PDRC Evaluation of Alveo Sense Poultry Avian Influenza Test on Infected Mallard Ducks: Results Summary

Overview

Alveo Technologies collaborated with Dr. Daniel R. Perez's influenza research lab at the University of Georgia's Poultry Diagnostic and Research Center (PDRC). Their joint exploratory study, conducted for research use only (RUO), evaluated the Alveo Sense Poultry Avian Influenza Test Type A H5 H9. The primary objective was to determine the test's effectiveness in detecting the **H5N1 virus** in experimentally infected ducks during the **early stages of infection**.

This collaboration supports broader efforts to understand how the Alveo Sense platform performs in real-world research settings and to gather additional data that can inform future sensitivity assessments of this qualitative assay.

1. Methodology

Animal studies were conducted at the University of Georgia's Animal Health Resource Center ABSL3-Ag facility, adhering to all regulations for highly pathogenic avian influenza virus (HPAIV) research. The Institutional Biosafety Committee and Institutional Animal Care and Use Committee of the University of Georgia approved the study.

For this study, five mallard ducks (2 weeks old) were experimentally infected with a prototypic strain of clade 2.3.4.4b H5N1 HPAIV at a dose of **1×10^4 $TCID_{50}$/duck** (500 µL via oculo-nasal-tracheal-cloacal routes). Clinical signs were monitored for five days. Daily, oropharyngeal and cloacal swabs were collected from each duck. These samples were then analyzed to determine **viral load (log_{10} $TCID_{50}$/mL)** and to assess the **sensitivity of the Alveo Sense Poultry Avian Influenza Test Type A H5 H9**.

PDRC determined viral titers by infecting Mammalian Madin-Darby Canine Kidney (MDCK) cells with serial dilutions of each individual swab[1]. $TCID_{50}$ values were calculated using the Reed and Muench method and expressed as log_{10} $TCID_{50}$/mL (Table 3)[2].

PDRC followed the intended user workflow of the Alveo Sense test in running the cartridges. The Alveo Sense test was run three times during the study, using pooled cloacal swabs from all five birds on days 1, 3, and 5 post-infection. Results via the mobile application were

reported qualitatively, in line with the current design of the Alveo Sense platform (Table 4). The raw data from all three runs were also extracted and analyzed for further evaluation (Table 5).

The Alveo Sense Poultry Avian Influenza Test Type A H5 H9 multiplexes six unique assays to detect general Type A influenza as well as differentiate H5 and H9 subtypes to ensure the greatest coverage of Avian Influenza strains (Table 1). As long as one of the assays per target group amplifies, the user will receive a qualitative positive result. The user will receive a negative result when there is no group-specific amplification and the test's internal process control amplifies successfully. The expected results of the test from infected H5N1 ducks are delineated in Table 2 below.

Table 1: Alveo Sense Poultry Avian Influenza Test Type A H5 H9 Assays and Genomic Targets

Group	Unique Assay	Target
Type A	Matrix_1	M gene (Broad Flu A)
	Matrix_2	M gene (Broad Flu A)
Subtype H5	H5_1	HA gene (2.3.4.4b + Eurasian)
	H5_2	HA gene (2.3.4.4b)
	H5_3	HA gene (American)
Subtype H9	H9	HA gene (H9 subtype)

Table 2: Expected Qualitative Results of the Alveo Sense Poultry Avian Influenza Test Type A H5 H9 Based on H5N1 Challenge

