

Healthy Dairy Cattle H5N1 Bird Flu Testing Protocols for
Monitored Herd, Pre-Movement, and Non-Monitored Herd Surveillance
FOR PRODUCERS

H5N1 Bird Flu virus is an emerging disease in cattle which continues to be an evolving situation as more information becomes available. The mode of introduction into a herd may be via wild birds, new additions of cattle to the herd, contaminated equipment or vehicles, or people. Surveillance for HPAI in the dairy herd may provide for early detection and containment of the disease. **The following guidance is specifically for surveillance in healthy dairy herds with no clinical signs of bird flu.**

Producer Alerts:

1. Producers with cattle showing clinical signs of bird flu need to contact their local Animal Health Branch District office for further information with respect to the clinical animal testing protocol.
2. **Lactating dairy cattle with clinical signs consistent with bird flu are ineligible for interstate movement, as per 9 C.F.R. 71.3(b).**
3. If pre-movement or surveillance testing is positive, the dairy/herd veterinarian will be contacted by a CDFA AHB or USDA veterinarian to initiate a Foreign Animal Disease (FAD) investigation.

Producers are invited to enroll their herds in either the state or federal programs detailed in Options 1 or 2 below. Enrollment and testing are free to producers and help producers verify their herd status.

I. HPAI in Livestock Surveillance Options for Producers:

Note: All laboratory submissions require a National Premises Identification Number (NPIN), which must be provided on the laboratory submission form. See [Location Number Request](#) or contact your local AHB district office for more information on obtaining a NPIN.

All HPAI tests on cattle must be performed by a lab in the National Animal Health Lab Network (NAHLN). CAHFS-Davis is the only laboratory conducting testing in California.

OPTION 1: CDFA HPAI in Livestock Monitored Herd Program

Purpose & Benefit: The Voluntary CDFA HPAI in Livestock Monitored Herd Pilot Program aims to create additional testing options for producers with herds that have tested negative for three weeks in a row. This program is primarily designed to provide assurance that dairies are conducting routine surveillance to monitor herd status.

How to enroll: Contact your local AHB District Office

Surveillance Protocols (producers can use either bulk tank or individual cow protocol)–

1. **Bulk Tank sampling** (preferred surveillance protocol):

Herd Size: Any size herd

Sample requirements:



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- The bulk tank sample needs to be representative of all cattle in a lactating herd in a 24-hour period
- Collect a minimum of seven (7) mls of milk from the Bulk Tank
- Collect sample(s) from the sick pen
- Sample collection vial required: 10-15 ml sterile screw top tubes/vials (Do NOT use snap cap vials or red top tubes)

Testing Frequency Healthy Strings:

Week 1 - 3: Once per week for the first 3 weeks

Continue every two (2) weeks thereafter

Testing Frequency Sick Pen:

Week 2 or 3: Collect a representative sample of milk from the hospital pen. The sample can be individual cow samples from all functional quarters from up to 30 head of cattle or a single bulk tank sample that represents the hospital pen.

Continue every four (4) weeks thereafter

2. Individual Cow Sampling

Herd & Sample Size:

| Milking Herd Size | Sample Size |
|-------------------|-----------------------------------|
| <33 head | Sample all lactating cows in herd |
| ≤ 1,000 head | 33 |
| > 1,000 head | 34 |

Sampling Requirements:

- Individual dairy cattle should be sampled in order of the following priority:
 1. Cows with clinical signs consistent with HPAI (H5N1)
 2. Cows in a hospital pen
 3. For dairies with activity monitors: cows with health alert/event on daily activity monitor
 4. Cows 30 to 150 days in milk (DIM)
 5. All other cows in the herd not mentioned above
- Wash and disinfect teats before sample collection
- Collect a minimum of seven (7) mls of milk from all functional quarters into one tube
- Sample collection vial required: 10-15 ml sterile screw top tubes/vials (Do NOT use snap cap vials or red top tubes)

Testing Frequency:

- **Weeks 1 – 3:** Once a week for the first 3 weeks
- **Every other week thereafter**

OPTION 2: USDA Voluntary HPAI Dairy Herd Status Program

Purpose and Benefit: The Voluntary H5N1 Dairy Herd Status Pilot Program aims to create



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additional testing options for producers with herds that have tested negative for three weeks in a row. Once herds have demonstrated being free of H5N1, they will need to conduct weekly tests on bulk tank milk from that herd to retain their status. They will then be able to ship their cows at the time they prefer and without additional pre-movement testing required of individual animals.

