Assessing the Performance of Diagnostic Tests in Detecting Low Pathogenic Avian Influenza Viruses in Pooled Swab Samples

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Background

- Early control of LPAI outbreaks is necessary to prevent possible mutations of the virus into HPAIV

- Pre-movement LPAIV testing to minimize chances of moving infected but undetected flocks may be desired for continuity of business during high risk periods

- Antigen detection tests can play a significant role during such LPAI surveillance and testing situations

- Proactive assessment of diagnostic test performances is necessary to standardize surveillance and testing protocols

Aims

- To determine the viral load in oropharyngeal (OP) swab samples of H5 and H7 LPAI virus subtypes from inoculated broilers

- To compare the performance of two antigen detection tests in detecting LPAI viruses in OP swab samples

- To assess the effect of swab pooling on the ability of diagnostic tests to detect LPAI viruses

Experiment Details

- Two experiments were conducted:
  - Single-swab samples experiment
  - Pooled-swab samples experiment

- 330 eleven-day old broilers inoculated intranasally with 10^6 EID_{50}/ml of LPAIV
  - Either Chicken/PA/13609/93, H5N2 or Guinea hen/MA/148081/2002, H7N2

- OP swabs were taken daily 5-8 dpi from all birds and pooled differently

- Tested using RT-PCR, FluDetect and VetScan

Controls   *Inoculated*

OP swab samples

Tested for LPAIV

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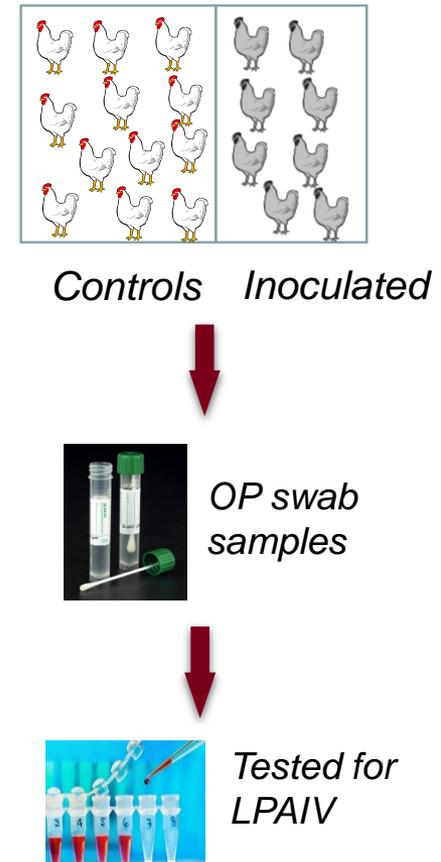
Sample Preparation

Swabs from inoculated and uninoculated birds were treated as follows:

- Single-swab samples:
  - Each swab from an inoculated bird was tested separately using RT-PCR, FluDetect and VetScan

- Pooled-swab samples:
  - One swab from an inoculated bird was pooled with either 4, 5 or 10 negative swabs from uninoculated birds and tested using RT-PCR and FluDetect

Data Analysis

- Test results were entered into spreadsheet for further analysis

- Statistical software R was used in the analysis

- Descriptive analysis involved:
  - Summarizing positive proportions and CT values by subtype, diagnostic test, and swab-pooling scheme

- Positive proportion comparison involved:
  - Using Fisher's exact test to compare FluDetect positive proportions for samples of 5 and/or 6 swab pools with those of 11 swab pools

Study Assumptions

- Only samples with CT ≤ 35 were considered PCR positive

- Non-detect samples (i.e., those whose reactions could not produce a minimum amount of signal) were assigned a CT = 45

- PCR positive samples were considered to be true positives (i.e., 100% specificity for PCR)