Strain	Type A Result	H5 Result	H9 Result
H5N1	Positive	Positive	Negative

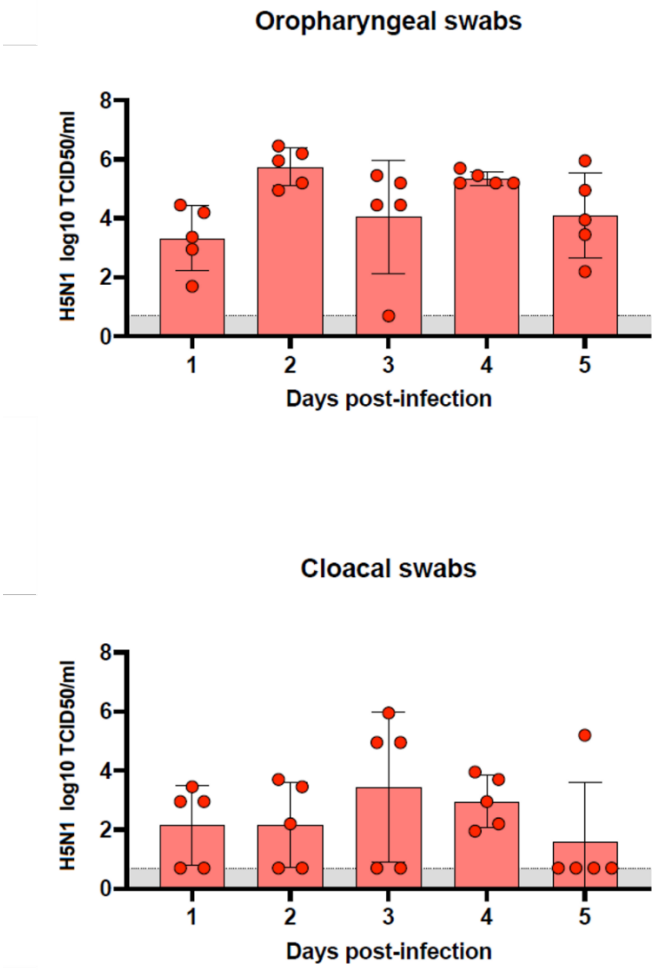
2. Results

This study employed a two-pronged approach that investigated: (1) the change in viral shedding in ducks in the five days following H5N1 infection, and (2) the sensitivity and genetic coverage of the Alveo Sense Poultry Avian Influenza Test Type A H5 H9 in detecting the infected H5N1 virus.

2.1. **Quantified daily H5N1 Shedding of Infected Ducks over Five Days Post-Infection**

The PDRC results indicate low viral loads on Day 1 post-infection, with increasing viral shedding observed between Days 2 and 4. Oropharyngeal swabs showed elevated viral levels on Day 5 relative to Day 1, while cloacal shedding was lowest on Day 5. Additionally, viral shedding was consistently higher in oropharyngeal swabs compared to cloacal swabs. See Figure 1 and Table 3 for the complete results.

Figure 1: Quantification of H5N1 Virus (log_{10} $TCID_{50}$/mL) per Individual Swab Collected During the First Five Days Post-Infection, for Oropharyngeal and Cloacal Swab Types



Each dot on the graph represents the $TCID_{50}$ value from an individual swab, while the bars indicate the mean value for that day. A dotted line denotes the limit of detection (0.699 log_{10} $TCID_{50}$/mL).

Table 3: Individual H5N1 Shedding Titers from Oropharyngeal (OP) and Cloacal (CL) Swabs Collected from Five Infected Ducks

Days Post-Infection	Swab Type	Individual Swab Titers (log_{10} $TCID_{50}$/mL)						
		Duck 1	Duck 2	Duck 3	Duck 4	Duck 5	**Daily Avg**	Std Dev
1	OP	4.199	4.449	1.699	2.949	3.366	**3.332**	1.097
	CL	2.949	0.699	0.699	3.449	2.949	**2.149**	1.339
2	OP	6.199	6.449	5.199	4.949	5.949	**5.749**	0.647
	CL	3.449	0.699	0.699	3.699	2.199	**2.149**	1.440
3	OP	5.199	5.449	0.699	4.449	4.449	**4.049**	1.925
	CL	4.949	0.699	0.699	5.949	4.949	**3.449**	2.543

4	OP	5.199	5.449	5.699	5.199	5.199	**5.349**	0.224
	CL	3.949	1.949	2.199	2.949	3.699	**2.949**	0.884
5	OP	2.199	3.949	5.949	4.949	3.449	**4.099**	1.432
	CL	5.199	0.699	0.699	0.699	0.699	**1.599**	2.012

