Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

FOI to US Department of Agriculture re "avian influenza virus"

Christine, an unincorporated woman <cmssyc@gmail.com> Sun, Jul 21, 2024 at 6:48 PM
To: foia.officer@aphis.usda.gov

July 21, 2024

To:
Tonya Woods, FOIA Officer
Department of Agriculture
4700 River Road
Unit 50
Riverdale, MD 20737
foia.officer@aphis.usda.gov

Dear Tonya,

This is an order for access to general records, per the Freedom of Information Act.

Description of Requested Records:

All studies in the possession/custody/control of the **Animal and Plant Health Inspection Service, Department of Agriculture**, authored by anyone, anywhere:

1.
- that scientifically prove/provide evidence of the existence of any alleged "**avian influenza virus**" (showing that the alleged particles exist, invade and replicate in "host" cells and cause the illness/symptoms that they are alleged to cause).

Note: Scientific proof/evidence is not opinions, speculation, declarations, review papers or descriptive studies; it requires use of the scientific method to test falsifiable hypotheses through valid, rigorous, repeatable controlled experiments.

2.
- that describe the purification of particles that are alleged to be "avian influenza virus" directly from bodily fluid/tissue/excrement of so-called "hosts" (without adding any sources of genetic material or proteins), with purification confirmed via EM imaging (the images must be available as well).

Purify = separate from everything else in the clinical sample.

I am aware that according to "virus" dogma a "virus" requires host cells in order to replicate. I am not seeking records describing the replication of a "virus" without host cells or that describe a suspected "virus" floating in a vacuum or a strict fulfillment of Koch's Postulates. I am simply seeking records that describe purification (separation from everything else in the "host" sample). I am not seeking private patient records.

3.
- wherein the purported "genome" of any alleged "avian influenza virus" was found intact in the bodily fluid/tissue/excrement of a "host" (as opposed to fabricated in silico, aka a computer model).

4.
- that scientifically demonstrate contagion of the illness / symptoms that are allegedly caused by purported "avian influenza viruses".

General Notes:

If any records match the above description and are publicly available, please provide enough information about each one so that I may identify and access it with certainty (i.e. title, author(s), date, journal, URL, DOI...).

This request is not limited to records that were authored by someone at the Department of Agriculture or that pertain to work done at/by the department; it includes any record(s) matching the above description authored by anyone, anywhere, ever.

If a timeframe is necessary, please use January 1, 1950 until the date of the search.

Format and conveyance:
Searchable pdf documents sent to me via email; please don't ship anything to me.

Fees:
Please let me know if there will be a fee greater than $40. If there will be a fee, I will be requesting a waiver given:
- that the topic is of urgent public health interest,
- countless animals have been killed over the years based on unsubstantiated claims of "viral infections", hence exposing the pseudoscientific nature of virology will prevent this from happening again - which will in turn save lives and vast sums of money, prevent baseless fear and stress, dispel any need for "vaccines" and protect the food supply.

Contact Information:
email: cmssyc@gmail.com

Thank you in advance and best wishes,
Christine

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

FOI to US Department of Agriculture re "avian influenza virus"

Christine, an unincorporated woman <cmssyc@gmail.com> Sat, Aug 17, 2024 at 2:53 PM
To: foia.officer@aphis.usda.gov

Hello,

Am I going to get a response soon?

Regards,
Christine
[Quoted text hidden]

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Regarding your FOIA Request 2024-APHIS-06103-F

yvonne.marquez@usda.gov <yvonne.marquez@usda.gov> Thu, Aug 22, 2024 at 6:44 AM
To: cmssyc@gmail.com

Dear Christine.,

My name is Yvonne Marquez, and I am a Government Information Specialist with Freedom of Information staff of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). I have been assigned to the above-mentioned Freedom of Information Act (FOIA) request. First, apologies, for the delay as we worked to find the appropriate office to search and collect records for your request. I assure you we will work as quickly as possible to provide you with a thorough response to your request.

APHIS processes FOIA requests by date of receipt, on a first-in, first-out basis. In an effort to identify and obtain the documents of interest to you, our intake staff previously forwarded your request to the program office(s) believed to have purview over the records. When that search has concluded, your case will be assigned a track (simple or complex), based on the complexity and/or volume of the documents identified. Any responsive documents that have been found will then be forwarded to me for review and possible release. I notice you do not have a Public Access Link (PAL) account. You may create you here: USDA Public Access Website-Home This allows for you to receive records directly to your email without file restriction on our end.

If you have questions about this e-mail or the processing of your request, please contact me directly at yvonne.marquez@usda.gov or 301-851-4058. I am on leave starting today and will be back at my desk on September 3, 2024. Please expect an update at that time. Have a wonderful rest of your week and weekend.

Sincerely,

Yvonne Marquez

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Regarding your FOIA Request 2024-APHIS-06103-F

Christine, an unincorporated woman <cmssyc@gmail.com> Thu, Aug 22, 2024 at 8:16 AM
To: yvonne.marquez@usda.gov

Thank you Yvonne.

It has already been 1 month since I filed the request. The Freedom of Information Act states in section(a) (3) (A) that:

> "*each agency, upon any request for records which (i) reasonably describes such records and (ii) is made in accordance with published rules stating the time, place, fees (if any), and procedures to be followed,* ***shall make the records promptly available*** *to any person".*

Further, the request relates to an urgent issue regarding public health, animal health, food safety, commerce, economics, etc. brought on in large part by supposed "experts" / "authorities" at the department who have published propaganda, "tested" and, from what I hear, encouraged state departments to pester farmers to provide samples, all over an alleged "virus" (**actually never shown to exist**), and they continue posting information regarding the **results** of fraudulent, impossible-to-validate "tests" that don't test for a virus.

Note that the CDC and other institutions have already been challenged to provide/cite records necessary to show the existence of this alleged "virus" and they all failed, because there has **never been** any valid scientific evidence of "its" existence. I will post links to these responses further below.

Thank you, however I don't want a Public Access Link (PAL) account and don't need one. I don't believe there will be any responsive records, and if the "experts" provide nonresponsive records they will be publicly accessible records with no privacy issues. Furthermore, I do wish to associate with the department in such a manner.

