



United States Department of Agriculture

NVSL AI/ND UPDATE



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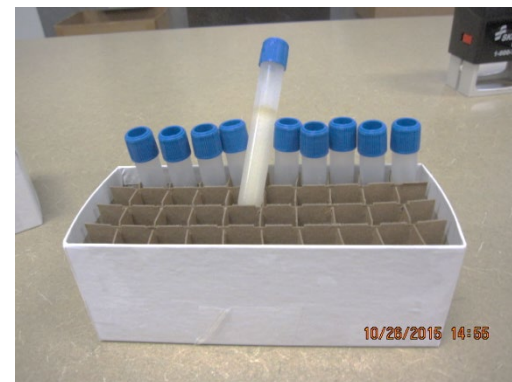
NPIP GCC
JUNE 2019
ALBUQUERQUE, NM

New Media Tubes for 5.5 ml

BHI – **blue cap-plastic tube 3ml** no abx for up to 5 OP/CL swabs from avian species

BHI – **white cap-plastic tube 5.5 ml** WITH abx for up to 11 OP swabs from gallinaceous poultry or environmental samples

Order form <https://www.aphis.usda.gov/library/forms/pdf/NVSLKitRequestForm.pdf>



3 ml BHI - no abx



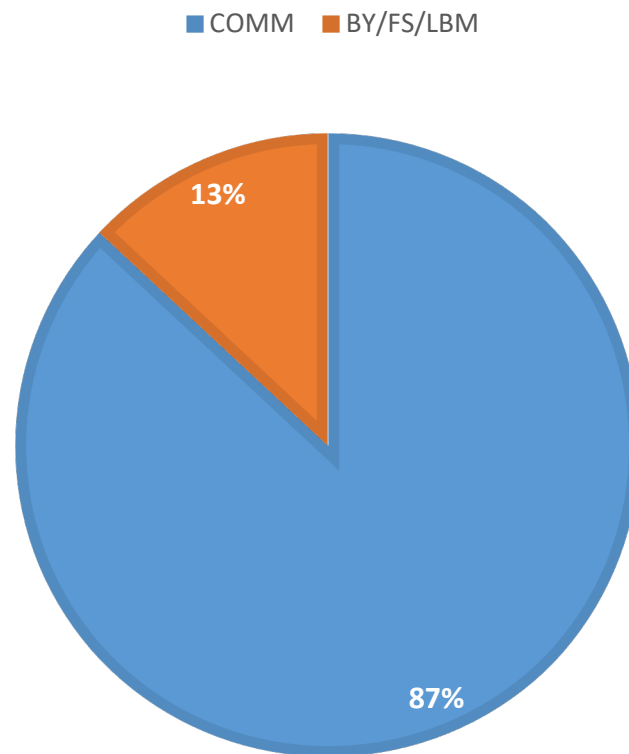
5.5 ml BHI + abx

Post C&D Environmental Testing

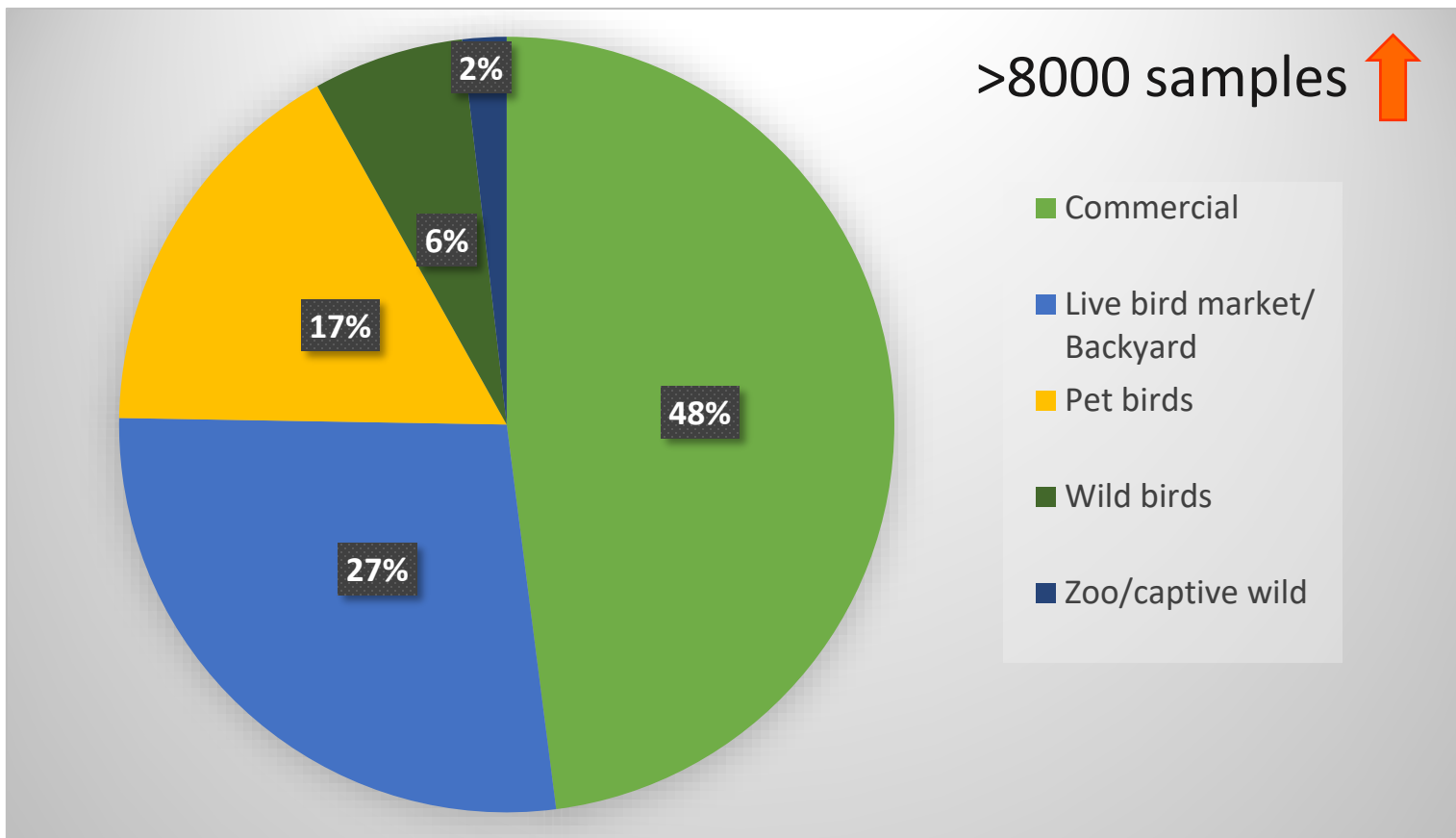
Since 2015, PCR use for official post C&D testing prior to virus recovery attempt has significantly reduced testing turnaround time and cost.

