# Use of ACIA as a screening test for AI Surveillance

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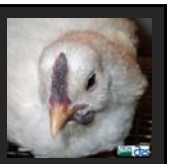
## Why use ACIA?

- Rapid, highly specific, on-site testing method
- ACIA in the NPIP
  - §145.14 and §146.13
    - *Paraphrase:* Use USDA licensed and OSA approved kits
    - *Paraphrase:* Use in accordance with manufacturer's instructions
    - "Chicken or turkey flocks positive on the ACIA must be further tested using the rRT-PCR or virus isolation."

# PROPERTIES OF ACIA


# Experimental Data



- Three replicates with different isolates in chickens
  - A/chicken/PA/1370/1983 H5N2 HPAIV
    - Long mean death time
    - One dose
  - A/chicken/Jalisco/12283/1012 H7N3 HPAIV
    - Short mean death time
    - Two doses
  - Had equal numbers of direct inoculates (n=50) and contact transmission birds (n=50) added to isolators 12hr PI

## HPAIV detection with ACIA: Clinical condition and timing

Challenge

Hours post challenge

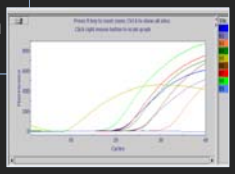
0 12 24 36 48 60 72 84 96 108

- Add contact transmission birds
- Collect oral swabs

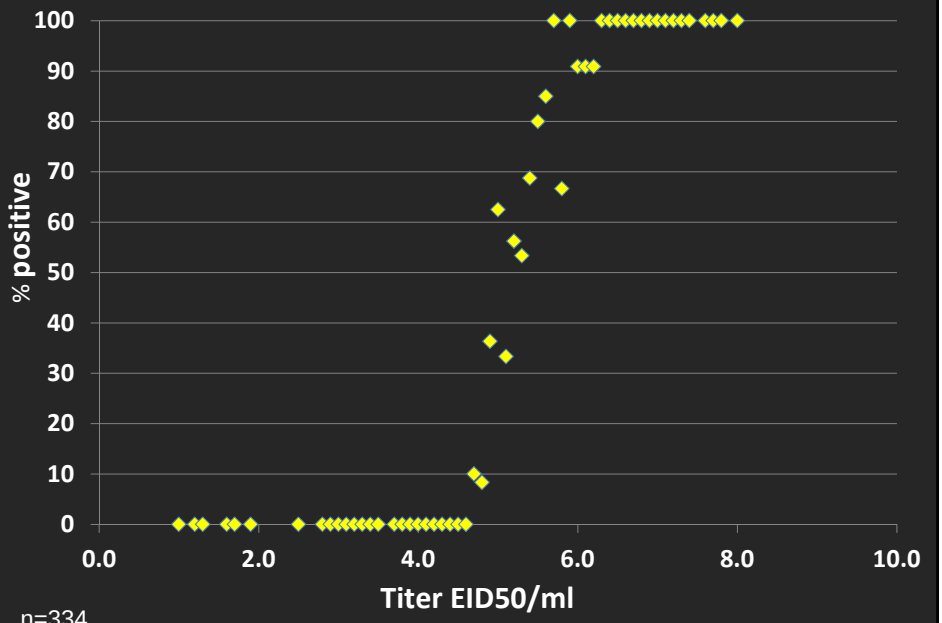
- Record clinical condition
- Collect OP swabs from live birds and 50% of dead/euthanized birds
- Test swabs immediately by ACIA
  - Results read by 2 people independently
- Hold 50% of dead birds until next sample time