Here are prior "avian influenza virus" FOI responses:

May 5, 2022:
U.S. CDC and Agency for Toxic Substances and Disease Registry confirmed that a search of their records failed to find any that describe anyone on Earth finding an alleged **"avian influenza virus"** in the bodily fluids of any diseased diseased host (animal or human) and purifying "it"… which is necessary so that "it" could be sequenced, characterized and studied with controlled experiments:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/05/CDC-avian-influenza-PACKAGE-redacted.pdf

July 29, 2024:
Sarah B. Kotler ("J.D.") acting as Director, Division of Freedom of Information, confessed that the **US Food and Drug Administration (FDA)** has no records authored by anyone, anywhere:
1. that **scientifically prove/provide evidence of the existence of any alleged** "**avian influenza virus**",
2. that describe the purification of particles that are alleged to be "avian influenza virus" directly from bodily fluid/tissue/excrement of so-called "hosts",
3. wherein the purported "genome" of any alleged "avian influenza virus" was found intact in the bodily fluid/tissue/excrement of a "host" (as opposed to fabricated in silico, aka a computer model), or
4. that scientifically demonstrate contagion of the illness / symptoms that are allegedly caused by purported "avian influenza viruses"…
[see **pg 12**].

May 20, 2022:
Public Health Agency of Canada confirmed confirmed that they have no record of any alleged "**avian influenza virus**" having been found in and purified from the bodily fluid/tissue/excrement of any diseased "host" on the planet (in order for "it" to be sequenced, characterized and studied with controlled experiments) by anyone, anywhere, ever.
Insanely, they insist that 1) "viruses" are in hosts despite their utter inability to find them there, 2) it's necessary to "grow them" in non-host cells (as if "they" would grow better there than they allegedly grew in the diseased host lol), and 3) they pretend that mixing complex substances together = purification.
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/05/PHAC-avian-influenza-PACKAGE-redacted.pdf

Japan's National Institute of Infectious Diseases has no confirmation of "**avian influenza virus**" pathogenicity using isolated/purified particles:
https://www.fluoridefreepeel.ca/wp-content/uploads/2024/04/Japan-National-Institute-of-Infectious-Diseases-avian-influenza-virus-PACKAGE.pdf
Related article:
https://www.fluoridefreepeel.ca/japan-natl-inst-of-infectious-diseases-has-no-scientific-evidence-of-viruses/

April 4, 2023:
European Centre for Disease Prevention and Control can't prove the existence of "**H5N1**":
Vicky Lefevre, Head of Unit, Public Health Functions, ECDC, responding to my colleague, failed to provide or cite even 1 record of any alleged "***H5N1 avian influenza virus*** being found in the bodily fluid/tissue of any bird that supposedly died from "the virus" and purified… in order for "it" to be sequenced, characterized and studied with controlled experiments, aka "science":
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/04/ECDC-H5N1-avain-influenza-PACKAGE-redacted.pdf

April 21, 2023:
UK Animal and Plant Health Agency (APHA) cannot show the existence of any "H5N1 virus" in birds
An anonymous man/woman with the Animal and Plant Health Agency (APHA), UK confessed to James Henderson that no one there has any record of anyone on Earth finding and purifying the alleged "H5N1 highly pathogenic avian influenza (HPAI) virus" from any dis-eased bird, ever, and thus they confessed to being unable to show "its" alleged existence since they know of no one obtaining a valid independent variable to study. According to the confession letter:
"*APHA is an Executive Agency of the Department for Environment, Food and Rural Affairs and also works on behalf of the Scottish Government, Welsh Government and Food Standards Agency to safeguard animal and plant health for the benefit of people, the environment and the economy.*"
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/06/UK-Animal-and-Plant-Health-Agency-H5N1-PACKAGE.pdf

April 11, 2022:
Thomas Piggott, the man who acts as Medical Officer of Health at **Peterborough Public Health** (Ontario, Canada) has no scientific studies to show that the alleged "avian flu virus" even exists. The health unit was unable to cite even 1 record of "it" ever having been purified from any alleged host anywhere on Earth (which would be a necessary step in proving that an alleged virus does exist)… and doesn't even have a copy of the testing protocol that was implemented to "confirm cases" in Peterborough.
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/04/Peterborough-Public-Health-avian-flu-PACKAGE-redacted.pdf

Regards,
Christine
[Quoted text hidden]

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Automatic reply: [EXTERNAL: Suspicious Link]Re: Regarding your FOIA Request 2024-APHIS-06103-F

Marquez, Yvonne - MRP-APHIS <yvonne.marquez@usda.gov> Thu, Aug 22, 2024 at 8:17 AM
To: "Christine, an unincorporated woman" <cmssyc@gmail.com>

Thank you for your message. I am out of the office today. I will return on Friday, August 30, 2024. If you need immediate assistance in my absence please contact my supervisor, Gerry Brooks via email at gerry.brooks@usda.gov. Regards, Yvonne Marquez Government Information Specialist Animal and Plant Health Inspection Service (APHIS) U. S. Department of Agriculture 301.851.4058 Yvonne.marquez@usda.gov

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Regarding your FOIA Request 2024-APHIS-06103-F

Christine, an unincorporated woman <cmssyc@gmail.com> Thu, Aug 22, 2024 at 9:04 AM
To: gerry.brooks@usda.gov

Hi Gerry,

Since Yvonne is suddenly out of the office, I'm forwarding this to you.

Regards,
Christine
[Quoted text hidden]

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Regarding your FOIA Request 2024-APHIS-06103-F

Christine, an unincorporated woman <cmssyc@gmail.com> Thu, Aug 22, 2024 at 11:06 AM
To: yvonne.marquez@usda.gov

p..s. correction: *I don't wish to associate....
[Quoted text hidden]

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Regarding your FOIA Request 2024-APHIS-06103-F

Christine, an unincorporated woman <cmssyc@gmail.com> Thu, Aug 22, 2024 at 11:06 AM
To: gerry.brooks@usda.gov

p..s. correction: *I don't wish to associate....

[Quoted text hidden]

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

Regarding your FOIA Request 2024-APHIS-06103-F

Christine, an unincorporated woman <cmssyc@gmail.com> Wed, Jan 29, 2025 at 6:03 PM
To: yvonne.marquez@usda.gov, foia.officer@aphis.usda.gov

Kindly send me the response to my FOIA order dated July 21, 2024, attached, and be sure to identify yourself with your name, job title and the organization that you work for.