Of nearly 1400 post C&D samples 13% have been from BY/FS/LBM sector.

For reportable events (H5/H7/vND outbreak) official post C&D samples must be tested per WI-AV-0045 which includes PCR testing.



FY2018 Samples Submitted by Sector



Non-H5/H7 Events 2018-19

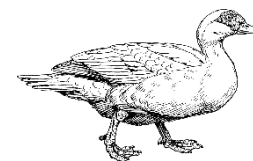
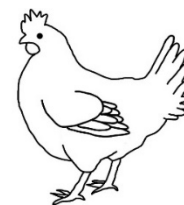
Wild bird viruses in turkeys

- MI H2N7 (Eurasian H2)
- PA, MD H2N3
- Swine lineage H1: MO; H3: NC



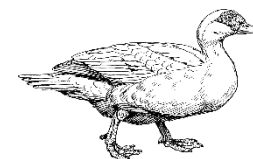
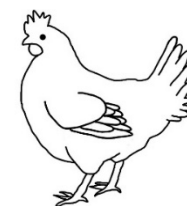
Other poultry

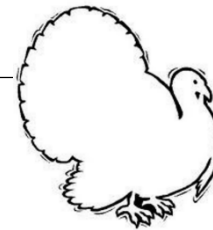
- AR H6N1 Broiler breeder
- FL H6N2 chicken LBM
- NJ, NY, PA H2N2 LBM



H5/H7 Events 2018-19

Date	State	Surv stream	initial sample	Subtype
Mar-18	MO, TX	commercial poultry (1 TX, 1 MO)	swab	H7N1 LPAI
Sep-18	CA	commercial turkeys	sera	H7N3 LPAI
Oct-18	MN	commercial turkeys	swab	H5N2 LPAI
Mar-19	PA, CT	LBM ducks	swab	H7N3 LPAI
Apr-19	CA	commercial ducks	swab	H5N2 LPAI

