INSTRUCTIONS FOR USE (IFU)

Alveo Sense Poultry Avian Influenza Test Type A H5 H7 or Type A H5 H9

REF ***A-FIN-000037 Alveo Sense Avian Influenza Test Type A H5 H7***

REF ***A-FIN-000031 Alveo Sense Avian Influenza Test Type A H5 H9***

These Instructions for Use (IFU) apply to the following products: A-FIN-000037 (Type A H5 H7) and A-FIN-000031 (Type A H5 H9). Both products share the same intended use and procedural steps.

For use with the Alveo Analyzer and the Alveo Sense Mobile App.

For veterinary use only.

For use with oropharyngeal and cloacal swab samples only.

Developed in partnership with Royal GD Animal Health

AHEAD IN ANIMAL HEALTH

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1 Intended Use

The Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) is a rapid molecular amplification test for the qualitative detection of Avian Influenza Type A viruses and differentiation of H5, H7, and H9 RNA subtypes from oropharyngeal and cloacal samples from symptomatic and asymptomatic chickens. Positive or presumptive positive test results with the Alveo Sense Poultry Avian Influenza Test should be submitted to an authorized reference laboratory for confirmatory testing.

All results are delivered directly to the user via the Alveo Mobile application, Alveo Sense, and saved to Alveo's secure, cloud-based system.

2 Summary and Explanation of the Test

Avian influenza, commonly referred to as bird flu, is a highly contagious viral infection that spreads among migratory and domesticated birds and can significantly impact domestic poultry and other animal species. Because viruses can mutate and reassort potentially giving rise to new strains, avian influenza outbreaks must be closely monitored to evaluate the risk to animal and human health. Rapid detection, surveillance, and control measures are crucial to managing and preventing the spread of avian influenza.

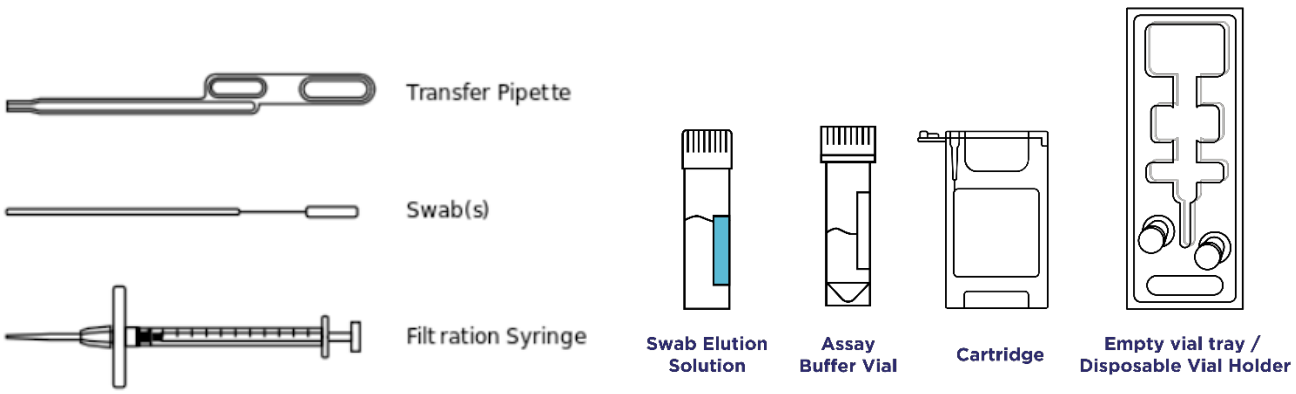
The Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) is a rapid molecular amplification test that detects and differentiates respective avian influenza strains commonly found in domesticated bird populations. The system uses loop meditated isothermal amplification method (LAMP) for rapid and specific detection of viral RNA targets.

3 Principles of the Test

The Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) is a multiplexed rapid molecular test which utilizes loop mediated isothermal nucleic acid amplification technology and electrical impedance sensors to qualitatively detect and differentiate respective avian influenza viral RNA strains in a single-use microfluidic Cartridge in approximately 45 minutes or less. The microfluidic Cartridge is equipped with 6 assays designed for broad detection of Avian Influenza Type A virus (Type A), and precise detection of H5, H7, and H9 RNA subtypes.

4 Materials Required

To complete an Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9), gather the following materials provided in the kit:



4.1 Alveo Sense Poultry Avian Influenza Test Kit Contents

(100) Swabs	(10) Filtration Syringes (Pre-assembled)	(10) Alveo Sense Poultry Avian Influenza Test Type A H5 H7 or Type A H5 H9 Cartridges
(10) Swab Elution Solution Vials (Blue Labels)	(10) Assay Buffer Vials	Quick Reference Instructions (QRI)
(10) Vial Tray Holders	(10) Transfer Pipettes	Electronic Instructions for Use (eIFU)

4.2 Additional Materials Required (Not Provided)

To complete an Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9), the following additional materials are required:

- Alveo Analyzer
- Smartphone with Alveo Sense App installed
    - **Note: The Alveo Sense Mobile App is supported on iPhone iOS 15 and above and Android OS 13 and above.** Certain features may be limited or unavailable on devices running on iOS 14 and below and Android OS 12 and below. Compatibility with other devices or OS versions is not guaranteed.

5 Storage & Handling of Alveo Sense Poultry Avian Influenza Test Kit Components

- Use all Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) Kit components between 15-30°C and in uncontrolled relative humidity, non-condensing.
- Change gloves between handling samples and setting up a new test.
- Store all Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) Kit components between 2-30°C.
- Do not reuse Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) Kit components.
- Use Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) Kit components before their individual expiration dates.
- Open packaging of Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) Kit components only when ready to begin a test.
    - Open Swab immediately before sample collection.
    - Open Cartridge from foil pouch immediately before use.

6 Warnings and Precautions

- For Veterinary Use Only.
- For use with oropharyngeal and cloacal swab samples only.
- Place the Analyzer on a flat, level surface to insert the Cartridge and run the test through to completion of the test.
- For single use only. Do not reuse test materials.
- Do not use test materials after their expiration date.
- Do not use damaged components.
- Avoid touching the electrical contacts on the Cartridge.
- Failure to follow the test instructions may alter test performance.
- Improper sample handling may lead to false negative, false positive, or invalid results.

7 Limitations

- Results should be interpreted in conjunction with other signs and observations.
- Negative results do not rule out infections or co-infections and should not be used as the sole basis for flock management decisions.
- Performance at the time of testing may vary depending on the sample collection techniques and circulating variants. This product is intended for poultry samples only.

8 Quality Control(s)

All Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) lots undergo Quality Control (QC) testing at Alveo Technologies, Inc. on every lot released. Each individual Alveo Sense Poultry Avian Influenza Test (Type A H5 H7 or Type A H5 H9) Cartridge contains an internal process control. Assuming the test is run properly, amplification in this well is expected and confirms a valid result.

Users should establish their own quality control frequency. All quality control requirements should be performed in conformance with local, state, country and/or federal regulations or accreditation requirements.