Christine
[Quoted text hidden]

US Dept of Agriculture avian influenza PACKAGE 2024 08.pdf
509K

Gmail Christine, an unincorporated woman <cmssyc@gmail.com>

Regarding your FOIA Request 2024-APHIS-06103-F

postmaster@usdagcc.onmicrosoft.com <postmaster@usdagcc.onmicrosoft.com> Wed, Jan 29, 2025 at 6:06 PM
To: cmssyc@gmail.com

Your message to yvonne.marquez@usda.gov couldn't be delivered.

yvonne.marquez wasn't found at usda.gov.

cmssyc	**Office 365**	**yvonne.marquez**
Action Required		Recipient

Unknown To address

How to Fix It

The address may be misspelled or may not exist. Try one or more of the following:

- Send the message again following these steps: In Outlook, open this non-delivery report (NDR) and choose **Send Again** from the Report ribbon. In Outlook on the web, select this NDR, then select the link "**To send this message again, click here.**" Then delete and retype the entire recipient address. If prompted with an Auto-Complete List suggestion don't select it. After typing the complete address, click **Send**.
- Contact the recipient (by phone, for example) to check that the address is correct.
- The recipient may have set up email forwarding to an incorrect address. Ask them to check that any forwarding they've set up is working correctly.
- Clear the recipient Auto-Complete List in Outlook or Outlook on the web by following the steps in this article: Fix email delivery issues for error code 5.1.1 in Office 365, and then send the message again. Retype the entire recipient address before selecting **Send**.

Was this helpful? Send feedback to Microsoft.

More Info for Email Admins

Status code 554 5.4.14

Typically this error occurs because the recipient email address is incorrect or doesn't exist at the destination domain. This can usually be fixed by the sender. However, sometimes the issue needs to be fixed by the recipient or the recipient's email admin. If the steps in the **How to Fix It** section above don't fix the problem, and you're the email admin for the recipient, try one or more of the following:

The email address exists and is correct - Confirm that the recipient address exists, is correct, and is accepting messages.

Synchronize your directories - If you have a hybrid environment and are using

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

USDA APHIS Freedom Of Information Act Request #2024-APHIS-06103-F

Kuralt, Wallace - MRP-APHIS <wallace.h.kuralt@usda.gov> Mon, Mar 17, 2025 at 2:15 PM
To: "cmssyc@gmail.com" <cmssyc@gmail.com>
Cc: "Ajua, David - MRP-APHIS" <David.Ajua@usda.gov>

Good afternoon,

Attached please find our Final Response to your USDA APHIS Freedom of Information Act (FOIA) Request #2024-APHIS-06103-F. If you have any questions, please don't hesitate to contact us at your convenience.

Best,

Hamilton Kuralt

Supervisory Government Information Specialist

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Freedom of Information Act & Privacy Act

4700 River Road, Riverdale, MD 20737

(301) 851-4010

2024-APHIS-06103-F - Final Response.zip
3998K

USDA Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Legislative and Public Affairs

Freedom of Information

4700 River Road Unit 50 Riverdale, MD 20737-1232

March 17, 2025

Christine Anonymous
cmssyc@gmail.com

RE: FOIA Request No. 2024-APHIS-06103-F

Dear Christine Anonymous:

This is in response to your Freedom of Information Act (FOIA) request dated July 21, 2024, received in this office on July 22, 2024, and assigned tracking number 2024-APHIS-06103-F. Please cite this number in any correspondence you send to APHIS regarding this request. Your request stated the following:

> *"All studies in the possession/custody/control of the Animal and Plant Health Inspection Service, Department of Agriculture, authored by anyone, anywhere:*
>
> *1. That scientifically prove/provide evidence of the existence of any alleged "avian influenza virus" (showing that the alleged particles exist, invade and replicate in "host" cells and cause the illness/symptoms that they are alleged to cause). Note: Scientific proof/evidence is not opinions, speculation, declarations, review papers or descriptive studies; it requires use of the scientific method to test falsifiable hypotheses through valid, rigorous, repeatable controlled experiments;*
>
> *2. That describe the purification of particles that are alleged to be "avian influenza virus" directly from bodily fluid/tissue/excrement of so-called "hosts" (without adding any sources of genetic material or proteins), with purification confirmed via EM imaging (the images must be available as well). Purify = separate from everything else in the clinical sample.*
>
> *I am aware that according to "virus" dogma a "virus" requires host cells in order to replicate. I am not seeking records describing the replication of a "virus" without host cells or that describe a suspected "virus" floating in a vacuum or a strict fulfillment of Koch's Postulates. I am simply seeking records that describe purification (separation from everything else in the "host" sample). I am not seeking private patient records;*
>
> *3. Wherein the purported "genome" of any alleged "avian influenza virus" was found intact in the bodily fluid/tissue/excrement of a "host" (as opposed to fabricated in silico, aka a computer model); and 4. That scientifically demonstrate contagion of the illness / symptoms that are allegedly caused by purported "avian influenza viruses".*

On July 23, 2024, we submitted your request to the Veterinary Services (VS) program to assist us in assessing whether a search for records could be conducted given the information provided in your request. Subsequent to consultation with VS subject matter experts and our own review of the request as written, it is our determination that your request lacks sufficient specificity as to the program personnel required for this search, any specific program areas to search for responsive records, or date range for records in order to conduct a search for records responsive to your request.

In response to our initial inquiry, and in order to attempt to best assist you, please note that VS program subject matter experts have provided two (2) documents, totaling twenty-nine (29) pages of records, appearing to be potentially responsive to your request. These records are being released to you in full.

Accordingly, due to the flaws in your original request rendering a reasonable search untenable, we are closing this request with this response and release. Please note that you may submit a revised request at any time. Please review the records provided with this response, and do not hesitate to contact us at your convenience if we can assist in any way or any future request.