How enroll: Producers can start enrolling in the Voluntary H5N1 Dairy Herd Status Pilot Program by contacting USDA Area Veterinarian in Charge and signing a Herd Monitoring Plan agreement. Contact Dr. Larry Rawson (Larry.rawson@usda.gov) or Dr. Adam Smith (adam.smith@usda.gov).

Surveillance Protocol –

Visit the USDA Website: [Detections of Highly Pathogenic Avian Influenza \(HPAI\) in Livestock \(usda.gov\)](#) for information on USDA's Dairy Herd Status Program

OPTION 3: Interstate Pre-Movement Surveillance for herds not enrolled in USDA Voluntary Program

Purpose and Benefit - Satisfies the requirements of the USDA Federal Order requiring that all lactating dairy cattle moving interstate have a Matrix-A negative HPAI test from a NAHLN lab, except when moving directly to slaughter. (Note: a Certificate of Veterinary Inspection (CVI) is also required within seven (7) days *before* interstate movement, with official individual identification listed for each animal in the shipment.)

How to enroll – No pre-enrollment needed

Surveillance Protocol –

Herd & Sample size

| Number of Dairy Cattle in Shipment | Sample Size |
|------------------------------------|-------------|
| ≤ 30 head | All animals |
| ≥ 30 head | 30 animals |

Sampling Requirements

- Samples to Collect: milk from all functional quarters of individual cows
- Wash and disinfect teats before sample collection
- Collect a minimum of seven (7) mls of milk from all functional quarters into one tube.
- If there are not enough lactating cows available to obtain 30 samples, you can collect nasal swabs to make up the balance of animals sampled and fulfill the sampling requirements.
- Sample collection vial required: 10-15 ml sterile screw top tubes/vials (Do NOT use snap cap vials or red top tubes)

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Testing Frequency

- Must test within seven (7) days *before* interstate movement.

Option 4: Herd Health Status Check (Not enrolled in any regulatory surveillance program)

For herds that do not wish to enroll in the CDFA HPAI Monitored Herd Program or the USDA Dairy Herd Status Program and still want to test for herd health status, follow the bulk tank or individual cow protocol in **Option 1: CDFA HPAI in Livestock Monitored Herd Program**.

Option 5: Herd Health Status Check for non-lactating cattle using nasal swabs

Purpose & Benefit: Nasal swabs can be used as a sample type when a producer wishes to sample non-lactating cattle for health status purposes. Nasal swab samples may aid producers in assessing the risk for movements of non-lactating dairy cattle.

Surveillance Protocol –

Herd & Sample size

- Collect samples from individual non-lactating cattle as needed.
- For statistically representative sampling of a herd:

| Herd Size | Sample Size |
|--------------|---|
| <33 head | Sample all non-lactating cows or calves in herd |
| ≤ 1,000 head | 33 |
| > 1,000 head | 34 |

Sample Requirements

- Use a single sterile swab with at least a 6-inch handle to sample both nostrils, firmly brushing the mucosa in the nasal passages with the swab.
- Place the swab in a 1.5 ml PrimeStore MTM tube, or as an alternative, BHI.
 - If no other option is available, sample with a swab pre-moistened with saline, place the swab in a sterile red top tube with a couple drops of saline, and seal the tube with the swab inside.
- If applicable, vigorously swirl the swab in the MTM or BHI media, express fluid by pressing the swab against the side of the tube and remove the swab. Do not leave the swab in the MTM or BHI media.

Testing Frequency:

- As needed

II. Submitting and Shipping Samples to the Laboratory:

1. Who can collect samples?
 - a. Accredited Veterinarians
 - b. Designated collector (dairy employee) trained and under direction of accredited veterinarians
2. Packaging Samples:
 - a. Label the tubes in order of sample collection that matches individual official IDs if

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collecting individual animal samples. Scan/input official IDs that correspond to tube labels into an Excel Spreadsheet file.