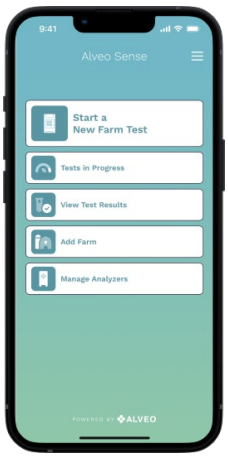
9 How to Run a Test

FOR FIRST TIME USE

Navigate to the Apple App Store or Google Play Store and download the **Alveo Sense Mobile App** on a **Smartphone** to register a New Account and agree to the Terms of Use. Ensure the Alveo Analyzer is powered on with 4 blue LED lights flashing, charged, nearby, and therefore ready for Bluetooth connection. *Note: The Alveo Sense Mobile App is supported on iPhone iOS 15 and above and Android OS 13 and above. It is not validated on Tablets or iPad.*

9.1 Log into Alveo Sense Mobile App.

a. Tap **“Start a New Test”**



b. Tap **“+Add New Farm”** and add relevant farm location details.

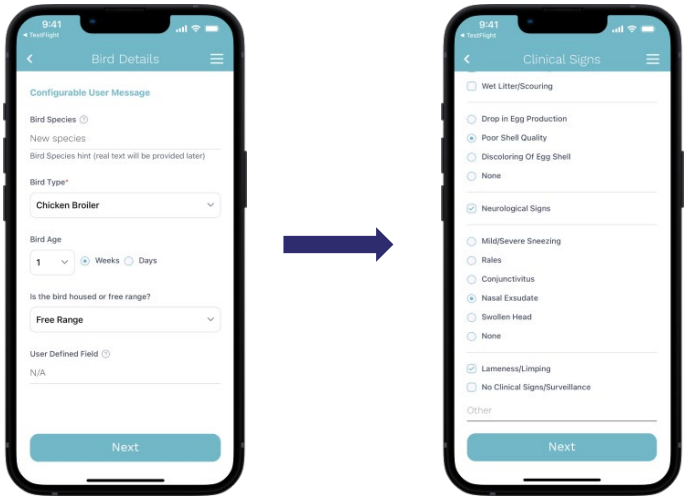
i. Note: Once a Farm location has been added, recently updated farms will appear under the Search Farms navigation bar.

c. Confirm Farm details, tap **“Next”**

d. Add **Bird Details**, including any notes for bird/flock if applicable, **tap “Next”**

i. Add **Clinical Signs** of bird/flock, if applicable, **tap “Next”**

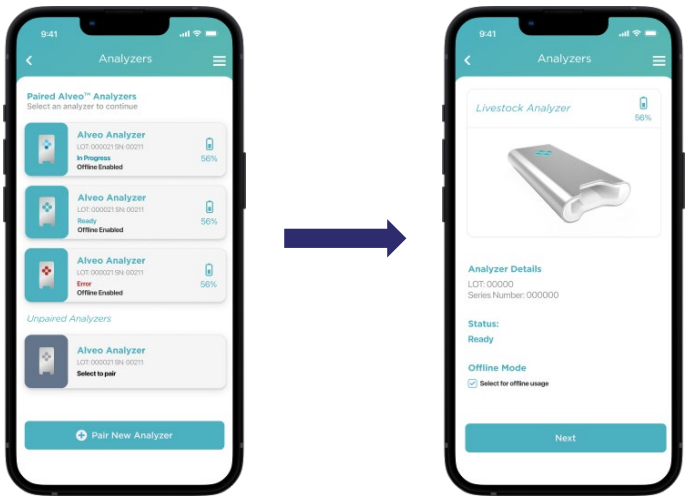
ii. Add **Postmortem Findings**, if applicable, **tap “Next”**



e. Select / Pair Alveo Analyzer

i. Tap **“+Pair New Analyzer”** if pairing Alveo Analyzer for first time.

ii. Tap Analyzer selected for testing once it has paired successfully. **Tap “Next”** to confirm. *(Note: Analyzer may need to update which may take ~2 minutes).*



f. Analyzer is now paired. Proceed to sample collection and testing procedure.

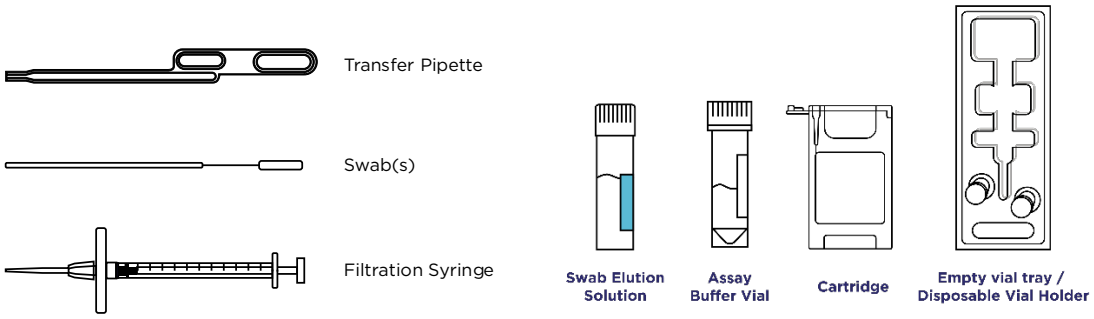
9.2 Gather Materials & Collect Sample

Note: *Read all instructions prior to sample collection and testing.*

Using Swab included in the Test Kit, collect oropharyngeal or cloacal sample from chicken. If pooling samples, collect sample and elute swabs one at a time into Swab Elution Solution. Pooled sample types may not be mixed between oropharyngeal and cloacal samples (all pools must be either oropharyngeal or

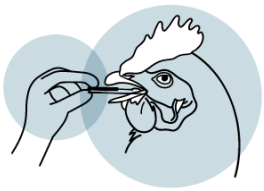
a. Follow in-App prompts: Gather all materials and place on a flat surface. **Tap "Next"**

Note: Do NOT open Cartridge pouch or swab until you are ready to collect and prepare sample, then perform test.

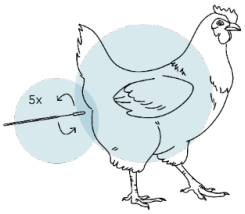


b. Open Cartridge from pouch and locate the Cartridge QR Code on the Cartridge label. **Tap "Scan Cartridge QR Code"**
c. Confirm Cartridge details and UDI. Select **"Yes"** to continue.
d. Collect sample(s).

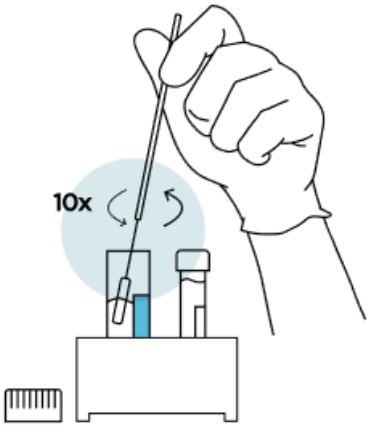
For oropharyngeal collection: Insert swab into mouth. Press swab tip against the roof of the oropharynx and rotate 5 times. On withdrawing the swab from mouth ensure mucosal material is visible on the swab.



For cloacal collection: Insert swab into the cloaca. Collect sample by rotating swab tip 5 times while pressed against the mucosa. Ensure mucosal material is visible on the swab.