You may contact David Ajua, the anaylst who process your request at (301) 851-4035 or by email at FOIA.Officer@usda.gov as well as Mr. Abbey Fretz, our FOIA Public Liaison, at (301) 851-4100 for any further assistance and to discuss any aspect of your request. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769. If you are not satisfied with this response, you may submit an administrative appeal by email to: FOIA.MRP.Appeals@usda.gov. Your appeal must be electronically transmitted within 90 days of the date of this response. Please reference case number FOIA 2024-APHIS-06103-F, and the phrase "FOIA APPEAL" in the subject line of your email. To assist the Administrator in reviewing your appeal, please provide specific reasons why you believe modification of this determination is warranted.

All fees have are waived.

Sincerely,

WALLACE KURALT

Digitally signed by WALLACE KURALT
Date: 2025.03.17 14:04:50 -04'00'

For:
Tonya G. Woods
Director
Freedom of Information & Privacy Act
Legislative and Public Affairs

Enclosures

National Veterinary Services Laboratories
1920 Dayton Avenue
Ames, IA 50010

Plum Island Animal Disease Center
P.O. Box 848
Greenport, NY 11944

Isolation of Avian Influenza and Avian Paramyxoviruses Viruses in Chicken Embryos from Avian Species

Document Number: **NVSL-SOP-0018** **Revision:** **05**

Previous Number: SOP-AV-0018.04

Author: MKILLIAN

Section/Area: Laboratory Activities

Release Date: 26 May 2022

Notes: Add References in next version
https://link.springer.com/protocol/10.1007/978-1-0716-0346-8_12
https://link.springer.com/protocol/10.1007/978-1-0716-0346-8_11#Sec3

Isolation of Avian Influenza and Avian Paramyxoviruses Viruses in Chicken Embryos from Avian Species

Table of Contents

1 Purpose/Scope

This protocol is used at the National Veterinary Services Laboratories (NVSL) for the isolation of influenza A virus (IAV) and avian paramyxoviruses (APMV) from embryonated chicken eggs. It includes the isolation of virulent Newcastle disease (vND) virus and highly pathogenic avian influenza (HPAI) virus as defined by World Organization for Animal Health (OIE) **NVSL-REF-0958**, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. The procedure is for the isolation of virus from avian specimens. For low pathogenicity avian influenza (LPAI) and low virulent Newcastle disease samples from the respiratory tract (trachea, air sac, lung, and sinus exudates) and digestive tract are suitable for isolation of the virus. Due to the systemic nature of HPAI and vND, virus can be isolated from a variety of tissues including liver, lung, spleen, heart or brain from clinically ill birds.

Tracheal and cloacal swabs can be pooled for isolation of virus from domestic poultry, however it is the policy of this laboratory to use swab pools for detection of viral RNA and for this reason tracheal and cloacal swabs should not be pooled together in one tube. If tissues or whole birds are submitted, a 10-20% suspension of the tissue is prepared in a Class II biological safety cabinet with a Tenbrock tissue homogenizer. Refer to **NVSL-WI-0023**, Avian Sample Collection for Influenza A and Newcastle Disease for additional sample collection information.

2 Definitions

- BSC – biosafety cabinet
- Candling – Evaluation of embryo viability (dead or live) status by viewing the mobility of the embryo and health of the chorioallantoic membrane through the eggshell with a microscope illuminator or other suitable light source.
- Allantoic route –Inoculation of a preparation of the diagnostic specimen or viral suspension into the allantoic sac of an embryonated egg.
- Nonspecific embryo death – dead embryos from which harvested allantoic fluid is negative for hemagglutinating virus and negative for bacterial contamination by the blood agar test or first day embryo deaths.
- Injury death – 24 hr embryo death due to inoculation injury
- AAF – amnio-allantoic fluid
- TBTB – tris-buffered tryptose broth
- Human-risk influenza A virus (IAV)—Regardless of the pathogenicity in animal species (LPAI, HPAI, mouse, swine, or ferret models), human-risk IAV are emergent or re-emergent IAVs that are isolated from human clinical cases and have caused human morbidity or mortality to the extent that they are considered a proximate threat to laboratory worker health or well-being. Human-risk IAVs

include but are not limited to Asian H5N1, reconstructed 1918 influenza, and the 2013 Chinese lineage of H7N9. When handling these viruses, follow the BSL-3E and ABSL-3E procedures outlined in **NVSL-WI-1207**, Biological Risk Assessments for Diagnostic Virology. Finally, human-risk IAVs must be separated from other agents, especially other IAVs; to avoid inadvertent cross-contamination. Follow the procedures outlined in the National Institute of Health (NIH) GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (Appendix G-II-C-5-a-(4), March 2013 revisions).

3 Special Precautions

Note: Personnel who work with human-risk IAV, HPAI, or vNDV select agents are required to wear a disposable wrap-around smock and shoe covers or select agent suite dedicated shoes. Any portion of the arms and hands entering the BSC will be sprayed down upon exit from the cabinet with 70% ethanol. Alternatively sleeve covers may be used but must be removed before removing arms and hands from the BSC. Gloves will still need to be sprayed with ethanol.

- **Personnel Requirements**Personnel will have read, understood, and agreed to abide by the contents of this protocol and its associated documents (including equipment).
  - Personnel performing the protocol will be aware of the potential hazards associated with the procedures performed. Specific hazards include biological hazard, chemical hazards, and the ergonomic hazard associated with repetitive motions.
  - Personnel will have completed safety training on biological safety cabinet (BSC) operation, autoclave safety, and must be familiar with any Safety Data Sheets (SDS) associated with chemicals required to perform this SOP.
  - Personnel will observe standard laboratory safety procedures, including using appropriate personal protective equipment (PPE): lab coat as per safety policy or disposable lab coat, safety glasses, and disposable gloves (per **NVSL-MAN-0018**, *Diagnostic Virology Laboratory Biosafety Security and Incident Response Plan*, for NVSL).

- **Disease agent-specific sample handling**
  - Guidance for Influenza A virus outlined in **NVSL-WI-1207**
    - Human-risk IAV[1]: Biosafety level 3 enhanced (BSL-3E) and Agricultural Biosafety level 3 enhanced (ABSL-3E) procedures**.**
    - Non-human-risk IAV[2]: Biosafety level 3 (BSL-3) or biosafety level 3-Ag (BSL-3AG) procedures will be followed.