Clinical conditions recorded as:
- Healthy
- Sick
- Dead

Virus titers were quantified from each swab by rRT-PCR

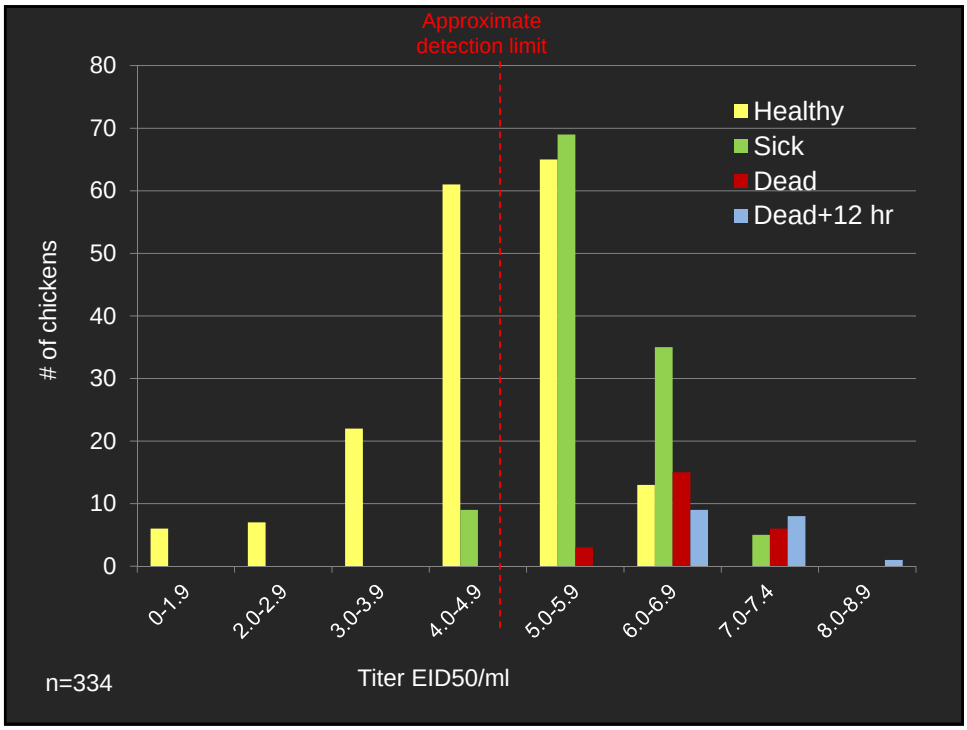


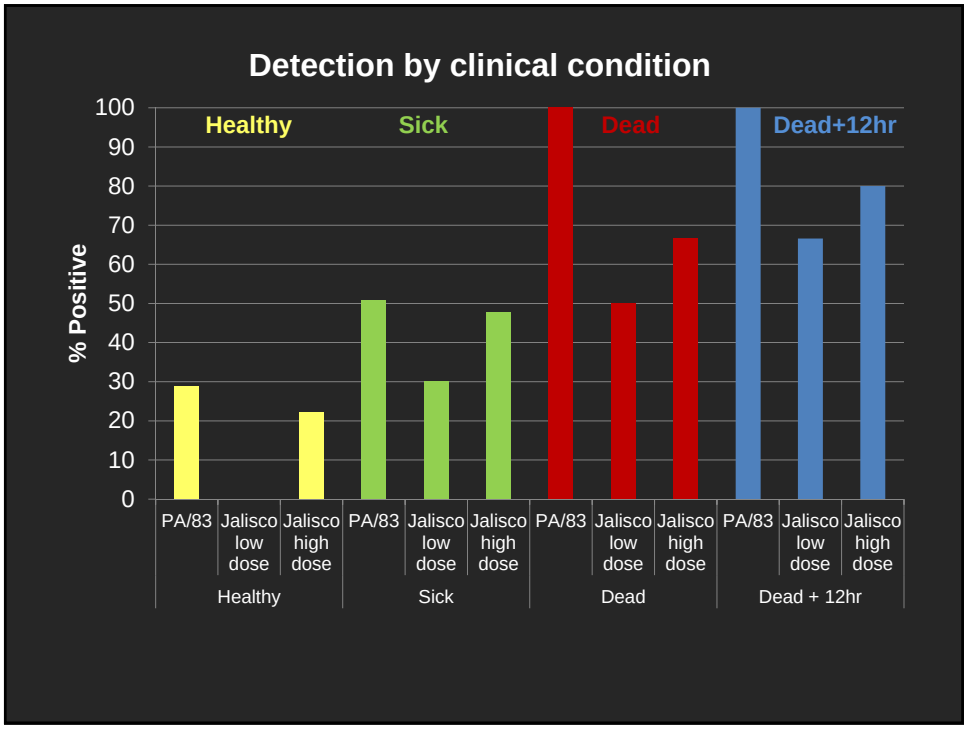
## Detection is titer based




n=334

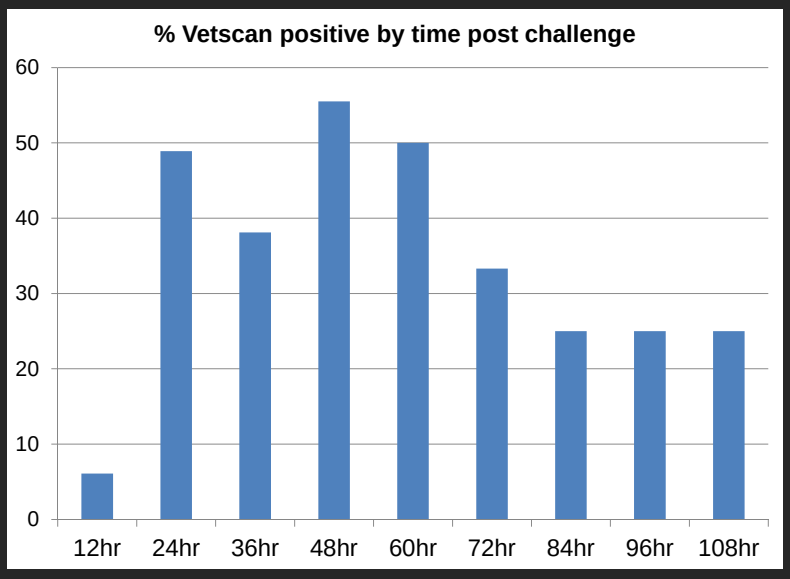
# How does HPAIV clinical presentation correlate with detection by ACIA?





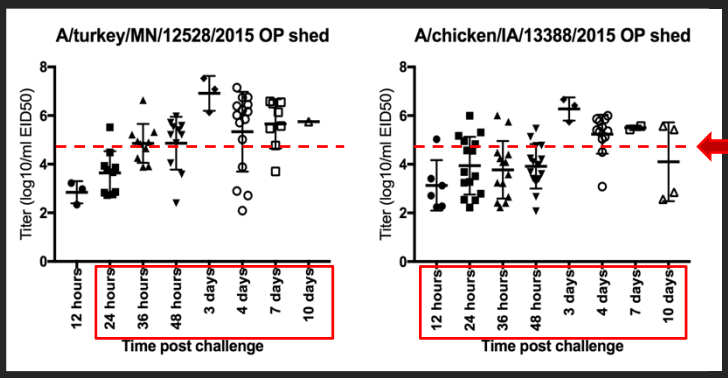

The difference in detection between the isolates is related to titers of virus shed.

# How soon can you detect virus after infection?



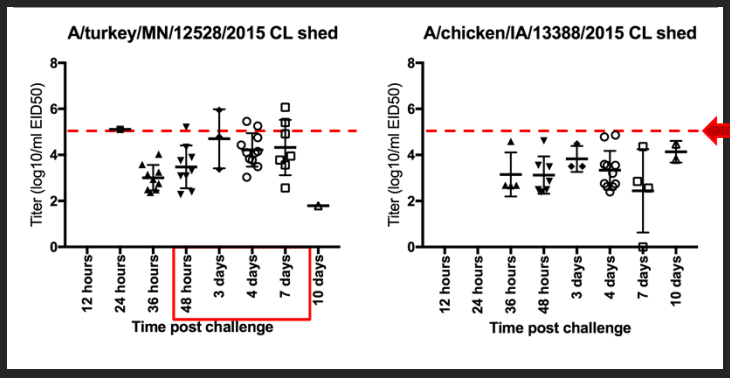

A/chicken/Jalisco/2012 H7N3 HPAIV dose =$10^5 EID_{50}$/bird n=203

## Another example: turkeys infected with the 2015 HP H5N2's oral shed




Approximate ACIA limit of detection

## Another example: turkeys infected with the 2015 HP H5N2's cloacal shed




Approximate ACIA limit of detection

## Does flu isolate matter?

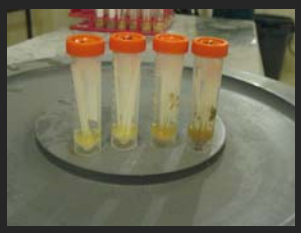
- Limit of detection tested with 4 isolates with diverse NP proteins (the ACIA targets the NP protein)
  - A/shearwater/Australia/2579/1979 H15N9
  - A/emu/NY/12716/1994 H5N9 (Eurasian strain from a quarantine station)
  - A/chicken/Jalisco/CPA-12283/2012 H7N3
  - A/chicken/PA/1370/1983 H5N2
- Evaluated both FluDetect and VetScan

Sensitivity was $10^{4.3}$-$10^{4.7}$ $\log_{10}$ for all strains.