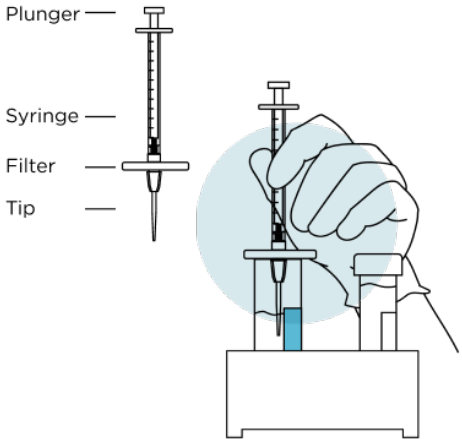
e. **Mix Sample:** Gather Swab Elution Solution (blue labeled vial) and Assay Buffer Vial (white labeled vial) and place each vial inside the Empty Vial Holder. Remove cap of Swab Elution Solution (blue label).
f. Insert the swab (with sample) into Swab Elution Solution Vial (blue label) and stir swab (10) times against the wall of the vial. Close cap.



g. Discard used swab(s) in appropriate biohazardous waste container. **Tap "Next"**

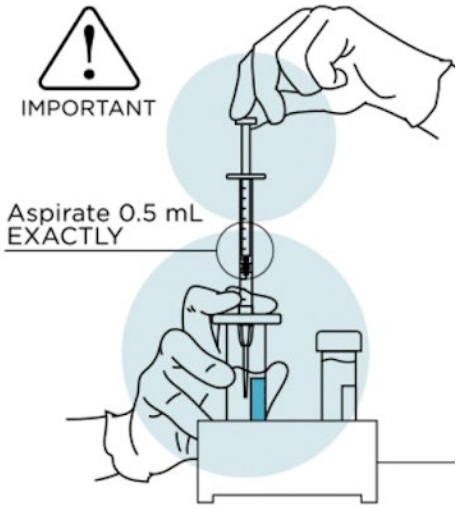
Note for Pooled Samples: *If pooling, stir each swab one at a time into Swab Elution Solution. Repeat process of sample collection and Swab Elution Solution Mixing in the same vial for subsequent swabs when preparing pools of 2-10 swabs for oropharyngeal samples, or 2-5 swabs for cloacal samples (as decided by the examining veterinarian).*

h. **Gather Filtration Syringe:** Obtain pre-assembled Filtration Syringe containing filter and tip from the test kit. Remove cap from Tip.

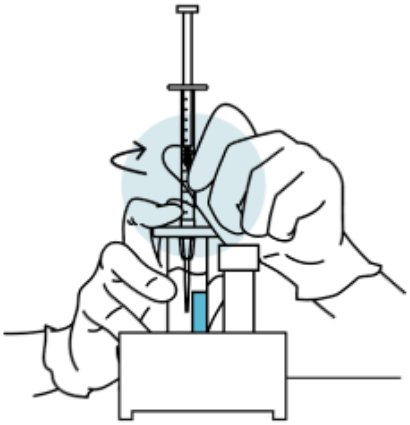


i. **Insert the Tip** of the filtration syringe into the **Swab Elution Solution** (blue label) until the filter contacts the vial opening. **Tap "Next"**
j. **Aspirate Sample** following in-App instruction and animation.

IMPORTANT! Perform reverse filtration step by gently pulling syringe plunger **<u>to collect 0.5mL of fluid</u>** in syringe. **Tap "Next"**



k. **Unscrew Syringe:** Watch in-App animation; **Hold filter in place and unscrew syringe counterclockwise** to detach syringe from filter and tip. Immediately discard the filter & tip in an appropriate biohazard waste receptable.



l. Re-cap the Swab Elution Solution Vial (blue label) and place to the side until test is completed successfully. **Tap "Next"**

m. **Dispense Sample into Assay Buffer Vial (white label):**

n. Unscrew cap from Assay Buffer Vial (white label). Depress syringe plunger to release sample into the Assay Buffer Vial (white label). Re-cap vial.

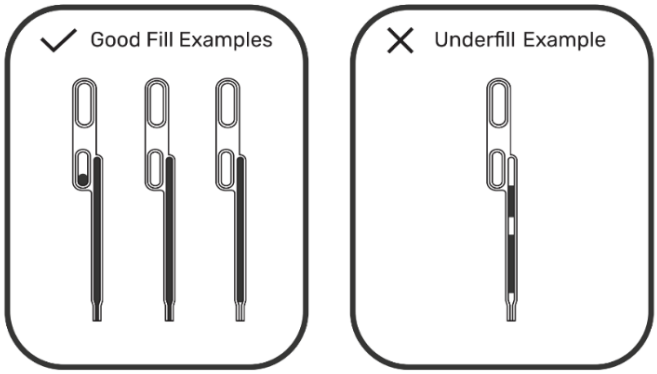
o. Discard syringe in appropriate biohazard waste receptable.

p. Invert Assay Buffer Vial (5) times to mix sample. **Tap "Next"**

9.3. Prepare Sample for Transfer into Cartridge & Running the Test

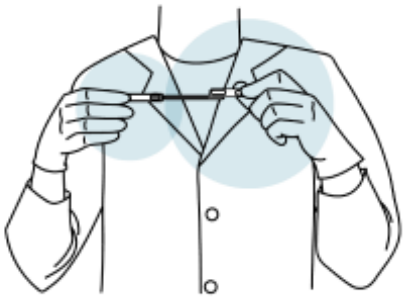
a. **Prepare Sample:** Obtain Transfer Pipette. Remove the cap of the Assay Buffer Vial (white label) with the mixed sample.
b. Squeeze the **top bulb** of the Transfer Pipette and insert the tip into the Assay Buffer vial, **until it reaches the bottom.**
c. Release the top bulb to fill the Transfer Pipette with the mixed Assay Buffer. Be sure to fill the Transfer Pipette stem completely.

Note: *If the stem is incompletely filled or air gaps or bubbles are present in the long stem of the Transfer Pipette, slowly empty the Transfer Pipette contents back into the Assay Buffer vial and refill slowly.* ***See examples on right of properly filled and underfilled Transfer Pipettes.***

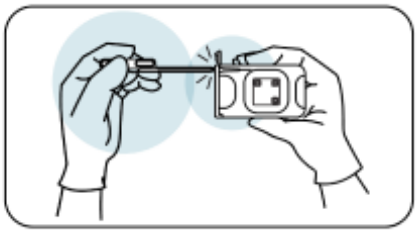


d. **Transfer Sample into Cartridge – Review In-App Animation**

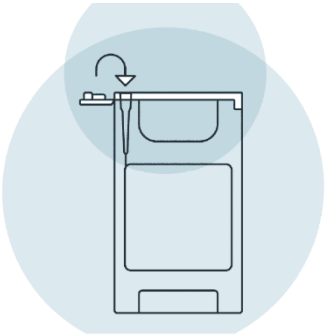
Eye Level View



Top Down View



e. **Hold Cartridge horizontally** along its sides with the **barcode facing up.** Avoid touching the area with the gold electrical contacts.
f. Insert the Transfer Pipette firmly into the sample port to form a strong seal between the Cartridge and the pipette.
g. Keep firm inward pressure on the Transfer Pipette and **firmly squeeze the top bulb** to dispense all liquid from stem into the Cartridge.
IMPORTANT! ROTATE CARTRIDGE.
h. Once filled, keep firm pressure on the top bulb and carefully **turn the Cartridge vertically** to the upright position.
i. Fully remove the Transfer Piette from the Cartridge **before releasing the top bulb** of the Transfer Pipette to avoid filling issues. Discard pipette in an appropriate biohazardous waste receptacle. **Tap "Next"**
j. **Close cap** by securely pressing the cap back into the Cartridge.

