

Rapid Immunoelectrophoretic Assays for Pen-Side Diagnostics

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Disruption of agrobusiness has large economic consequences

US livestock is extremely vulnerable to intentional (agroterrorism) and natural outbreaks of infectious diseases and agroterrorism poses a huge threat to economical and sociopolitical well-being

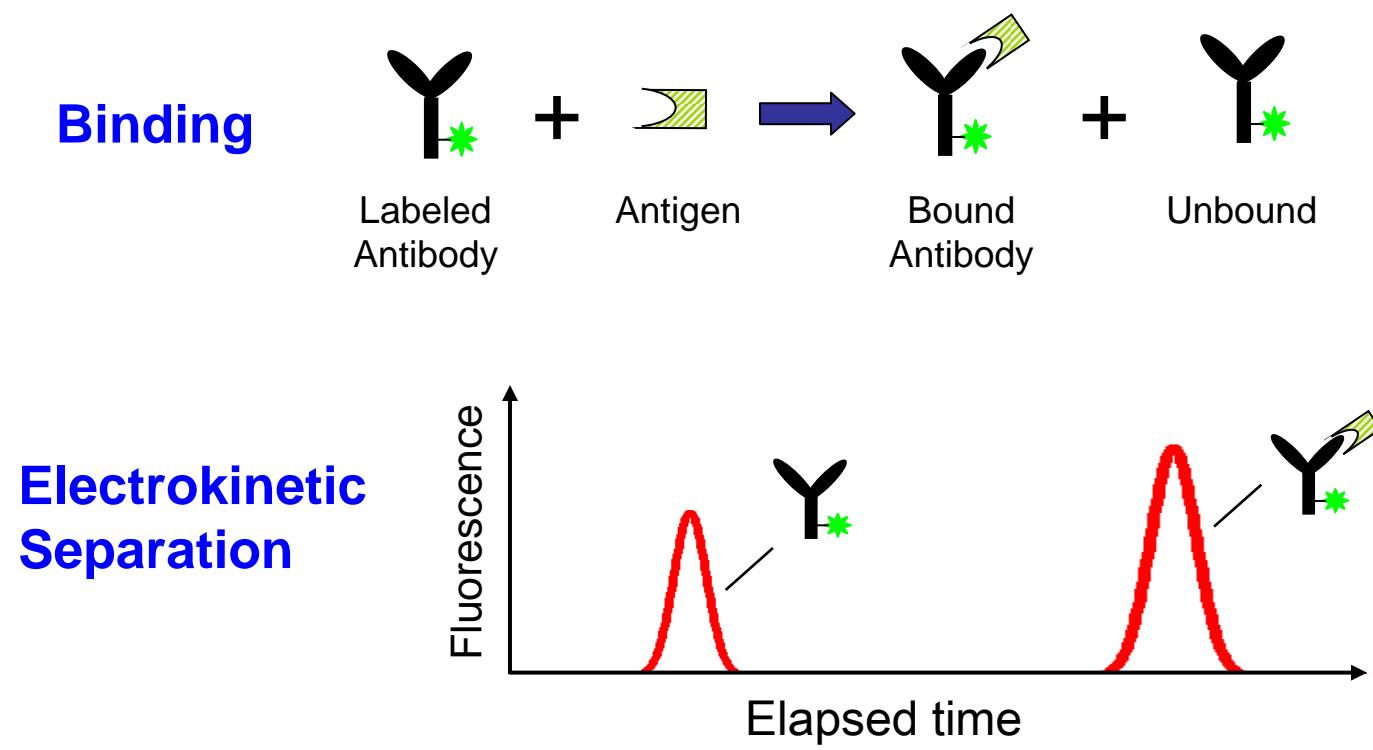


2001 Foot-and-Mouth Disease (FMD) outbreak in UK led to the slaughter of 4 million animals and \$40B in lost revenue

"Agroterrorism is not about killing cows...it's an economic assault on national security and infrastructure"
Jerry Jaax, Ex-Chief of Vet. Med., USAMRIID

Pen-side diagnostic devices are needed for early detection and consequently, containment of an outbreak

Microchip electrophoretic immunoassay allows rapid analyte quantification



1. Mix labeled antibody and antigen
2. Separate out bound and unbound antibody using electrokinetic methods
3. Measure the peaks for bound vs. unbound

photopolymerized A. Horwitz et al., J. Am. Chem. Soc., 2005, 127, 8000-8001. [DOI 10.1021/ja044750u](http://dx.doi.org/10.1021/ja044750u)

6% acrylamide gel Acknowledgments: This work was funded by Sandia National Laboratories, Louise Barrett, Amy Horwitz, Jill Pischke, and Bruce Gharib. Directed Research and Development funding was provided by Sandia National Laboratories. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Security Administration under Contract DE-AC04-94AL85000.

Foot-and-mouth disease is dangerous to US livestock industry

- A highly communicable viral disease of cattle and swine
- Also affects sheep, goats, deer.

Symptoms: Fever, blisters and erosions on the tongue, lips, in the mouth, and between the hooves.

