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

Amos Ssematimba, Sasidhar Malladi, Peter Bonney, Cristian Flores, Jeannette Muñoz, David A. Halvorson, Carol J. Cardona

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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
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 \( \leq 35 \) 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

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

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