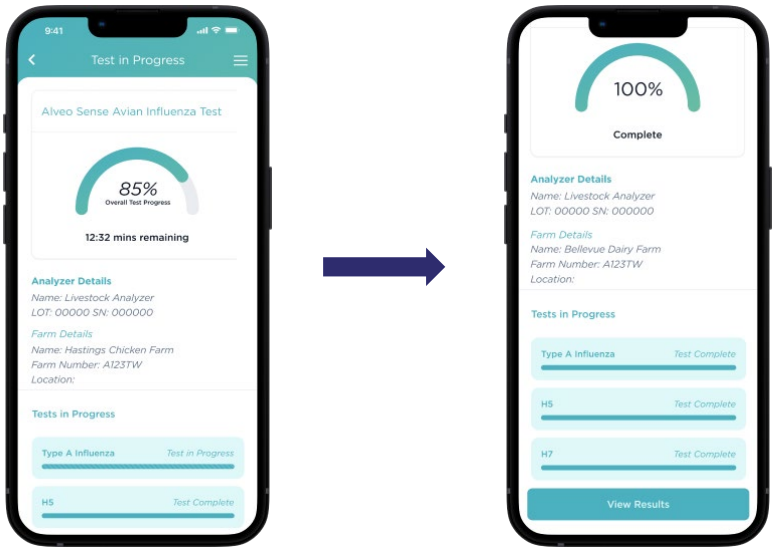


Important: Visually inspect the Cartridge for bubbles. Please note the first well on the Left is empty by design. In Wells 2-8, if you notice any significant bubbles (occupying >20% of the well volume), you are at a risk of an invalid test result. Dispose of the Cartridge, re-collect sample from same vial, and start again. **Tap "Next"**

9.4 Inserting Test Cartridge into Analyzer

a. **Insert Cartridge** into Analyzer.
   - Ensure the Analyzer is on a clean, flat surface with the 4 blue LED indicator lights visible on top.
   - Fully insert the Cartridge with the QR code facing upwards into the glowing Alveo Analyzer port.
   - ***Note:*** *The blue dots will change from solid to a circular light pattern when the Cartridge is fully inserted, and the Test will begin.*

b. Test is in progress as soon as Cartridge is inserted into Analyzer.



Note: If needed, a test can be canceled while in progress. Click the **'Cancel Test'** button and confirm you want to cancel the test. App will report result(s) as "CAN" for target(s) that were canceled before the test completed.

c. App screen will notify when the test is complete:
   - Click **'View Results'** to access recent and past test results. Results may be shared by clicking 'Share Result' button.
   - Click **'View Results Key'** for details on how to interpret test results.
   - Once test results are complete, discard remaining Swab Elution Solution Vial (blue label) in an appropriate biohazardous waste receptacle.
   - **Note:** If you are unsure how to interpret a result or require technical support, please contact GD Animal Health support, support@gdanimalhealth.com. For product quality issues, please contact Alveo support, support@alveotechnologies.com.

10 Interpretation of Results

- Results will be reported through the Alveo Sense Mobile App as **Positive, Negative, or Invalid**.
- **Positive or Presumptive Positive** test results with the Alveo Sense Poultry Avian Influenza Test should be submitted to an authorized reference laboratory for confirmatory testing.
- **Invalid** test results with the Alveo Sense Poultry Avian Influenza Test indicate that the result cannot be interpreted. A repeat test with a new sample should be performed, if needed. Invalid results for one or more target combinations are possible and are reported specifically for each target.
- In cases where a test was Canceled or an Error occurred, results will display as Canceled or Error.

Interpretation of Results Tables

For REF# A-FIN-000037 Alveo Sense Poultry Test Type A H5 H7:

Type A	H5	H7	Interpretation
NEG	NEG	NEG	Negative; Target RNA is not detected.
POS	NEG	NEG	Type A Positive; Type A RNA target is detected.
POS	POS	NEG	H5 Positive; Type A RNA and subtype H5 target RNA are detected.
POS	NEG	POS	H7 Positive; Type A RNA and subtype H7 target RNA are detected.
POS	POS	POS	Type A, H5, H7 Positive; Type A target RNA, subtype H5 target RNA, and subtype H7 target RNA are detected.
NEG	POS	NEG	H5 Presumptive Positive; Type A is not detected but subtype H5 target RNA is detected and is a presumptive positive.
NEG	NEG	POS	H7 Presumptive Positive; Type A is not detected but subtype H7 target RNA is detected and is a presumptive positive.
NEG	POS	POS	Presumptive H5 H7 Positive; Type A is not detected but subtypes H5 target RNA and H7 target RNA are detected and are a presumptive positive.
INV	INV	INV	Invalid; cannot be interpreted. Repeat test.

For REF# A-FIN-000031 Alveo Sense Poultry Test Type A H5 H9:

Type A	H5	H9	Interpretation
NEG	NEG	NEG	Negative; Target RNA is not detected.
POS	NEG	NEG	Type A Positive; Type A RNA target is detected.
POS	POS	NEG	H5 Positive; Type A RNA and subtype H5 target RNA are detected.
POS	NEG	POS	H9 Positive; Type A RNA and subtype H9 target RNA are detected.
POS	POS	POS	Type A, H5, H9 Positive; Type A target RNA, subtype H5 target RNA, and subtype H9 target RNA are detected.
NEG	POS	NEG	H5 Presumptive Positive; Type A is not detected but subtype H5 target RNA is detected and is a presumptive positive.
NEG	NEG	POS	H9 Presumptive Positive; Type A is not detected but subtype H9 target RNA is detected and is a presumptive positive.
NEG	POS	POS	Presumptive H5 H9 Positive; Type A is not detected but subtypes H5 target RNA and H9 target RNA are detected and are a presumptive positive.
INV	INV	INV	Invalid; cannot be interpreted. Repeat test.

11 Performance Characteristics

11.1 Limit of Blank (LoB):

Limit of Blank testing was conducted with four unique negative cloacal pools (equivalent 5 swabs per pool) and four unique negative oropharyngeal pools (equivalent 10 swabs per pool). Each pool was tested in replicates of five, yielding 20 valid cartridge results per matrix type. Testing was performed using one lot of A/H5/H7 cartridges and one lot of A/H5/H9 cartridges.

As shown in Table 1, a 100% negativity rate was observed across both matrices and cartridge types (A/H5/H7 and A/H5/H9). Note that the individual Type A and H5 results are summative, as the assays are shared across cartridge configurations.