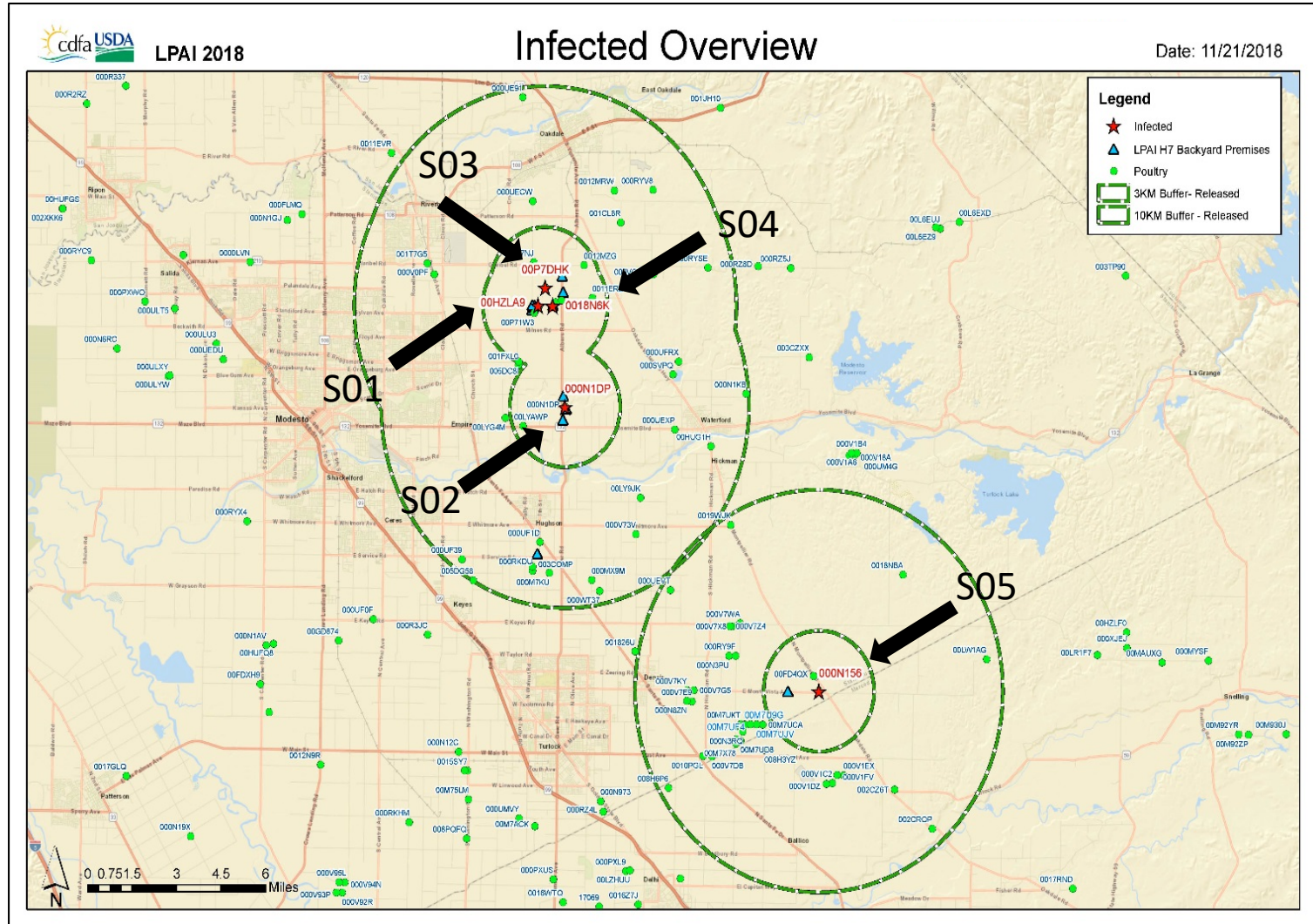


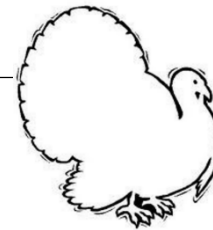


2018 Sept: H7N3 LPAI

- Birds initially submitted for testing in response to slight elevation in mortality and some respiratory signs.
- 6 Sept: CAHFS reported AI antibody and presumptive H7.
- 8 Sept: North American wild bird H7N3 LPAI confirmed at NVSL.
- 14 Sept: second flock confirmed with H7N3.
- Two additional flocks identified through incident area surveillance (9/14, 9/18).
- 11 Oct: fifth and final flock confirmed with H7N3 LPAI.