[1] Emergent or re-emergent IAVs that are isolated from human clinical cases and have caused human morbidity or mortality and are considered a proximate threat to laboratory worker health or well-being, regardless of the pathogenicity in animal species (low pathogenicity non-H5/H7, LPAI H5/H7, HPAI), are categorized as “human-risk IAV,” and include but are not limited to Asian H5N1, reconstructed 1918 influenza, and the 2013 Chinese lineage of H7N9.
[2] All IAV that do not meet the definition of human-risk IAV.

- NVSL-specific guidance for virulent Newcastle disease virus (vNDV) is zoonotic agent which can cause conjunctivitis in humans; take appropriate safety precautions to avoid accidental introduction of the virus into the eyes.
  - Virulent NDV is a zoonotic agent which can cause conjunctivitis in humans; take appropriate safety precautions to avoid accidental introduction of the virus into the eyes. Biosafety level 3 (BSL-3) or biosafety level 3-Ag (BSL-3AG) procedures will be followed.

- **Disinfection and sharps handling**
  - All surfaces and equipment that come in contact with infected materials must be disinfected with an appropriate disinfectant such as iodine, 70% ethanol, or oxidizing agents prepared according to manufacturer's recommendations. All contaminated instruments, containers and fluids must be autoclaved before reuse or disposal.
  - Recapping syringes may cause accidental needle sticks, dispose of needles immediately without recapping. If recapping is necessary, do not bend or break the needle and do not remove the needle by hand.

4 Equipment and Materials Required

- **Equipment Required**
  - Sterile scissors, forceps, and tissue grinders (mortar and pestle, Tenbrock tissue homogenizer or equivalent)
  - Serologic pipettes (1 ml, 2 ml, 5 ml, 10 ml [approximate volume ± 2%])
  - Certified class II microbiological BSC
  - Sterile latex or nitrile examination gloves
  - Sterile polyester swabs with plastic or metal shafts (for whole birds)
  - Bags: Whirl-pack bags, zip lock bags or equivalent (for freezing tissues) and small bags for discarding wrappers etc. within safety cabinet
  - 3” x 5” note cards or equivalent
  - Receptacle with disinfectant for decontaminating used scissors, forceps, tissue homogenizer and pipettes
  - Covered pan containing disinfectant solution placed within easy reach of safety cabinet for disposal of contaminated glassware from BSC
  - Pro-pipette bulb, pipette aid or equivalent
  - Centrifuge (refrigerated) capable of attaining a force of 2,000 x g Beckman J-HC centrifuge or equivalent
  - Vortex mixer
  - 1-dram screw-cap vials
  - Electric jewelry engraving drill or equivalent
  - Light source such as microscope illuminator sufficient for viewing viability of embryo
  - 15 ml 17 x 120 mm centrifuge tubes
  - 3 cc syringe with 25 gauge 5/8 inch needle and with 23 gauge 1 inch needle
  - Egg and tube labels for identification of accession and sample number
  - 36-39C incubator with humidity
  - Ultra-low freezer (-60°C or below)
  - Egg flats

- **Reagents and Materials**
  - Unicide 256, (NVSL warehouse order #56158)
  - BioSentry Iodine, (NVSL warehouse order #42100)
  - 70% Ethanol (NVSL warehouse order #42011)
  - 7% iodine solution (NVSL media 30262)
  - 50% ethanol/iodine solution, prepared by mixing equal volumes of 7% iodine and 70% ethanol
  - TBTB containing 10T antibiotics in 1.8, 2.0, and 3.5 ml volumes and 33T (NVSL media 10410) antibiotics in 1-dram screw cap vial in 1.3 ml volume. Antibiotics are prepared according to current version of **NVSL-SOP-0013**, Preparation of Antibiotics to Treat Inoculums for Virus Isolation in Embryonated Eggs
  - Quick drying glue for sealing eggs (Duco Cement or equivalent)
  - 9-11 day old specific-pathogen free (SPF) embryonated chicken eggs (8 or 12-day-old SPF eggs may be used for occasions when 9-11 day eggs are not available such as Sundays)

5 Procedure

5.1 Procedure for Sample Receipt, Verification, and Log-In

In the Diagnostic Virology Laboratory (DVL), specimen containers and submission forms are removed from the NVSL transport plastic bag. All submission packages with evidence of leakage should be opened within a BSC with proper PPE. Specimen tube identification is compared to information provided on the NVSL 10-4 submission form for accuracy. All discrepancies should be noted on the original 10-4 submission form by the technical staff during the sample log-in process. Sample identification, number of samples submitted, and number of animals sampled needs to be checked for accuracy. If tube identification is different from the identification listed on the 10-4 submission form, the tube ID is noted on the VI worksheet and/or submission form as the official specimen identification. Sample information is entered into the laboratory information management information system (LIMS) for the creation of virus isolation (VI) worksheets, egg, and tube labels. The VI worksheet should include the following information if available: accession number, owner's name and address, date received, and individual specimen identification. Additional isolation procedures or request if outside of the standard procedures described below should be noted on the worksheet. The submission form is stapled to the worksheet and both sheets are placed in the case notebook in numerical accession order.

5.2 Procedure for Processing Swabs

5.2.1 Prepare and affix the client sample tube label (accession number and sample number) to the swab specimen tube and a duplicate antibiotic blank label to a 1-dram screw-cap vial containing 1.3 ml TBTB with 33T antibiotics.

5.2.2 Vortex the specimen tube and centrifuge at approximately 1,500 x g (2,500 rpm in Beckman J-6 centrifuge with JS 4.2 rotor) for 20-30 min at approximately 4°C.

5.2.3 Place the following equipment in a BSC: waste container with disinfectant, 2 ml serological pipette, pro-pipette bulb or equivalent pipette tool, centrifuged specimen tubes, and 33T 1-dram vials. Equipment should be arranged to allow a clean to dirty work flow. Note: Run the safety cabinet fan for approximately 3 min before using.

5.2.4 Aseptically remove 1.8 – 2.2 ml of supernatant with a 2 ml pipette and dispense into the corresponding 1-dram vial with 33T antibiotics. Note: If there is less than 1.8 ml of supernatant in the specimen tube, TBTB (without antibiotics) should be added to bring the total specimen volume to approximately 2.0 ml; otherwise the final antibiotic concentration will change. The TBTB should be added to the 1 dram vial and not to the original specimen tube. Record the volume of TBTB added (if ≥ 1 ml) to each specimen on the 10-4 submission form or accompanying VI worksheet.