Table 1

Matrix Type	Matrix Pool	Qualitative Results by Target *(# Positive | # Tested)*				Qualitative Results by Cartridge *(# Positive | # Tested)*	
		Type A	H5	H7	H9	A/H5/H7	A/H5/H9
Oropharyngeal *(Pool of 10)*	1	0 | 10	0 | 10	0 | 5	0 | 5	0 | 20 (0%)	0 | 20 (0%)
	2	0 | 10	0 | 10	0 | 5	0 | 5		
	3	0 | 10	0 | 10	0 | 5	0 | 5		
	4	0 | 10	0 | 10	0 | 5	0 | 5		
Cloacal *(Pool of 5)*	1	0 | 10	0 | 10	0 | 5	0 | 5	0 | 20 (0%)	0 | 20 (0%)
	2	0 | 10	0 | 10	0 | 5	0 | 5		
	3	0 | 10	0 | 10	0 | 5	0 | 5		
	4	0 | 10	0 | 10	0 | 5	0 | 5		
Qualitative Results by Target *(# Positive | # Tested)*		0 | 80	0 | 80	0 | 40	0 | 40		

11.2 Analytical Sensitivity (Limit of Detection)

Limit of Detection was defined as the lowest concentration where ≥ 95% of the replicates tested positive for the hemagglutinin-specific test (H5, H7, H9). Testing was conducted using four viral samples: an H5N2 strain of the American non-GsGD clade, an H5N8 strain of the 2.3.4.4b clade, an H7Nx strain of the Eurasian lineage, and an H9N2 strain of the G lineage. Testing of H5N2, H5N8, and H7Nx was conducted using two lots of A/H5/H7 cartridges, while testing of H9N2 was conducted using one lot of A/H5/H9 cartridges.

Testing was executed by spiking the inactivated Influenza A viruses into assay buffer containing filtered cloacal (equivalent 5 swabs per pool) or oropharyngeal (equivalent 10 swabs per pool) matrix, and was confirmed if the H5, H7, or H9 indicator achieved ≥ 95% positivity rate across 20 valid cartridge replicates (≥ 19/20). The obtained Limit of Detection dilutions were correlated with Royal GD's Influenza A TaqMan PCR assay to obtain equivalent Ct values.

As shown in Table 2, the LoD for H5N2 corresponds to Ct 33.5 in cloacal matrix and Ct 33.0 in oropharyngeal matrix. The LoD for H5N8 corresponds to Ct 30.6 in cloacal matrix and Ct 31.9 in oropharyngeal matrix. The LoD for H7Nx corresponds to Ct 28.5 in cloacal matrix and Ct 29.0 in oropharyngeal matrix. The LoD for H9N2 corresponds to Ct 31.6 in both cloacal and oropharyngeal matrices.

Table 2:

Sample	**Matrix Type**	**Equivalent *PCR Ct*** *GD TaqMan PCR*	**Qualitative Results by Target** *(# Positive | # Tested)*			
			Type A	**H5**	**H7**	**H9**
H5N2 (Am_nonGsGd)	Cloacal *(Pool of 5)*	33.5	20 | 20	20 | 20	0 | 20	*N/A*
	Oropharyngeal *(Pool of 10)*	33.0	20 | 20	20 | 20	0 | 20	*N/A*
H5N8 (2.3.4.4b)	Cloacal *(Pool of 5)*	30.6	20 | 20	20 | 20	0 | 20	*N/A*
	Oropharyngeal *(Pool of 10)*	31.9	19 | 20	20 | 20	0 | 20	*N/A*
H7Nx (Eurasian)	Cloacal *(Pool of 5)*	28.5	20 | 20	0 | 20	20 | 20	*N/A*
	Oropharyngeal *(Pool of 10)*	29.0	20 | 20	0 | 20	20 | 20	*N/A*

H9N2 (G lineage)	Cloacal *(Pool of 5)*	31.6	19 | 20	0 | 20	*N/A*	20 | 20
	Oropharyngeal *(Pool of 10)*	31.6	17 | 20	0 | 20	*N/A*	20 | 20

11.3 Avian Influenza Virus Inclusivity and Exclusivity

Inclusivity and specificity (exclusivity) testing was conducted with the following 12 Influenza A strains: H3N1, H5N1 (European non-GsGD), two H5N2 strains (one EU-non-GsGD, one AM-non-GsGD), two H5N3 strains (both EU-non-GsGD), H5N8 (2.3.4.4b), H6N1, H7N1 (Eurasian lineage), H7Nx (Eurasian lineage), and two H9N2 strains (G lineage). Testing was performed by spiking the inactivated Influenza A viruses into assay buffer containing filtered cloacal (CL, equivalent 5 swabs per pool) or oropharyngeal (OP, equivalent 10 swabs per pool) matrices, with a minimum of three cartridge replicates per matrix and cartridge type. The Influenza A Ct-value in the final assay buffer suspension was determined with the GD TaqMan Influenza A PCR assay. Testing was performed using one lot of A/H5/H7 cartridges and one lot of A/H5/H9 cartridges.

As shown in Table 3, all avian influenza virus strains were correctly detected across Type A, H5, H7, and H9 results. Note that the individual Type A and H5 results are summative, as the assays are shared across cartridge configurations.

Table 3:

Strain *(subtype/lineage/clade)*	**Matrix Type**	**GD TaqMan PCR (Ct)** *A/H5/H7 | A/H5/H9*	**Qualitative Results by Target** *(# Positive | # Tested)*			
			Type A	**H5**	**H7**	**H9**
H3N1	OP	28 | 32	8 | 8	0 | 8	0 | 4	0 | 4
	CL	25 | 25	6 | 6	0 | 6	0 | 3	0 | 3
H5N1 (EU_nonGsGd)	OP	30 | 28	6 | 6	6 | 6	0 | 3	0 | 3
	CL	27 | 27	6 | 6	6 | 6	0 | 3	0 | 3
H5N2 (AM_nonGsGd)	OP	29 | 31	6 | 6	6 | 6	0 | 3	0 | 3
	CL	28 | 28	7 | 7	7 | 7	0 | 4	0 | 3
H5N2 (EU_nonGsGd)	OP	28 | 29	6 | 6	6 | 6	0 | 3	0 | 3
	CL	25 | 25	6 | 6	6 | 6	0 | 3	0 | 3

H5N3 (EU_nonGsGd)	OP	28 | 29	7 | 7	7 | 7	0 | 4	0 | 3
	CL	26 | 25	6 | 6	6 | 6	0 | 3	0 | 3
H5N3 (EU_nonGsGd)	OP	29 | 28	6 | 6	6 | 6	0 | 3	0 | 3
	CL	26 | 26	6 | 6	6 | 6	0 | 3	0 | 3
H5N8 (2.3.4.4b)	OP	31 | 25	6 | 6	6 | 6	0 | 3	0 | 3
	CL	27 | 24	6 | 6	6 | 6	0 | 3	0 | 3
H6N1	OP	28 | 30	8 | 8	0 | 8	0 | 4	0 | 4
	CL	26 | 26	6 | 6	0 | 6	0 | 3	0 | 3
H7N1 (Euasian)	OP	29 | 31	6 | 6	0 | 6	3 | 3	0 | 3
	CL	26 | 26	6 | 6	0 | 6	3 | 3	0 | 3
H7Nx (Eurasian)	OP	29 | 31	6 | 6	0 | 6	3 | 3	0 | 3
	CL	27 | 24	6 | 6	0 | 6	3 | 3	0 | 3
H9N2 (G-lineage)	OP	28 | 29	6 | 6	0 | 6	0 | 3	3 | 3
	CL	25 | 25	6 | 6	0 | 6	0 | 3	3 | 3
H9N2 (G-lineage)	OP	26 | 28	7 | 7	0 | 7	0 | 4	3 | 3
	CL	26 | 26	6 | 6	0 | 6	0 | 3	3 | 3