Single-Swab Experiment Results

*Assuming PCR specificity ≈ 100% and CT ≤ 35 implies PCR positive

| Outcome	Subtype	FluDetect	VetScan
		No. AC$^+$ (% based on given PCR results)	
No. AC$^+$ that are PCR$^+$ (% of PCR$^+$ samples)*	H5	16 (37%)	12 (28%)
	H7	36 (58%)	26 (42%)
No. AC$^+$ with CT ≤ 30 (% of samples with CT ≤ 30)	H5	16 (64%)	12 (48%)
	H7	36 (68%)	26 (49%)
		Summary CT values of AC$^+$ samples	
Highest CT detected by AC tests	H5	28.12	27.91
	H7	29.31	29.31
Mean CT detected by AC tests	H5	26.10	25.94
	H7	26.07	25.69

Results on:

POOLED-SWAB EXPERIMENT

CT Value by Virus Subtype

For merged pool sizes:

- 11/227 H7 and 116/298 H5 samples had CT > 35 and none was positive in FluDetect

- Overall positive proportions by FluDetect were 114/227 for H7 and 52/298 for H5 subtype

- Six H7 and 53 H5 subtype samples had non-detectable PCR signal
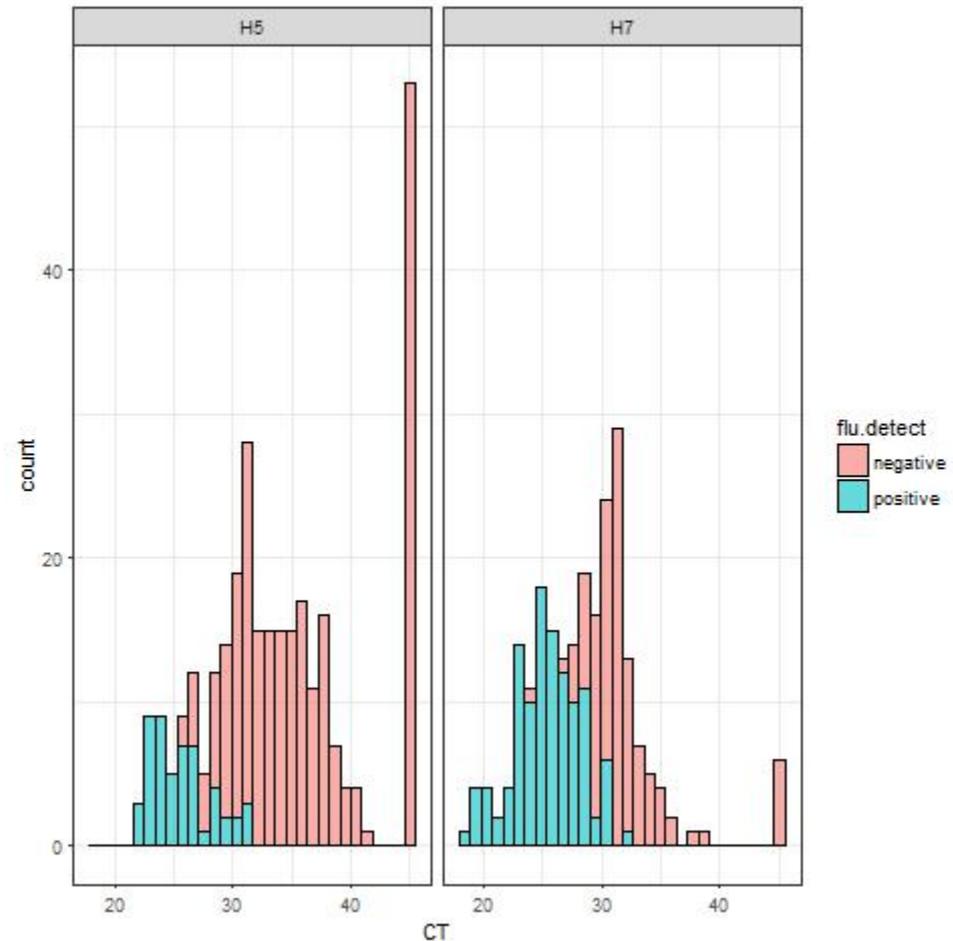
CT Value Distribution by Virus Subtype