5.2.5 Incubate specimen-antibiotic mixture for approximately 1 hr at room temperature before inoculating into embryonated eggs (section 5.4).

5.3 Procedure for Processing Tissues and Tissues Collected from Whole Birds

5.3.1 Remove the appropriate number of 3.5 ml 10T antibiotic blanks and/or BHI tubes from freezer and place in a rack to thaw. Label with accession and sample number (client sample label).

5.3.2 Place the bird/tissues and any necessary supplies (10T, and BHI tubes, sterile Tenbrock homogenizer, tube rack, polyester swabs, sterile scissors and forceps, sterile gloves, containers with disinfectant, and plastic bags) in a clean BSC. Note: Run the safety cabinet fan for approximately 3 min before using.

5.3.3 Remove sterile gloves from the wrapper and put the gloves on. Place the glove wrapper, sterile side up, on the cabinet tray to provide a sterile working surface.

5.3.4 Specimens collected from individual birds should be processed separately (not pooled) unless tissue sections were pooled in a single specimen bag upon receipt.

5.3.5 If collecting samples from a whole bird, collect tracheal/oropharyngeal and cloacal swabs before opening the carcass.

5.3.5.1 Do not pool tracheal/oropharyngeal swabs with cloacal swabs if PCR is requested.

5.3.5.2 Insert swab into the cloacal or oropharyngeal and/or tracheal area, and swab thoroughly. Be sure to swab the tracheal opening and draw the swab through

the choanal cleft on the upper palate when collecting swabs from the respiratory tract.

5.3.5.3 Place each swab in a 5.5 ml tube of BHI media or 3.5 ml tube of 10T antibiotic TBTB.

5.3.5.4 Vigorously swirl swabs in the media to expel contents from swab fibers.

5.3.5.5 Lift the swab out of the media; press the swab firmly against the side of the tube to remove any remaining liquid from the swab.

5.3.5.6 Discard the swab in disinfectant.

5.3.5.7 Thoroughly vortex swab samples and centrifuge for 20-30 min at approximately 1,500 x g (2,500 rpm in Beckman J-6 centrifuge with JS 4.2 rotor) at approximately 4°C.

5.3.5.8 If swabs were collected in a 3.5 ml 10T tube, aseptically remove the supernatant and place in a sterile 1-dram screw-cap vial. If swabs were collected in BHI tubes, 1.8 – 2.2 ml of swab supernatant should be transferred into a 1.3 ml 33T antibiotic blank as described in section 5.2.4.

5.3.5.9 Incubate swab suspension for approximately 1 hr. at room temperature before inoculating into eggs.

5.3.6 To collect tissues from whole birds, open the carcass as follows: Position the bird on its back. Using a pair of sterile scissors, cut through the skin at the distal end of the keel and reflect the skin forward as far as possible. Next, cut through the muscle at the distal end of the keel and extend the cut laterally (toward the neck) through the rib cage on each side to the base of the wing. Reflect the keel upward and forward as far as possible to expose the visceral organs.

5.3.7 Collect appropriate tissues for disease replication. For IAV and APMV lung and spleen are collected and pooled into a single sample. If neurologic signs are reported, brain should also be collected and processed separate from other tissues. Collect sufficient quantity of the appropriate tissue to make a 10-20%

suspension (0.3 to 0.7 g) and confirmation or repeat testing. Refer to Appendix 8.1 for other tissue systems and guidance on pooling.

5.3.8 Place the organ or representative tissue that is being retained for confirmation testing in a clean zip lock or whirl pack bag with identification of tissue, accession and sample number.

5.3.9 Homogenize tissue(s) in 10T antibiotic solution with a Tenbrock tissue homogenizer (or equivalent).

5.3.10 Transfer tissue homogenate back into the labeled tube(s), cap tightly, and place homogenizing equipment in disinfectant.

5.3.11 When finished, place the bird in a clean plastic bag and label (accession number and date received) for storage.

5.3.12 Cover and transfer contaminated homogenizing equipment to a covered pan containing disinfectant for autoclaving. Store homogenate and original tissue specimens at -60°C or colder.

5.3.13 Centrifuge tissue suspensions for 20-30 min at approximately 1,500 x g (2,500 rpm in Beckman J-6 centrifuge with JS 4.2 rotor) at approximately 4°C.

5.3.14 Working within a BSC, transfer tissue supernatant into a clean, empty 1-dram vial labelled with the accession and sample number. Incubate homogenized tissue supernatant approximately 1 hr at room temperature before inoculating into eggs.

5.4 Inoculation of Specific-Pathogen Free Embryonated Eggs by the Allantoic Route Method A Procedure

5.4.1 Candle 9-11 day-old specific-pathogen-free (SPF) embryonated chicken eggs for viability. Discard dead and inferior quality eggs. Inferior quality includes eggs with poorly centered air cells and weak or uneven chorioallantoic membranes (CAM). Eggs are arranged on an egg flat according to accession, sample number, route of inoculation, passage level, and standard DVL procedures. Four eggs are inoculated per specimen for first passage and reprocess (RP) specimens and 3 eggs per specimen for second (ar2) and third passage (ar3) specimens. Each flat is marked with disease, route, date inoculated, and refrigeration date. Each egg is identified with an adhesive label with the accession number, specimen number, disease, passage level/route, and date inoculated.

5.4.1.1 The broad end or air cell end of the egg is swabbed with disinfectant (50% iodine/ethanol solution recommended).

5.4.2 Using an electric drill, or equivalent, make a small hole in the eggshell along the center axis at the top of the egg being careful not to rupture the eggshell

membrane. Disinfect drill tip prior to drilling eggshell (50% iodine/ethanol solution recommended).

5.4.3 Drilled eggs, inoculums, Duco cement, sharps container, and syringes are placed within a BSC.

5.4.4 Eggs from first passage and RP specimens are inoculated with 0.3 (±0.01) ml of specimen-antibiotic mixture via the allantoic sac route.

5.4.4.1 Check the 33T and egg labels for accuracy. Using a syringe fitted with a 25-guage 5/8-inch (16-mm) needle, draw up >1.5 ml of specimen-antibiotic mixture. Position the needle vertically so all air bubbles flow up to the top of the syringe barrel. Insert needle into specimen tube and remove air bubbles from syringe barrel and inoculum by pushing plunger toward the needle being careful not to create an aerosol or viral droplets.