11.4 Non-Avian Influenza Virus Cross-Reactivity

DNA and RNA from the following nineteen non-influenza viruses and bacteria were evaluated: four strains of Infectious bronchitis virus (D388_GI-19, D274_GI-12, M41_GI-1, and 4/91_GI-13), Infectious laryngotracheitis virus, avian met-apneumovirus subtypes A, B and C (AMPV-A, AMPV-B, AMPV-C), Avian paramyxovirus type 1 (Newcastle Disease virus (NCD)), two *Mycoplasma synoviae* strains, two *Mycoplasma gallisepticum* strains, three *Avibacterium paragallinarum* serovars (A1, B1, C4), *Pasteurella multocida*, *Gallibacterium anatis, Ornithobacterium rhinotracheale*, and *Riemerella anatipestifer*. Testing was performed in duplicate per cartridge type by spiking the nucleic acid extracts into assay buffer containing filtered oropharyngeal matrix (equivalent 10 swabs per pool). Testing was performed using one lot of A/H5/H7 cartridges and one lot of A/H5/H9 cartridges. Ct-values were determined with the pathogen specific TaqMan GD PCR assay, targeting < Ct 25 in the final reaction. For two bacterial samples (*Ornithobacterium rhinotracheale* and *Riemerella anatipestifer*), a >4.0 McFarland suspension was used without determination of the Ct-value.

As shown in Table 4, none of the organisms tested interfered with the A/H5/H7 test or A/H5/H9 test by generating false positive results. Note that the individual Type A and H5 results are summative, as the assays are shared across cartridge configurations.

Table 4:

Non-Avian Influenza Pathogen ***(serotype or subtype)***	***GD TaqMan PCR (Ct)*** *A/H5/H7 | A/H5/H9*	**Qualitative Results by Target** *(# Positive | # Tested)*				**Qualitative Results by Cartridge** *(# Positive | # Tested)*	
		Type A	**H5**	**H7**	**H9**	**A/H5/H7**	**A/H5/H9**
Infectious bronchitis virus, D388	22 | 22	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Infectious bronchitis virus, D274	21 | 21	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Infectious bronchitis virus, M41	23 | 23	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Infectious bronchitis virus, 4/91	27 | 27	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Infectious laryngotracheitis virus (vaccine like)	24 | 24	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avian metapneumovirus A (AMPV-A) (TRT)	25 | 26	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avian metapneumovirus B (AMPV-B) (TRT)	21 | 23	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avian metapneumovirus C (AMPV-C) (TRT)	27 | 27	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avian paramyxovirus type 1 (NCD)	22 | 22	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Mycoplasma synoviae	21 | 21	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Mycoplasma synoviae	26 | 26	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Mycoplasma gallisepticum	22 | 22	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avibacterium paragallinarum A1	23 | 23	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avibacterium paragallinarum B1	23 | 23	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Avibacterium paragallinarum C4	24 | 24	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Pasteurella multocida	24 | 24	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Gallibacterium anatis	22 | 22	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Ornithobacterium rhinotracheale (ORT)	*N/A*	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
Riemerella anatipestifer	*N/A*	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2

11.5 Reproducibility and Precision

Reproducibility and precision (repeatability) was conducted across two laboratories divided among four operators over three days. Each operator tested three replicates per condition, per day, including a no template control (NTC) and a combined positive control (PC). For the A/H5/H7 test, the PC included H5N2, H5N8, and H7Nx strains, while the PC for the A/H5/H9 test included H5N2, H5N8, and H9N2 strains, each at a concentration of 3x LoD. Testing was conducted by spiking TE Buffer (negative sample) or the combined Influenza A viruses (positive sample) into assay buffer containing filtered cloacal matrix (equivalent 5 swabs per pool). Testing was performed using one lot of A/H5/H7 cartridges and one lot of A/H5/H9 cartridges.

As shown in Table 5, Reproducibility and Precision was executed with 100% agreement with the expected result for both A/H5/H7 and A/H5/H9 cartridges.

Table 5:

Laboratory	Test Day	Operator	Qualitative Results by Cartridge Type *(# Positive | # Tested)*			
			A/H5/H7		A/H5/H9	
			Negative	Positive *(H5N2/H5N8/H7Nx)*	Negative	Positive *(H5N2/H5N8/H9N2)*
Laboratory 1	Day 1	Operator 1	0 | 3	3 | 3	0 | 3	3 | 3
		Operator 2	0 | 3	3 | 3	0 | 3	3 | 3
	Day 2	Operator 1	0 | 3	3 | 3	0 | 3	3 | 3
		Operator 2	0 | 3	3 | 3	0 | 3	3 | 3
	Day 3	Operator 1	0 | 3	3 | 3	0 | 3	3 | 3
		Operator 2	0 | 3	3 | 3	0 | 3	3 | 3
Laboratory 2	Day 1	Operator 3	0 | 3	3 | 3	0 | 3	3 | 3
		Operator 4	0 | 3	3 | 3	0 | 3	3 | 3
	Day 2	Operator 3	0 | 3	3 | 3	0 | 3	3 | 3
		Operator 4	0 | 3	3 | 3	0 | 3	3 | 3
	Day 3	Operator 3	0 | 3	3 | 3	0 | 3	3 | 3
		Operator 4	0 | 3	3 | 3	0 | 3	3 | 3
Overall Qualitative Results by Cartridge *(# Positive | # Tested)*			0 | 36	36 | 36	0 | 36	36 | 36

11.6 Validation with Clinical Negative Field Samples

Oropharyngeal swabs from eight postmortem influenza-negative flocks were pooled at a ratio of five (5) swabs per vial per flock and tested with a minimum of two cartridge replicates following the end-to-end workflow. All field samples were confirmed Influenza A negative by the Wageningen BioVeterinary Research reference institute laboratory prior to testing. Testing was performed using one lot of A/H5/H7 cartridges and one lot of A/H5/H9 cartridges.

As shown in Table 6, a 100% negativity rate was observed for all negative clinical field samples for both A/H5/H7 and A/H5/H9 cartridges.

Table 6:

Sample	Matrix Type	Qualitative Results by Target *(# Positive | # Tested)*				Qualitative Results by Cartridge *(# Positive | # Tested)*	
		Type A	H5	H7	H9	A/H5/H7	A/H5/H9
01	Oropharyngeal	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
02	Oropharyngeal	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
03	Oropharyngeal	0 | 5	0 | 5	0 | 3	0 | 2	0 | 3	0 | 2
04	Oropharyngeal	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
05	Oropharyngeal	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2
06	Oropharyngeal	0 | 7	0 | 7	0 | 3	0 | 4	0 | 3	0 | 4
07	Oropharyngeal	0 | 5	0 | 5	0 | 3	0 | 2	0 | 3	0 | 2
08	Oropharyngeal	0 | 4	0 | 4	0 | 2	0 | 2	0 | 2	0 | 2

The evaluation of the Alveo Sense Poultry Avian Influenza H5, H7, and H9 subtype tests showed an NPA of ≥99% in combination for both OP and CL samples.

