression), reproductive disorders, BVDV, acute mucosal disease, chronic mucosal disease
Salmonella typhimurium: Gram-negative bacteria

- Cylindrical rod
- 2-3 µm (length); 0.6-0.9 µm (diameter)
- Food poisoning (gastroenteritis)
• Detection limits of current methods

ELISA: 50 pg

PCR: 10-100 TCID_{50}/ml
Lab-on-a-chip (LOC)

- A semiconductor chip used to analyze the composition of a person’s body fluids (blood, saliva, urine) for medical purposes. It combines microfluidics and semiconductor processing on the same chip.
- Eliminate human effort such as pipetting.
- Many biological reactions are diffusion-limited; Miniaturization can speed up the reaction.
- Hundreds of assays can be performed simultaneously, saving considerable time and effort.
- We will use LOC for veterinary diagnostics
- **Latex immunoagglutination assay (LIA)**

- LIA has NOT been demonstrated in LOC (diffusional mixing issue)
- First demonstrated by UA Biosensors Lab in 2006
  
**Procedure**

- Antibody-conjugated structured particles
- 390-, 510- or 920-nm (mostly 510-nm) Highly carboxylated polystyrene particles

Duplicate experiments w/ two-well slide (75 µl, 800 µm depth)

Light scattering detection w/ optical fibers

Target solution

380-nm LED

Miniature spectrometer

Syringe pump

Positioning stages

45 degree forward light scattering detection
- Bovine Viral Diarrhea Virus (BVDV): 2-well

**Detection limit**
1~2 TCID$_{50}$/ml

**Monoclonal antibody**
Tissue: MDBK
Washed by centrifuging

• BVDV: microfluidic

Detection limit: 1 TCID_{50}/ml
BVDV: RT-PCR

- **Denaturation**: 95°C
- **Annealing**: -50°C
- **Extension**: 72°C
Gel electrophoresis of BVDV PCR products ranging from 1-10^4 TCID_{50}/mL

Bands observed as low as 10^3 TCID_{50}/mL

Porcine Reproductive & Respiratory Syndrome Virus (PRRSV): 2-well

Live vaccine (attenuated virus)
Similar to BVDV
• PRRSV: microfluidic

Detection limit: $10^{-3} \text{ TCID}_{50}/\text{ml}$
• PRRSV: time-course detections

Maximum signal at 2 min.
Gel electrophoresis of PRRSV PCR products ranging from $10^{-1}$-$10^5$ TCID$_{50}$/ml

Bands observed as low as $10^2$ TCID$_{50}$/ml
• Salmonella typhimurium: 2-well

Salmonella: 2-well (MES, KPL antibody)

Salmonella: 2-well (PBS, KPL antibody)

Cultured in media, washed by centrifuge
Polyclonal antibodies
Salmonella: 2-well & microfluidic

Detection limit: 0.5-50 CFU/ml
- Salmonella: immunoagglutination images with 910-nm beads
• Other targets under testing

- *Brucella abortus*
- Influenza A (H3N2)
• **Conclusion**

- Detection limits: BVDV <1 TCID$_{50}$/ml (MAb)
  PRRSV $10^{-3}$ TCID$_{50}$/ml (PAb)
  Salmonella <5 CFU/ml (PAb)

  - Maximum signal achieved at 2 min
  - Total assay time <5 min

• **Future work**

- Integrate optical fibers (optical waveguide)
- Replace miniature spectrometer with Avalanche photodiode (APD) and computer/software with op-amp circuit/voltmeter
- Test for field sample
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