2.2. **Evaluating the Sensitivity and Genetic Coverage of the Alveo Sense Poultry Avian Influenza Test Type A H5 H9 in Detecting the Infected H5N1 virus**

The Alveo Sense Poultry Avian Influenza Test Type A H5 H9 successfully detected the presence of Type A and H5 targets in all three tests conducted using pooled cloacal samples from Days 1, 3, and 5 (Table 4). Further analysis of the raw data confirmed successful amplification of both Type A assays, as well as the H5 assays capable of detecting the 2.3.4.4b clade. Since the H5N1 virus used in this study belonged to the 2.3.4.4b clade, amplification of the H5_3 assay – designed to detect the American lineage – was not expected and, as anticipated, was not observed. Notably, the early time-to-result (TTR) values for the Matrix_1 and H5_2 assays (Table 5) indicate strong sensitivity, particularly on Day 5, when cloacal $TCID_{50}$/mL levels were at their lowest.

Table 4: Qualitative Results of Alveo Sense Poultry Avian Influenza Test Type A H5 H9 on Pooled Cloacal Duck Samples

Days Post-Infection	Swab Type	Number of Pooled Swabs	Type A Result	H5 Result	H9 Result	Expected Result?
1	Cloacal	5	Positive	Positive	Negative	Yes
3	Cloacal	5	Positive	Positive	Negative	Yes
5	Cloacal	5	Positive	Positive	Negative	Yes

Table 5: Time to Result (TTR) of Each Assay per Cartridge of the Alveo Sense Poultry Avian Influenza Test Type A H5 H9*

Days Post-Infection	Sample	Unique Assay and Targeted Clade				
		Type A Matrix_1	Type A Matrix_2	H5_1 2.3.4.4b + Eurasian	H5_2 2.3.4.4b	H5_3 American
1	Pooled Cloacal	19.07	42.02	20.3	22.6	No Amp
3	Pooled Cloacal	19.02	29.13	20.17	14.63	No Amp
5	Pooled Cloacal	22.08	33.17	36.25	19.77	No Amp

*Note that the H9 assay was not included in the above table as there was no amplification across all runs, as expected.

Conclusion

This RUO study demonstrates both the dynamic nature of H5N1 viral shedding in ducks over a five-day post-infection period and the high sensitivity and genetic coverage of the Alveo Sense Poultry Avian Influenza Test Type A H5 H9.

The Alveo Sense test successfully detected Type A and H5 targets even in pooled cloacal samples from Day 5, when the pooled viral load was at its lowest. The early time-to-result values and robust amplification of relevant assays, particularly Matrix_1 and H5_2, underscore the platform's strong analytical sensitivity and its suitability for detecting viruses from the 2.3.4.4b H5N1 clade.

According to the PDRC $TCID_{50}$ data, viral loads rose from Day 1 to a peak between Days 2 and 4, then declined by Day 5, most notably in cloacal swabs. Across all time points, oropharyngeal swab pools yielded higher average $\log_{10}$ $TCID_{50}$/mL titers than cloacal pools, which suggests similarly pooled oropharyngeal samples would produce comparable or improved detection using the Alveo Sense test, particularly in low-titer scenarios.

Taken together, these findings validate the utility of the Alveo Sense test for detecting low levels of H5N1 virus in avian samples and support the feasibility of sample pooling strategies in field-based surveillance efforts.

References

1 Webster, R., Cox, N. & Stohr, K. *WHO Manual on Animal Influenza Diagnosis and Surveillance*, <http:/ / csr/ resources/ publications/ influenza/ whocdscsrncs20025rev.pdf> (2005).

2 Reed, L. J. & Muench, H. A simple method for estimating fifty percent endpoints. *Am. J. Hyg.* **27**, 493-497 (1938).

Note: This study was conducted by the Poultry Diagnostics and Research Center in collaboration with University of Georgia and Alveo Technologies for Research Purposes Only, test components were not for sale.