2018 Sept: AM H7N3 LPAI

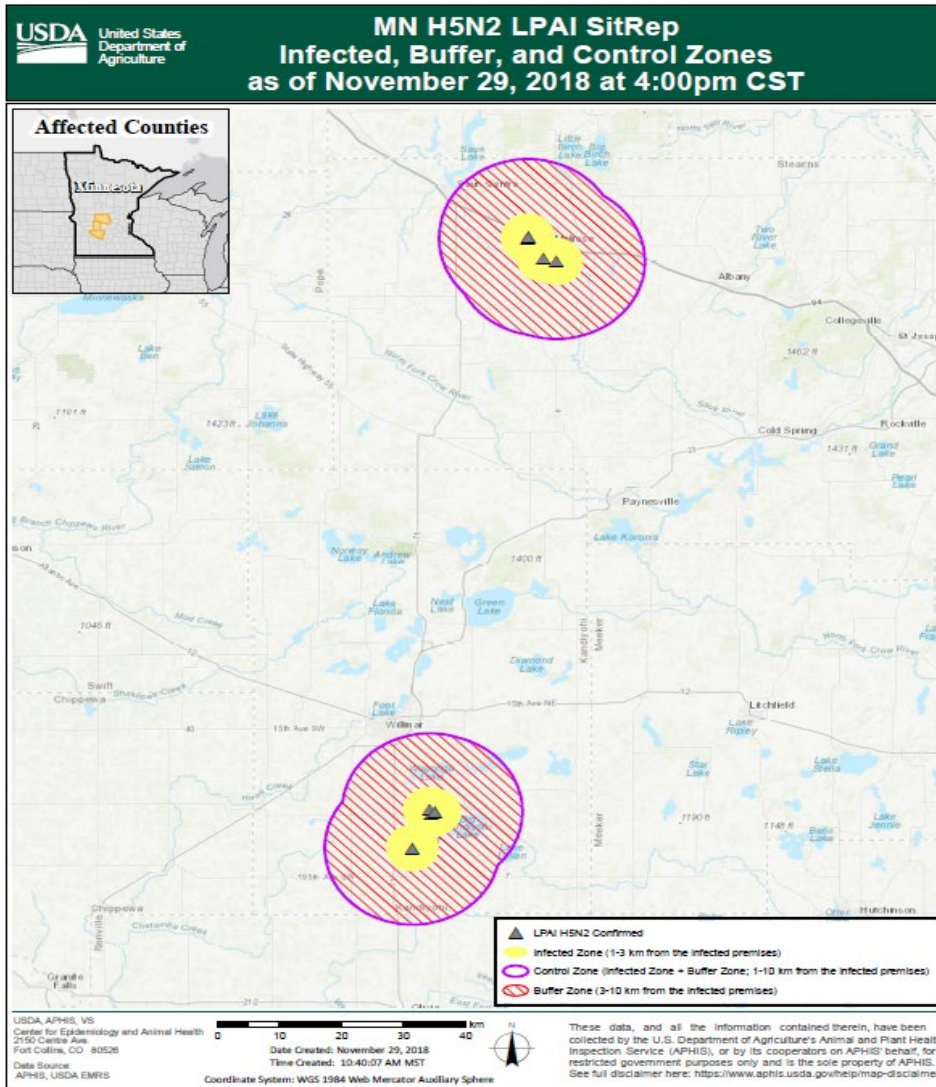




2018 Oct: H5N2 LPAI

- State and industry have defined high risk periods for enhanced surveillance in specific counties.
- 19 Oct: a non-negative drinker biofilm sample triggered collection of samples from the turkeys.
- Within 24 hours H5N2 LPAI was confirmed from the turkey samples.
- Another in a different county confirmed ~10 days later.
- Controlled marketing was pursued in accordance with Minnesota's Initial State Response and Containment Plan (ISRCP) and VS agreement.