- b. Barcodes can be used to label tubes and must correspond to individual official ID when collecting from individual animals or National Premise Identification Number (NPIN) if collecting bulk tank samples.
 - c. Place each tube into a whirl-pak bag.
 - d. Place in a shipping container. Do not pack the shipping container too tight, as tubes can unscrew themselves by rubbing.
 - e. Maintain cold chain for all samples (ship on ice). Samples should never be stored in the freezer portion (-20°C) of a standard refrigerator/freezer unit. Rather store milk in the refrigerator (4°C) until shipping.
3. Complete the [Laboratory Submission Form](#):
- a. Depending on the reason for surveillance testing, select one of the following options in the top left section of the submission form:
 - i. Select “Monitored Herd Surveillance” if participating in either the CDFA or USDA monitored herd program
 - ii. Select “Non-monitored Herd Surveillance” if conducting routine surveillance not associated with a monitored herd or interstate movement
 - iii. Select “Pre-Movement” if testing for interstate movement and not enrolled in the monitored herd program
 - b. Scan or take a picture of the lab submission form.
 - c. Email submission form and Excel spreadsheet with IDs to (include tracking number for shipment when available): hpai.labcoordinator@cdfa.ca.gov
4. Ship samples **PRIORITY or FIRST OVERNIGHT** to:
- CAHFS-Davis
University of California, Davis
620 W. Health Sciences Dr.
Davis, CA 95616
530-752-8700

III. Receipt of Surveillance Results (expect at least a 3-day turnaround for results once samples are received by CAHFS-Davis)

1. **If testing is negative:**
 - a. Results will be sent to the sample submitter
 - b. Movement can proceed as planned
2. **If testing is positive:**
 - a. Results will be sent to the sample submitter
 - b. CDFA or USDA will contact the dairy and initiate “CA Response to HPAI in Cattle Protocol.”
 - c. Herd will be placed under quarantine

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d. Movement permits will be issued based on risk-assessments



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Table 1: Testing Protocol Options for HPAI H5N1 in Livestock

| | Pre Movement Testing for Interstate Movement for herds NOT enrolled in USDA Monitored Program | CDFA Monitored Herd Testing and Non Monitored Herd Testing | USDA Monitored Herd Testing | Nasal Swab Testing for Non lactating cattle |
|-------------------------------------|---|---|---|---|
| Specimen Type (s) | Milk from all functional quarters ¹ | Bulk Tank Milk (BTM) and/or Milk from all functional quarters | Bulk Tank Milk (BTM) | Nasal swab from both nasal passages |
| Collection Vial | 10-15 mls sterile clean screw cap tube/vial | 10-15 mls sterile clean screw cap tube/vial | 10-15 mls sterile clean screw cap tube/vial | 1.5 ml PrimeStore MTM tube, BHI or pre-moistened swab with saline- collect sample and place swab in a sterile red top tube with a couple drops of saline. |
| Submission Form Box to Check | Pre-Movement | Monitored Herd Surveillance Or Non-Monitored Herd Surveillance | Monitored Herd Surveillance | Non-monitored Herd Surveillance |
| Premises ID Required | Yes | Yes | Yes | Yes |
| Frequency of Sampling | Within 7 days <i>before</i> interstate movement (need CVI and official ID) | Weekly x 3 weeks Then every other week (if using bulk tank protocol test hospital pen once in the first 3 weeks then once every 4 weeks unless clinical signs develop) | 3 consecutive weekly tests (sample from hospital/sick pen on week 3) Then test BTM at weekly intervals and hospital/sick pen once every 4 weeks | As needed |
| Number of Cows to Sample | Shipping ≤30 head = all animals Shipping ≥30 head = 30 animals | Bulk Tank sample size: 1 BTM representative of all lactating cows in herd in a 24-hour period Individual animal sample size: Herds <33 head = all lactating cows in herd Herds ≤ 1000 head = 33 head Herds > 1000 head = 34 head | 1 BTM sample for each bulk tank emptying in 24-hour period or a single collection cycle- whichever is longer | Individual animal sample size: Herds <33 head = all lactating cows in herd Herds ≤ 1000 head = 33 head Herds > 1000 head = 34 head |

¹ If there are not enough lactating cows available to obtain 30 samples, you can collect nasal swabs to make up the balance of animals sampled and fulfill the sampling requirements.

Note: samples can be collected by an Accredited Veterinarian (AV) or a trained designated collector under supervision of an AV.