For merged pool sizes:

- Median CT value for the H5 subtype was higher than the 75th percentile for the H7 subtype

- Overall, H7 subtype samples had lower CT values than H5
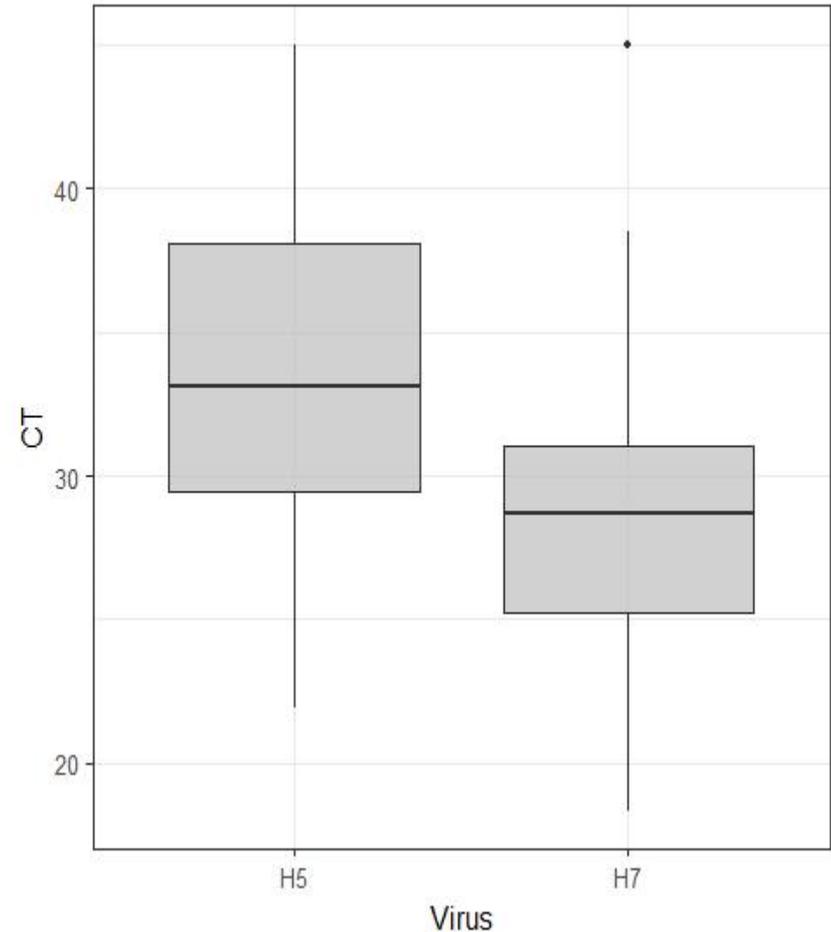
CT Value by Virus Subtype and Pool Size

For pools of 5, 6, 11 respectively:

- Total no. samples: H5- 98, 91, 109 and H7- 74, 80, 73

- No. FluDetect positive: H5- 17, 18, 17 and H7- 40, 43, 31

- No. samples with CT > 35: H5- 38, 38, 40 and H7- 5, 2, 6

- No. samples with no detectable PCR signal: H5- 16, 15 , 22 and H7- 3, 1, 2
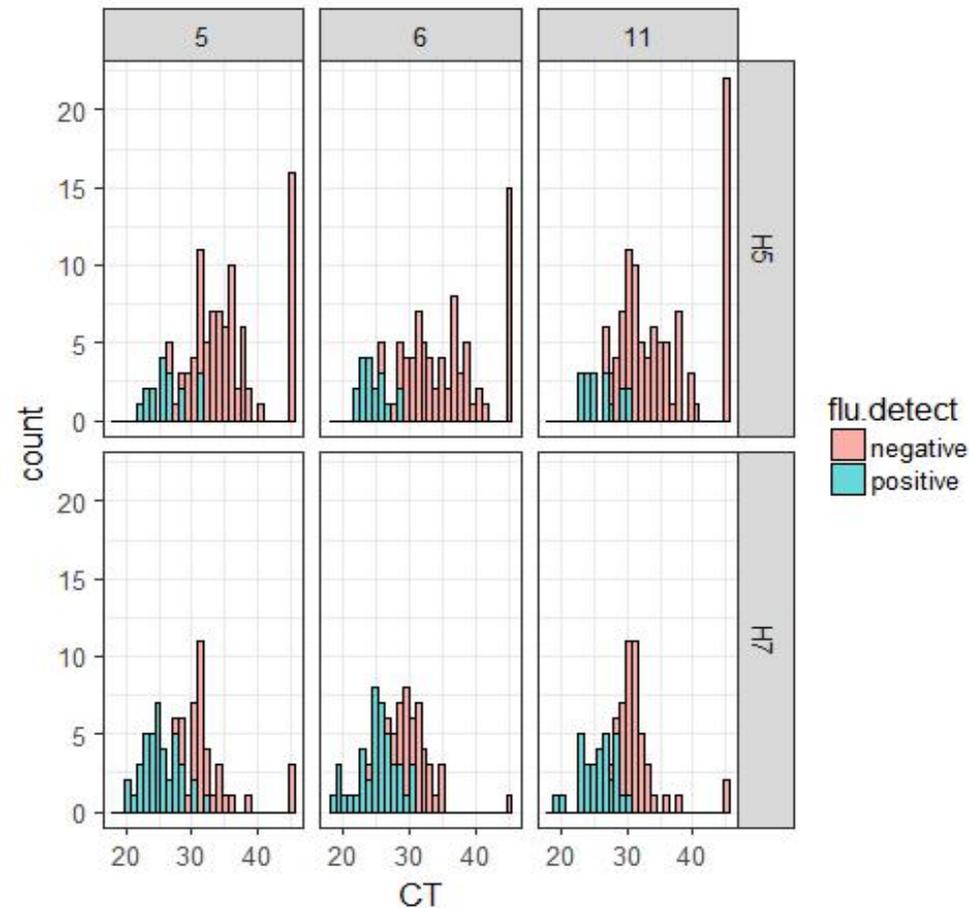
Pooled-Swab Experiment Results

Testing the significance of differences in FluDetect positive proportions for different pool sizes using one-sided Fisher's exact test for only the samples with CT ≤ 35

Subtype	Pool Size	Fraction FluDetect positive (%) : Fraction for 11-swab pools (%)	Test: AC positive proportion greater for pools of 11
H7	5 or 6	83/147 (56.5%) : 31/69 (44.9%)	0.075
	5	40/69 (58.0%) : 31/69 (44.9%)	0.086
	6	43/78 (55.1%) : 31/69 (44.9%)	0.142
Merged H5 & H7	5 or 6	118/260 (45.4%) : 48/138 (34.8%)	0.026
	5	57/129 (44.2%) : 48/138 (34.8%)	0.074
	6	61/131 (46.6%) : 48/138 (34.8%)	0.033

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Overall Results Summary

- H7 subtype was shed in higher titers than the H5 subtype- does that imply better replication and/or adaptation?

- FluDetect detected more PCR positive samples than VetScan with differences of 9% and 16% for H5 and H7 subtype respectively

- In some cases, pooling 1 swab from an inoculated bird with 10 negative swabs resulted in reduced detection rates compared to pooling it with 4 and/or 5 negative swabs

Concluding Remarks

- Swabbing all inoculated birds irrespective of their clinical status may lead to an underestimation of sensitivities for antigen detection tests

- Percent detection or diagnostic sensitivity of antigen capture tests is correlated with CT value and virus concentration

- Extrapolating this study's findings to LPAI field surveillance requires further studies

- Do we need to rethink the cutoff of CT ≤ 35 for PCR positivity, especially for periods when prevalence is still low?

Acknowledgement

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