Based on this and data with other isolates detection is similar regardless of flu strain

## Swab pooling

- Not much data for ACIA
- The question is: Will the extra material (i.e. mucous) in the sample affect the results?



## What about LPAIV?

- Is LPAIV shed at lower titers than HPAIV?
- Regarding ACIA, the main difference is clinical presentation.

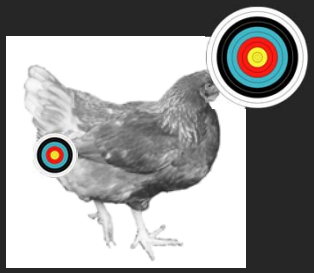
# Does species matter?

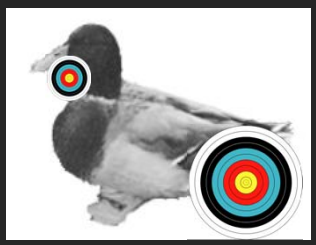
- Chickens vs. turkeys vs. ducks
  - The difference is in clinical presentation not necessarily shed.
- Same for HPAIV or LPAIV



# Which end to target?

Same as other tests





# What if there are no sick or dead birds to sample?

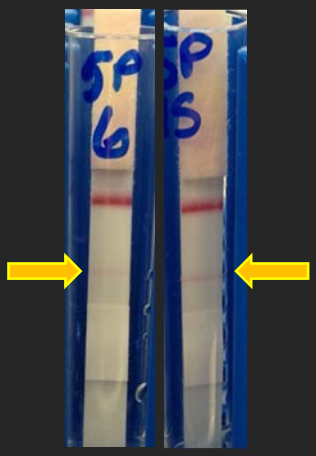
- That's a good thing.
- Ideally:
  - Random birds should be tested by rRT-PCR
  - More samples should be collected
- We don't live in an ideal world for AIV detection:
  - Random birds from throughout the house
  - Test more birds and by rRT-PCR if possible

## Sample Size as a Function of Population Size and Minimum Probability of Detection

| Confidence interval | Prevalence | Number of birds in the flock | | | | |
|---|---|---|---|---|---|---|
| | | 50 | 100 | 500 | 1,000 | 10,000 |
| 95% | 1% | 48 | 96 | 225 | 258 | 294 |
| | 5% | 31 | 45 | 56 | 59 | 59 |
| | 10% | 22 | 25 | 28 | 29 | 29 |
| 99% | 1% | 50 | 99 | 300 | 386 | 448 |
| | 5% | 39 | 59 | 83 | 68 | 90 |
| | 10% | 29 | 36 | 42 | 43 | 44 |

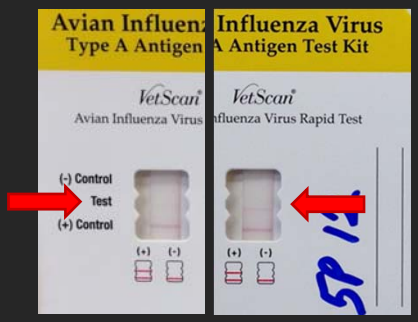
Slide: Dennis Senne

$10^{4.7}$ $10^{5.0}$



## Training can improve accuracy

$10^{4.7}$ $10^{5.0}$

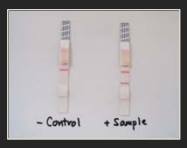



$10^{4.7}$ ⟵ 2-fold difference ⟶ $10^{5.0}$

## Do not cheat on incubation time!

- Strong positives may be visible in a few minutes
- Weak positives need the whole time to develop
  - The result is stable, the band does not fade after 15minutes.
    - In experiments we have left them out at ambient temperature (~72-76F) for up to 24hrs and the results are identical to fresh tests.

## Take-home message, which you already know




- It's all about the virus titer
- You can usually trust a positive ACIA result
  - Negative results are less reliable
- Weak positives can be difficult to read
  - Users should be trained
- Test as many samples as you can
- The difference between LPAI and HPAI is that LPAI may not give you the severe clinical "warning"

## Questions or Comments?