5.4.4.2 Insert the needle vertically through the hole the entire length of the needle and inject 0.3 ml (fig. 1). Avoid moving the syringe sideways once the needle is inserted to prevent tearing of the CAM, which could cause bleeding and death of the embryo.

5.4.5 Second and successive passage eggs (ar2 and ar3) are inoculated with 0.2 (±0.01) ml of specimen-antibiotic mixture prepared using the procedure described in section 5.4.4.1 and 5.4.4.2.

5.4.6 Seal the hole with Duco cement or quick drying glue. The tip of the glue container should not touch the eggshell.

5.4.7 If a droplet of inoculum drips onto the eggshell or bubbles out of the egg, disinfect droplet and bubbles with 70% ethanol or 50% iodine/ethanol solution before removing from hood or proceeding with egg inoculation.

5.4.8 Inoculated embryos are incubated in a humid incubator at 36-39°C for 5 days.

5.4.9 The remaining specimen-antibiotic mixture is frozen at -60°C or colder until needed for reisolation or RP or for 90 days as indicated in sections 5.6 and 5.7.

5.4.10 Eggs should be candled daily to determine embryo viability. All egg deaths are recorded in NVSL LIMS and specimen virus isolation worksheet.

5.4.11 AAF is harvested according to procedures outlined in NVSL-SOP-0022 (Candling and Harvesting Embryonated Eggs for Isolation and Identification of Avian Pathogens) and tested for evidence of virus (NVSL-SOP-0009, Hemagglutination and Hemagglutination-Inhibition Test for Influenza A Virus or NVSL-SOP-0010, Hemagglutination and Hemagglutination-Inhibition Tests for Avian Paramyxovirus (APMV) Identification) or by rRT-PCR (NVSL-SOP-0068, Real-time RT-PCR Detection of Influenza A Virus and Avian Paramyxovirus Type-1) or lateral flow

devices (LFD) NVSL-REF-0009, Avian Influenza Virus Type A Antigen Test Kit Flu Detect.

5.4.12 Specimens with three or more first day deaths are reprocessed according to the procedures outlined in section 5.7.

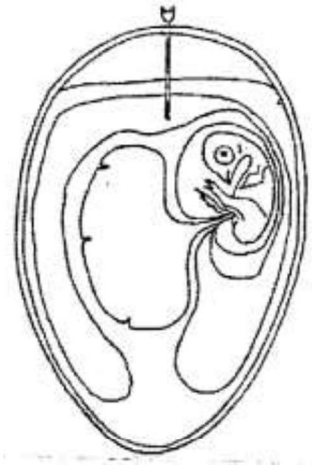

Figure 1. Method A - Allantoic route of embryo inoculation

Procedure should be conducted with a 25 gauge 5/8 inch needle.

5.5 Alternative Routes of Egg Inoculation

5.5.1 The method B route of allantoic sac inoculation has been shown to be more sensitive than method A with certain specimens and species. Method B has been shown to be a more sensitive method for the propagation of influenza viruses that are not well adapted to the chicken embryo.

5.5.1.1 Candle 9-11 day-old embryonated eggs and mark the position of the air cell.

5.5.1.2 Place eggs on an egg flat, air cell up, and disinfect the marked area. Drill a small hole through the eggshell just above the air cell being careful not to rupture the eggshell membrane.

5.5.1.3 Using a syringe fitted with a 25 gauge 5/8 inch needle, inoculate 0.3 (±0.01) ml of inoculum per egg by inserting the needle, to its full length, vertically through the hole or at a slight angle away from the center of the egg and inject the specimen (fig. 2).

5.5.1.4 Seal hole and incubate eggs in a humid incubator at 36-39°C for 5 days.

5.5.1.5 Eggs should be candled daily to determine embryo viability. All egg deaths are recorded in NVSL LIMS and specimen virus isolation worksheet.

5.5.2 AAF is harvested according to procedures outlined in SOP-AV-0022 and tested for evidence of virus by the HA test, rRT-PCR, or LFD.

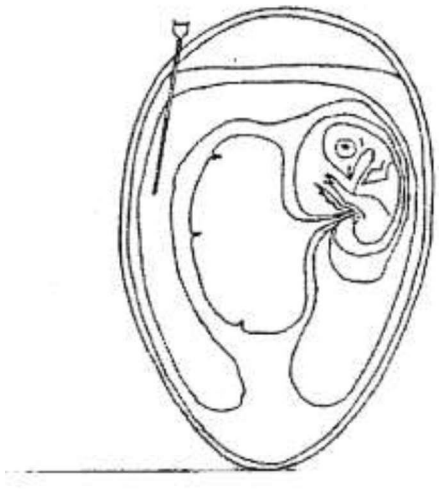

Figure 2. Method B - Allantoic route of embryo inoculation

Procedure should be conducted with a 25 gauge 5/8 inch needle.

5.5.3 The dropped chorioallantoic membrane (CAM) and yolk sac (YS) routes of inoculation are not recommended as the primary procedure for the isolation of IAV or APMV-1, but can be used as an alternative route when isolation of other viruses are requested (example: infectious laryngnotracheitis virus). For additional information on the dropped CAM and YS routes of inoculation refer to NVSL-REF-0008 the American Association of Avian Pathologists manual for the Isolation, Identification, and Characterization of Avian Diseases.

5.6 Processing Specimens for Repassage or Allantoic Route 2 (ar2) Passage

Specimens with one or more nonspecific dead embryos on days 2-5 post inoculation or harvested from specimens testing positive by rRT-PCR for IAV or APMV-1 are processed for a second blind passage. Nonspecific deaths are defined as those embryos from which harvested amniotic allantoic fluid (AAF) is negative for hemagglutinating virus and negative or inconclusive for bacterial contamination by the blood agar test (see **NVSL-SOP-0009** or **NVSL-SOP-0010**). AAF from dead embryos testing negative or inconclusive by HA, rRT-PCR, or LFD and blood agar (BA) test are routinely processed for a second passage.