Outcome: -Many affected animals recover, but are debilitated
-severe loss in the production of meat and milk
-fatal in newborn animals

Inactivated swine influenza was used in place of FMD for this work

Live FMD work can only be performed at the USDA lab at Plum Island, NY

Swine flu virus was chosen to test our ability to detect a virus, as Influenza antibodies and viral samples are available

Bovine enterovirus was used a negative control for swine influenza experiments

Rapid
Why is it a threat?
Fleecelike MDV, cross-reactive
Hand-held device
Extremely contagious
Quite stable (can survive in food, water, wate

er, wate

FCID₅₀ (est.)

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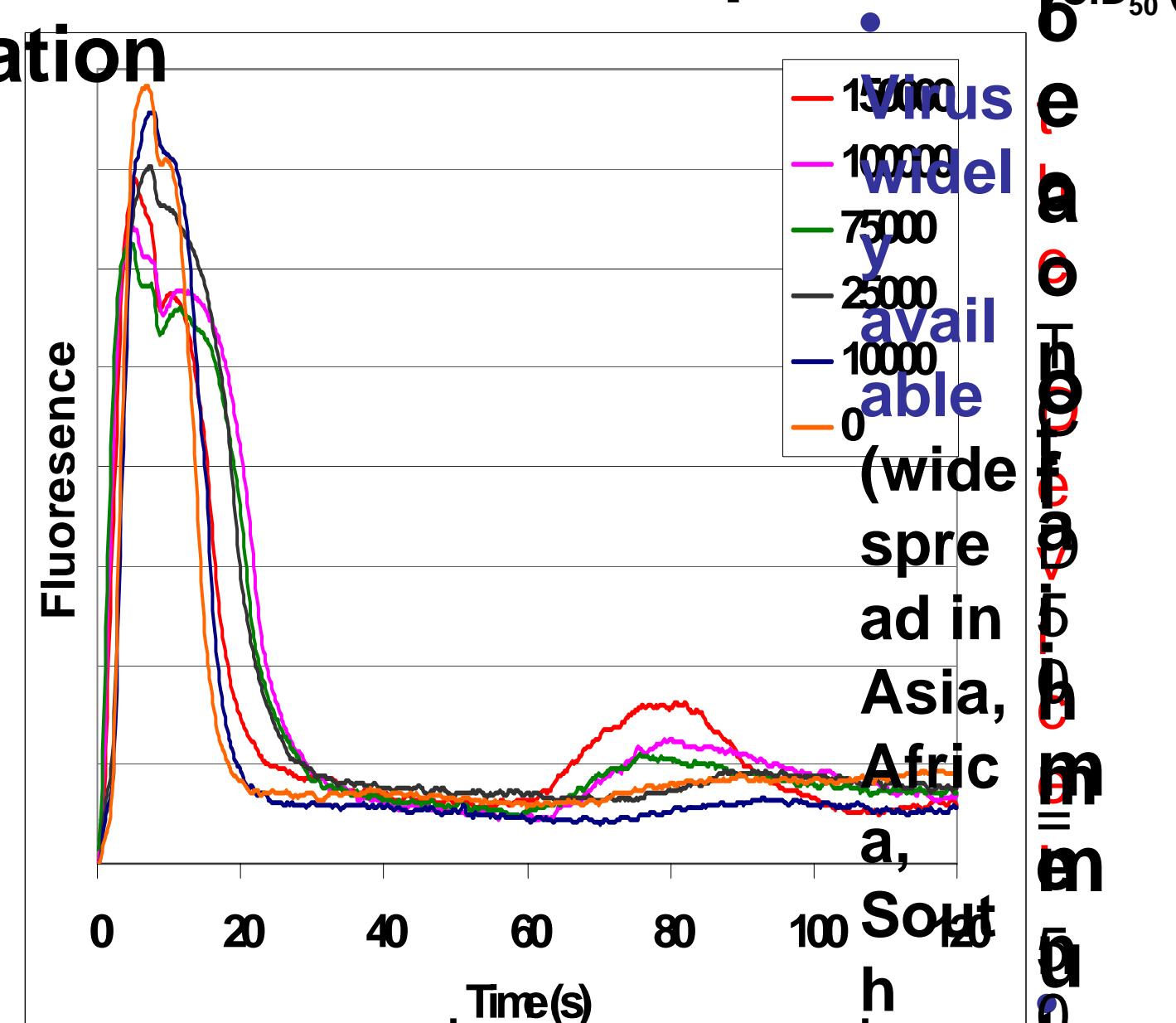
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Swine flu virus forms a complex with antibody and is detectable in an open channel separation

- Complex peak increases with increasing amount of virus.
- Separation of bound and unbound virus in 2 min
- Open channel format more difficult than gel electrophoresis



Fluorescence
Time(s)
Labeled + Unlabeled
15,000 TCID₅₀ (est.)
10,000
7,500
5,000
2,500
1,000
0 (wide spread in Asia, Africa, South America, Europe, and US)

Sample controls: Bovine enterovirus alone does not fluoresce. Antibody alone produces minimal fluorescence. Antibody + antigen produces a complex peak that is proportional to antigen concentration.

Bovine enterovirus was used as a negative control, and gave similar results to antibody alone.

Sample concentrated for 60 seconds, membrane flushed for 180s (4 min time). Off-chip antibody-antigen incubation in ~5min.