11.7 Validation with Contrived Positive Samples

Validation using clinical positive samples was not feasible in the laboratory setting due to avian influenza testing restrictions. Positive sample validation was thus performed by spiking the viruses used in the LoD experiment (H5N2, H5N8, H7Nx, H9N2) into the sample

matrix prior to testing. The A/H5/H7 test was evaluated with H5N2, H5N8, and H7Nx, while the A/H5/H9 test was validated with H5N2, H5N8, and H9N2. Positive sample testing was concurrently evaluated with pool size validation: oropharyngeal samples were tested in pools of 1, 3, 5, and 10, while cloacal samples were tested in pools of 1, 3, and 5. The virus samples were spiked into the matrix pools and tested following the end-to-end workflow, with a minimum of two cartridge replicates per condition. Testing was performed using one lot of A/H5/H7 cartridges and one lot of A/H5/H9 cartridges. The influenza A Ct-value in the diluted matrix/virus suspension was determined with the GD TaqMan Influenza A PCR assay.

As shown in Table 7, the contrived positive samples yielded a 95.7% (44 | 46) agreement for the A/H5/H7 cartridge type, with 100% (46 | 46) of cartridges showing Influenza A detection. One H5 false negative was observed in an H5N2 sample from an oropharyngeal pool of 10 with an Influenza A Ct 36 (Type A was successfully detected). One Type A false negative was observed in an H5N8 sample from a cloacal pool of 5 with an Influenza A Ct 30 (H5 was successfully detected). The Ct values ranged from Ct 24 to 36.

Table 7:

Strain	Matrix Type	Pool Size	GD TaqMan PCR (Ct)	Qualitative Result by Target *(# Positive | # Tested)*		
				Type A	H5	H7
H5N2 (Am_nonGsGd)	Cloacal	1	29	2 | 2	2 | 2	0 | 2
		3	29	2 | 2	2 | 2	0 | 2
		5	35	2 | 2	2 | 2	0 | 2
	Oropharyngeal	1	33	2 | 2	2 | 2	0 | 2
		3	34	2 | 2	2 | 2	0 | 2
		5	34	2 | 2	2 | 2	0 | 2
		10	36	2 | 2	1 | 2	0 | 2
H5N8 (2.3.4.4b)	Cloacal	1	30	2 | 2	2 | 2	0 | 2
		3	29	2 | 2	2 | 2	0 | 2
		5	30	2 | 3	3 | 3	0 | 3
	Oropharyngeal	1	28	2 | 2	2 | 2	0 | 2
		3	28	2 | 2	2 | 2	0 | 2
		5	30	2 | 2	2 | 2	0 | 2
		10	31	3 | 3	3 | 3	0 | 3
H7Nx (Eurasian)	Cloacal	1	28	2 | 2	0 | 2	2 | 2
		3	30	2 | 2	0 | 2	2 | 2
		5	27	3 | 3	0 | 3	3 | 3
	Oropharyngeal	1	24	2 | 2	0 | 2	2 | 2
		3	25	2 | 2	0 | 2	2 | 2

		5	25	2 | 2	0 | 2	2 | 2
		10	28	3 | 3	0 | 3	3 | 3

As shown in Table 8, the contrived positive samples yielded a 100% (45 | 45) agreement for the A/H5/H9 cartridge type across Type A, H5, and H9 targets. The Ct values ranged from Ct 26 to 33.

Table 8:

Strain	Matrix Type	Pool Size	GD TaqMan PCR (Ct)	Qualitative Result by Target *(# Positive | # Tested)*		
				Type A	H5	H9
H5N2 (Am_nonGsGd)	Cloacal	1	30	2 | 2	2 | 2	0 | 2
		3	30	2 | 2	2 | 2	0 | 2
		5	31	2 | 2	2 | 2	0 | 2
	Oropharyngeal	1	31	2 | 2	2 | 2	0 | 2
		3	33	2 | 2	2 | 2	0 | 2
		5	31	2 | 2	2 | 2	0 | 2
		10	30	3 | 3	3 | 3	0 | 3
H5N8 (2.3.4.4b)	Cloacal	1	29	2 | 2	2 | 2	0 | 2
		3	29	2 | 2	2 | 2	0 | 2
		5	28	2 | 2	2 | 2	0 | 2
	Oropharyngeal	1	29	2 | 2	2 | 2	0 | 2
		3	30	2 | 2	2 | 2	0 | 2
		5	30	2 | 2	2 | 2	0 | 2
		10	28	3 | 3	3 | 3	0 | 3
H9N2 (G lineage)	Cloacal	1	28	2 | 2	0 | 2	2 | 2
		3	29	2 | 2	0 | 2	2 | 2
		5	29	2 | 2	0 | 2	2 | 2
	Oropharyngeal	1	26	2 | 2	0 | 2	2 | 2
		3	27	2 | 2	0 | 2	2 | 2
		5	28	2 | 2	0 | 2	2 | 2
		10	28	3 | 3	0 | 3	3 | 3

The evaluation of the Alveo Sense Poultry Avian Influenza H5, H7, and H9 subtype tests showed a PPA of ≥99% in combination for both OP and CL samples with a Ct range of approximately 30-32.

12 Frequently Asked Questions (FAQs) & Troubleshooting

How to manually reset the Analyzer:

To manually reset the Analyzer, hold the power button until the lights turn off. The Analyzer will turn back on momentarily after.

Responding to Errors During Testing:

If the Analyzer LEDs turn red or other error notices are displayed on the Mobile App **immediately upon insertion of the Cartridge into the Analyzer or mid-test,** remove the Cartridge, restart the Analyzer restart testing procedure.

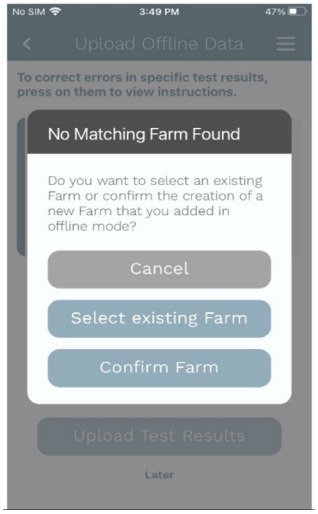
Running Multiple Tests at Once:

The system allows for up to six (6) Alveo Analyzers to be connected to a single Smartphone. If running multiple tests at once, the Analyzers should be uniquely named. Follow instructions in the Mobile App on renaming the Alveo Analyzer. Please contact Alveo Technologies Customer Support for assistance.

What happens if cellular or Wifi disconnect during testing?

The system supports “offline mode.” If cellular or WiFi are lost during the testing process, the system saves up to 10 results that will display in the Mobile App as soon as cellular or WiFi are restored.