2018 Oct: H5N2 LPAI

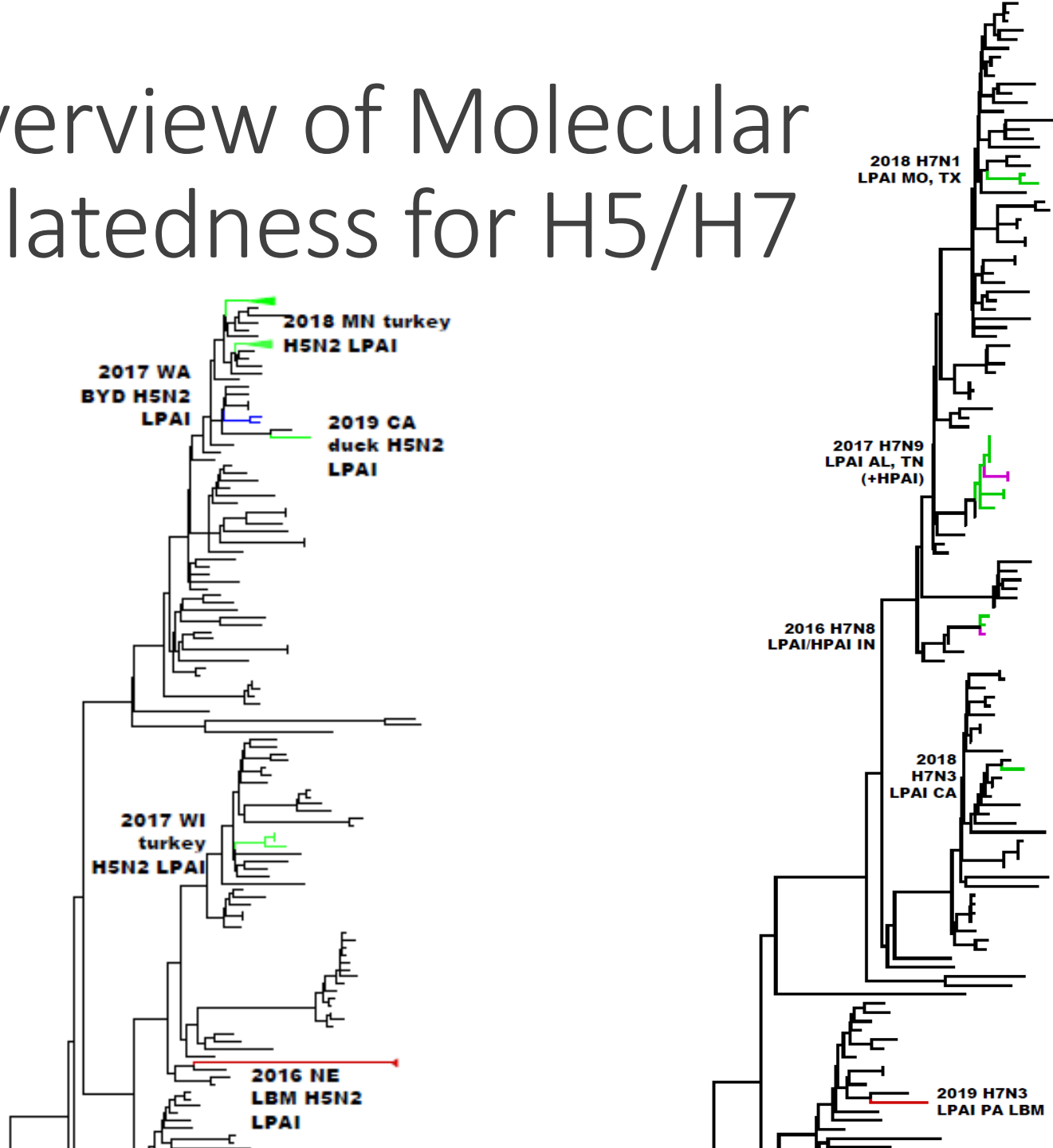


Independent introductions

Phylogenetic analysis suggests independent introduction by county followed by secondary spread within county.

	Virus from County 1	Virus from County 2
PB2	96%	95%
PB1	94%	95%
PA	99%	89%
HA	99%	99%
NP	99%	93%
NA	99%	99%
MP	99%	97%
NS (MALL)	99%	78%
NS(NOPI)	79%	99%

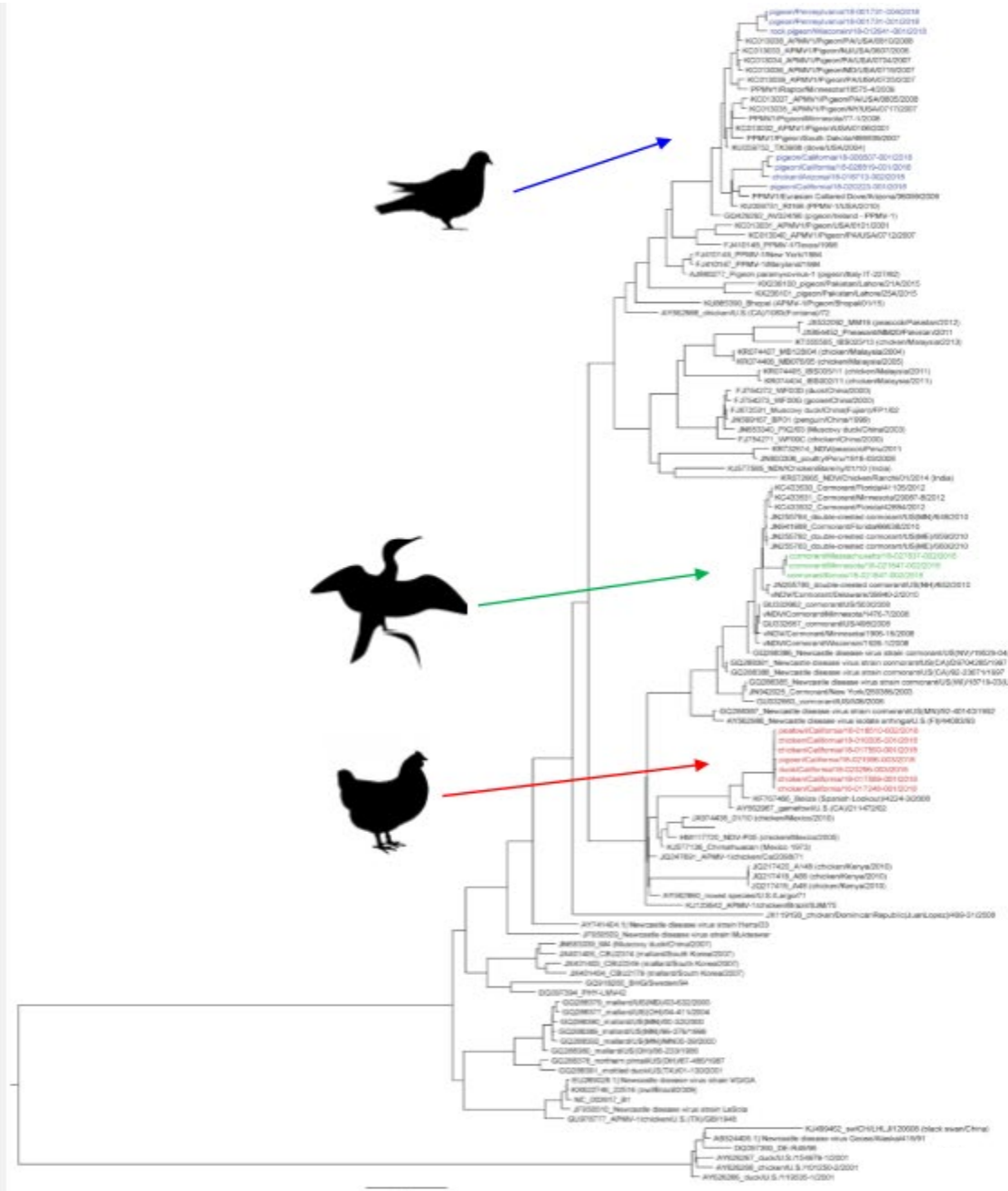
Overview of Molecular Relatedness for H5/H7



APMV-1 in 2018

Phylogeny of representative viruses

- 2018 pigeon/dove
PPMV-1 from AZ, CA, ID, ME, MN, MT, NV, PA, TX, WA, and WI
- 2018 **cormorant** from IL, MA, and MN – also reported in NY
- 2018 **vNDV CA backyard exhibition birds**



California vND Incident 2018

On **May 16, 2018**, the California Department of Food and Agriculture (CDFA) detected virulent Newcastle disease in a small flock of backyard exhibition chickens.