Notifications/Errors in Alveo Sense App

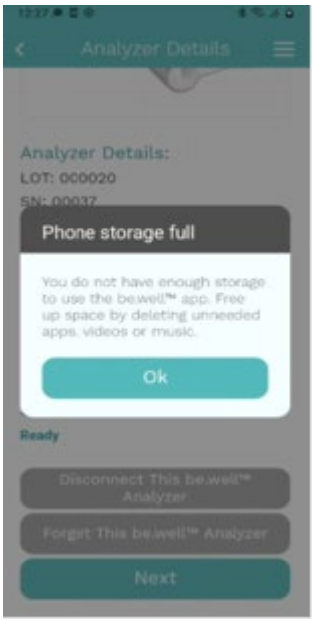


No Matching Farm Found

The No Matching Farm Found popup informs the user to select an existing farm or create a new farm.

User selects

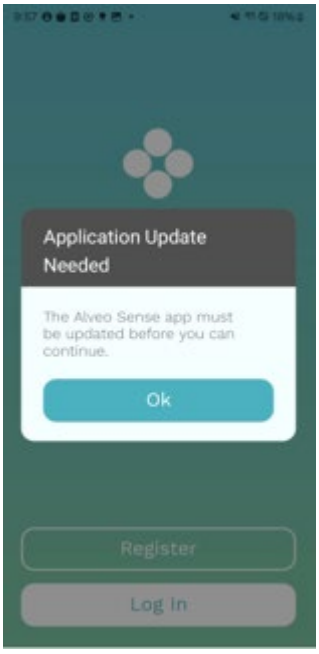
- Cancel
- Select existing farm
- Confirm farm



Phone storage full

The Phone storage full pop-up window informs the USER that there is not enough storage to use the App and to free up space.

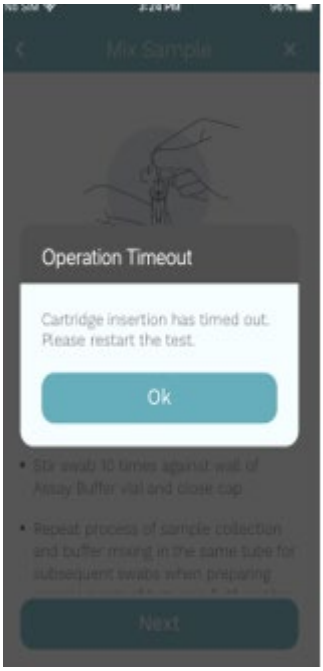
The USER clicks “Ok”.



Application Update Needed

The Application Update Needed pop-up window informs the USER that the application needs to be updated before they can continue.

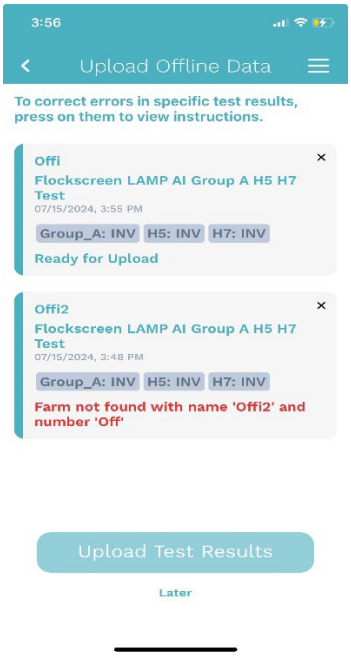
The USER clicks “Ok”.



Operation Timeout

The Operation Timeout pop-up window informs the USER that the time to install the cartridge to start the test has expired.

The USER clicks “Ok”.



Upload Offline Data

The Upload Offline Data screen displays all the offline tests that are ready for upload to the cloud.

Any tests that have an error to be resolved are displayed with the error message in red text. After the errors are resolved, the tests may be uploaded.

The User chooses

- Upload Test Results
- Later

Upload Test Results button will be activated once all errors are resolved.

When User clicks Upload Test Results, the screen shows the tests being uploaded and their status.

If there is an issue with upload, Upload Failed will be displayed next to the test.

In this case, the User chooses

- Verify
- Continue

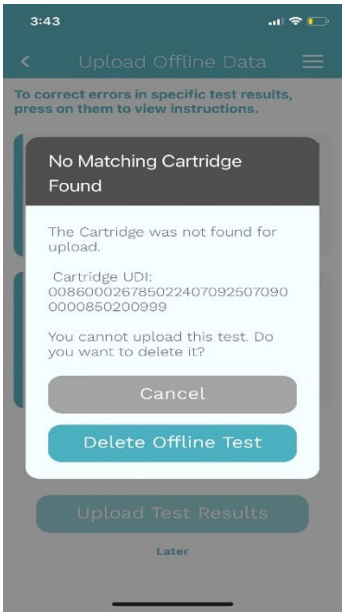
Verify will allow User to check the offline tests and resolve any issues.

If there is no internet during upload, the upload will fail and user gets a message to turn on internet and retry upload.

User can turn on internet and upload offline data or click Continue.

When the tests are uploading, the Uploading message is displayed next to the test. This message changes to Upload Completed once the upload is successful.

The User can click the “Continue” button once upload is completed. It is greyed out while the tests are being uploaded.

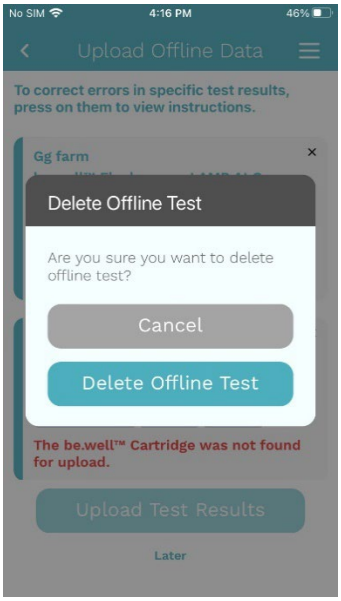


No Matching Cartridge Found

The No Matching Cartridge Found popup informs the user that the Cartridge was not found for upload and the test cannot be uploaded.

User selects

- Cancel
- Delete Offline Test



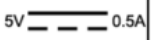
Delete Offline Test

The Delete Offline Test popup confirms with the user if they want to delete the offline test.

User selects

- Cancel
- Delete Offline Test

13 Symbols

	Manufacturer		Caution
	Batch code		Consult Instructions For Use
	Part number		Use-by YYYY-MM-DD
	Serial number		Do not use if packaging is damaged
	Keep dry		Single use, do not re-use
	Temperature limit		Sterilized using ethylene oxide
	Dispose of material		One (1) box contains materials for ten (10) tests
	Input rating		Electrical and Electronic Equipment Waste: Discard product at separate collection facility for recovery and recycling.
			Compliance to FCC Part 15 Subpart B

14 Customer Support

For assistance for ordering product or technical support, please contact GD Animal Health support, support@gdanimalhealth.com. For product quality issues, please contact Alveo support at support@alveotechnologies.com.

15 Product Descriptions

REF A-FIN-000037 Alveo Sense Poultry Avian Influenza Test Type A H5 H7

REF A-FIN-000031 Alveo Sense Poultry Avian Influenza Test Type A H5 H9

REF A-FIN-000039 Alveo Analyzer

REF Alveo Sense Mobile App

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