Since May 18, USDA has confirmed more than 440 cases of vND in backyard birds in California, one in Utah, and one in Arizona.

Since December 18, 4 cases have been confirmed in commercial operations.

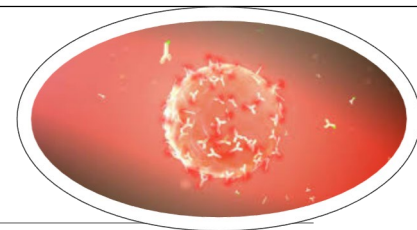
Phylogenetic Analysis CA 2018

The CA 2018 virus (genotype Vb) is related to Mexican-lineage viruses from Central America village poultry (Belize 2008, Honduras 2007), and the U.S. (smuggled parrot 1996, backyard CA 2002).

- Preliminary analysis of CA2018 virus isolates supports a single introduction followed by secondary spread.
- Lack of epidemiologic and contemporary sequence data contribute to the uncertainty surrounding the origin of the outbreak.
- Evolutionary analysis of available sequences with the CA2018 and CA2002 viruses suggest ongoing circulation of the virus; however, where and in what population remains unclear.

The virus is not related to classic vaccine strains, available data from vaccinated poultry in Mexico (2000-2010), species-adapted viruses from columbids (pigeons, doves), nor closely related to those from double crested cormorants.

AI Antibody Surveillance



Based upon Type A influenza: antibody to conserved proteins M, NP

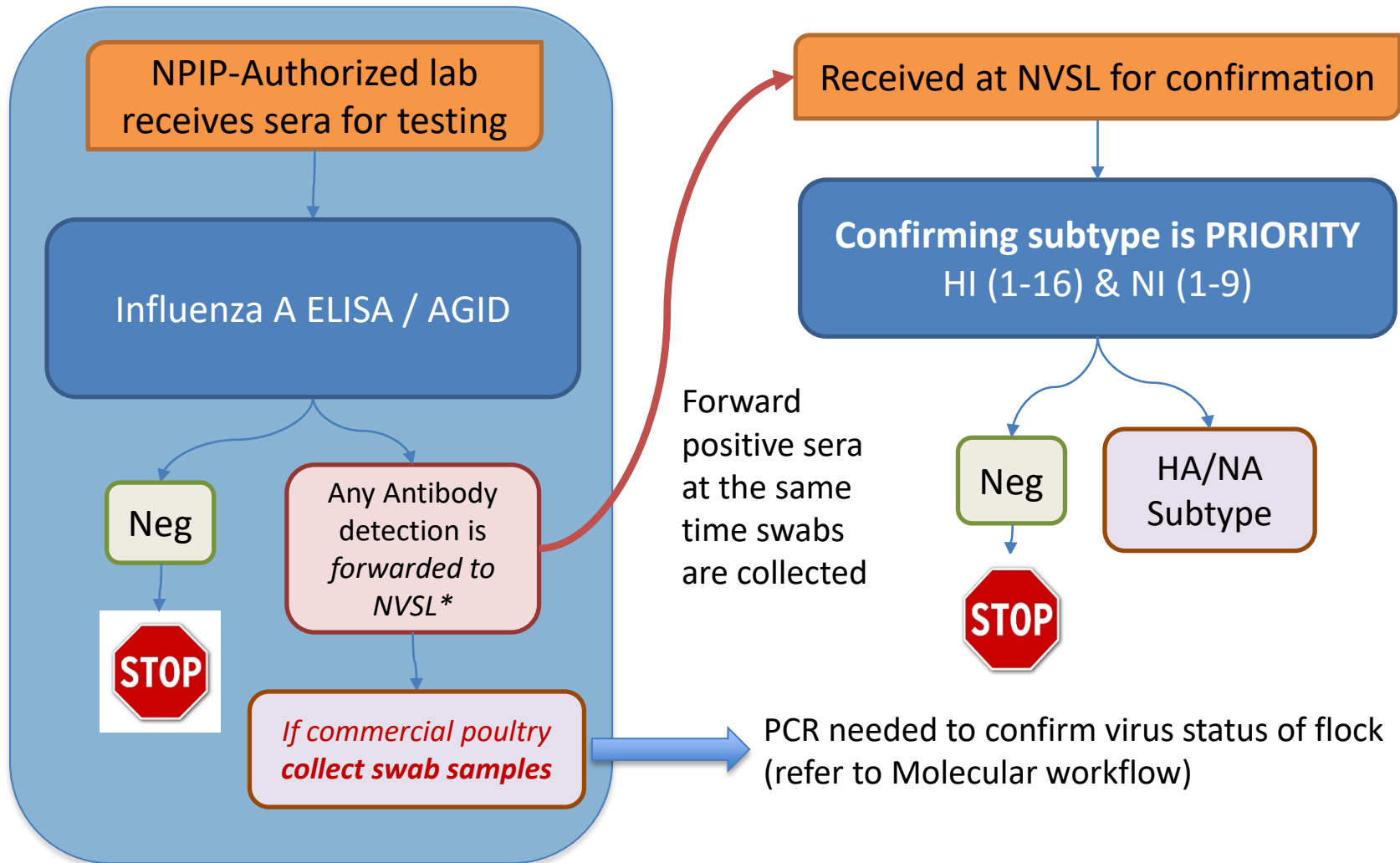
Indicator of prior exposure – useful to demonstrate disease freedom in longer lived birds

Typically used pre and post-outbreak

If vaccine is used...*e.g. swine lineage H1/H3 for turkey breeders*

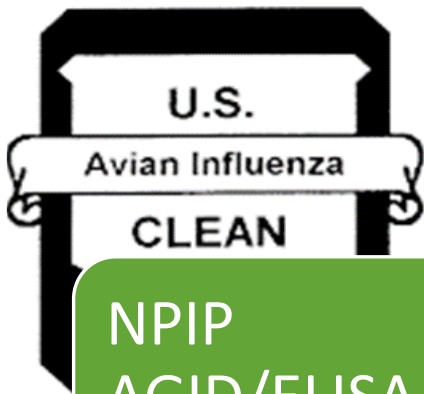
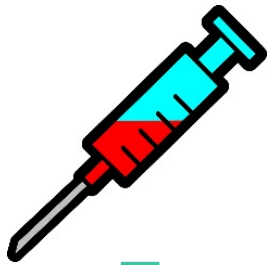
- Antigen testing is recommended to address movement testing needs
- Does not exclude the potential use of serology to determine prior exposure as part of routine health or program monitoring

NPIP Serologic Workflow (AI)



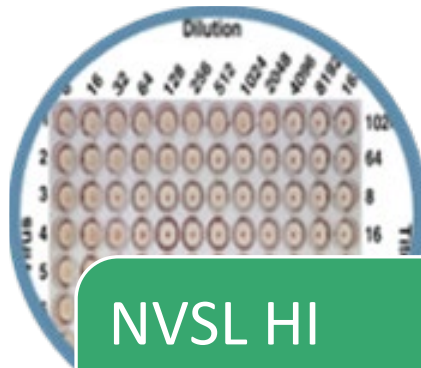
Serologic Test Protocol (AI)

Exposure confirmed through repeatable detection targeting antibody to different parts of the virus
Test cannot determine virus status or pathotype



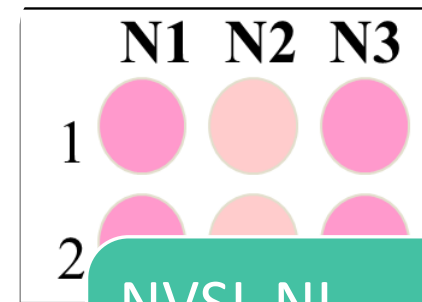
NPIP
AGID/ELISA

- Detect antibody to NP



NVSL HI

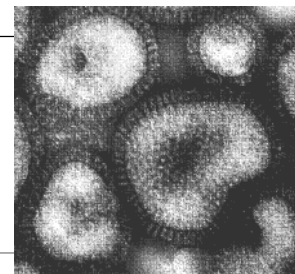
- H1-H16
- Confirm antibody to HA



NVSL NI

- N1-N9
- Confirm antibody to NA

Antigen/RNA Surveillance



Primary tests target conserved proteins/
genes: e.g. matrix gene

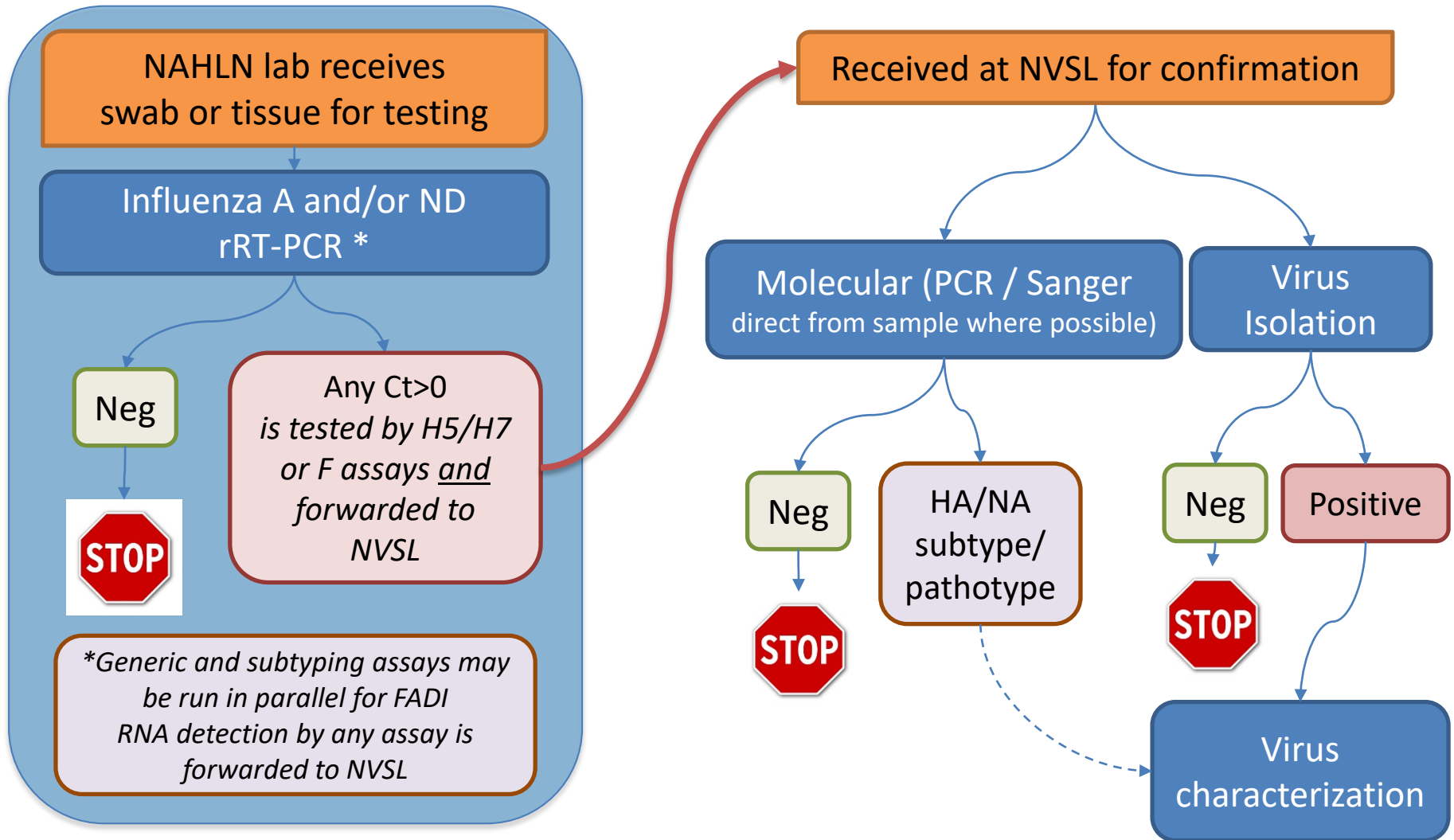
**Provides concrete evidence of virus – useful
to demonstrate active virus shedding**

Used pre, during, and post-outbreak

Vaccine use does not impact the test

Recovery of virus for characterization

Molecular Workflow



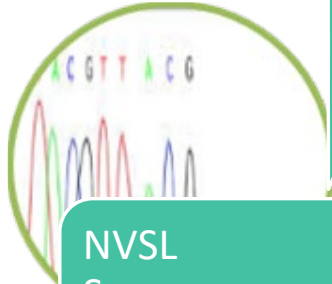
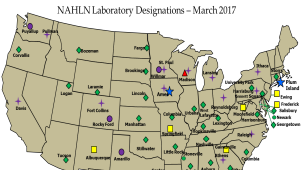
Molecular Test Protocol

Disease confirmed through repeatable detection targeting different parts of the virus



PCR confirms H5/H7

Cleavage site sequence confirms vNDV



NAHLN lab rRT-PCR

- Generic (e.g. matrix)
- Specific (H5/H7/ND)

NVSL repeats rRT-PCR

- Specific (H5/H7/ND)
- +/- generic (e.g. matrix)

NVSL Sequence

- Partial (H&N for IAV, F for ND)
- Full genome

NVSL Virus Isolation

- In vivo test
- Gen/ant.char



The Road to Confirmation...

For flu, don't forget to collect swabs for PCR testing if detecting antibody or using ACIA

NAHLN / NPIP FORWARDS
non-negative samples

NVSL Confirmation
For AI = H5/H7 PCR
For ND = partial F gene sequence

Virus Characterization
Full genome sequence,
In vivo testing

➔ Where HPAI/vND suspected, depopulation may be initiated if:

- **H5/H7/fusion-gene RNA** is detected at a NAHLN lab, from a flock that meets the **USDA case definition**, AND there is **agreement** between state and federal officials

AND forward samples to NVSL

Handy Resources

Avian Sample Collection [WI-AV-0020](#)

[H5/H7 Avian Influenza Case Definition](#)

[Newcastle Disease Case Definition](#)

[Post C&D Environmental Sampling Guide](#)

APHIS FAD PReP website

<http://www.aphis.usda.gov/fadprep>

Secure Poultry Supply Plan

www.securepoultrysupply.com

FAD PReP

Special Thanks to:

- CAHFS, MPTL
- USDA ARS SEPRL
- SAHOs, NPIP and NAHLN Labs
- Team Avian, Diagnostic Virology Lab

Remember to send duplicate samples to your NAHLN lab and to NVSL in parallel for the fastest confirmation!