5.6.1 Following the completion of all dead and live HA testing (or alternative rRT-PCR or IAV LFD detection methods) for a single specimen, identify specimens with nonspecific deaths between 2-5 days or specimens inoculated with rRT-PCR positive swab material, and schedule for repassage. Specimen should be

scheduled for an ar2 passage in the NVSL LIMS system. Associated worksheets and labels are printed for identification of tubes and record of testing.

5.6.2 Locate the AAF harvested from any first passage egg and thaw. AAF harvested from specimens with dead embryos on more than one day should be pooled together to create a single second passage specimen.

5.6.3 Label a 1.8 ml 10T TBTB antibiotic blank for each specimen with specimen accession and sample number.

5.6.4 Vortex thawed AAF and centrifuge at approximately 1,500 x g (2,500 rpm in Beckman J6 centrifuge) for approximately 10 min at approximately 4°C.

5.6.5 Prepare a 1:10 dilution (final dilution of AAF from all dead embryos) of the AAF by transferring 0.2 ml of clarified AAF into the 1.8 ml TBTB blank (ex. If there are pools from 2 different days, transfer 0.1 ml from each pool for a total of 0.2 ml.).

5.6.6 Vortex the 1:10 TBTB/specimen suspension and incubate at room temperature for approximately 30 min to 1 hr.

5.6.7 Following incubation disinfect eggshell and drill a small hole through the eggshell just above the air cell as described in sections 5.4.2.

5.6.8 Inoculate with 0.2 ml of specimen-antibiotic mixture using the same procedure described in section 5.4.4.

5.6.9 Seal with Duco cement or equivalent and incubate at in humid incubator at 36-39 °C for 5 days. Eggs should be candled daily to determine embryo viability. All egg deaths and repassage records are recorded in NVSL LIMS and specimen virus isolation worksheet.

5.6.10 Harvest AAF from all eggs that die between days 2-5 according to NVSL-SOP-0022 and tested for evidence of virus by the HA test, rRT-PCR, or LFD.

5.7 Processing Specimens for Reprocess or Repeat (RP) Passage

All 24 hr or first day deaths are contributed to injury, contamination or nonspecific factors unless the specimens were collected from clinically ill birds with clinical signs indicative of HPAI or vNDV. If HPAI, human-risk IAV, or vNDV is suspected and specimens have multiple first day deaths, the AAF from the embryos dead within 24 hours should be tested by the HA, rRT-PCR, or LFD test for detection of a hemagglutinating virus. Specimens with 3 or more embryos dead within 24 hours should be processed for a RP passage according to the procedure outlined below. In addition, specimens with either 3 or more eggs testing positive for bacteria by the BA test or have a combination of 3 or more eggs either dead within 24 hours and/or positive by the BA test should be treated with additional antibiotics and processed for a repeat passage as outlined below.

5.7.1 The 33T containing the specimen-antibiotic mixture should be retrieved from the freezer (section 5.4.9) and thawed.

5.7.2 Specimen should be scheduled for a RP passage in the NVSL LIMS system. Associated worksheets and labels are printed for identification of tubes and record of testing.

5.7.3 A 2.0 ml 10T antibiotic blank should be thawed for each specimen requiring a RP passage. Tube labels with accession and sample number should be attached to the 2.0 ml 10T antibiotic blank and a 17 x 120 mm (15 ml conical) centrifuge tubes.

5.7.4 In a BSC, transfer the entire volume remaining in the 33T specimen-antibiotic mixture into the 17 x 120 mm centrifuge tube and centrifuge for approximately 30 min at approximately 1,500 x g (2,500 rpm in Beckman J6 centrifuge) at approximately 4°C.

5.7.5 Following clarification, transfer specimen supernatant (being careful not to transfer the pellet) into the 2.0 ml 10T antibiotic blank and incubate at room temperature for approximately 1 hr.

5.7.6 Inoculate 0.3 ml of RP specimen into each egg using the method A route of allantoic sac route (AR) inoculation as outlined in sections 5.4.1-5.4.4.

5.7.7 Incubate in a humid environment for 5 days at 36-39°C.

5.7.8 Eggs should be candled daily to determine embryo viability. All egg deaths are recorded in NVSL LIMS and specimen virus isolation worksheet.

5.7.9 AAF is harvested according to procedures outlined in NVSL-SOP-0022 and tested for evidence of virus by the HA test, rRT-PCR, or LFD.

5.8 Confirmation of Virus Isolation Results

Reisolation (confirmation of virus isolation from the original specimen) and reinoculation **(**confirmation of virus isolation from the original 33T specimen-antibiotic mixture) must be conducted for any import/export specimen or submission from which isolation will deny importation. In addition, reisolation and/or reinoculation can be scheduled at the discretion of the technical and/or managerial staff to confirm the isolation of a virus or to rule-out laboratory contamination.

5.8.1 Reisolation

Specimen processing and egg inoculation is conducted as outlined in sections 5.2, 5.3, and 5.4 from the original specimen tube or tissue. TBTB is

added to the original tube and remaining specimen as outlined in section 5.1.4 to bring the volume to approximately 1.8 ml. The specimen is vortexed well to rinse all remaining specimen from the side of the tube and centrifuged as described in section 5.2.2.

5.8.2 Reinoculation

Retrieve the original 33T specimen-antibiotic mixture from the -70°C freezer, thaw, and reinoculate into embryos as described in section 5.4. In the event there is insufficient 33T specimen for the inoculation of 4 embryos with 0.3 ml of 33T specimen it is preferential to inoculate less than 4 embryos than to change the concentration of integrity of the 33T specimen

6 Associated NVSL Quality Documents/References

- **NVSL-REF-0008**, A Laboratory Manual for the Isolation, Identification, and Characterization of Avian Pathogens.
- **NVSL-REF-0945**, Biosafety in Microbiological and Biomedical Laboratories (BMBL)
- **NVSL-REF-0958**, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019
- **NVSL-MAN-0018**, Diagnostic Virology Laboratory Biosafety Security and Incident Response Plan
- **NVSL-REF-0009**, Avian Influenza Virus Type A Antigen Test Kit Flu Detect
- **NVSL-SOP-0009**, Hemagglutination and Hemagglutination-Inhibition Test for Influenza A Virus
- **NVSL-SOP-0010**, Hemagglutination and Hemagglutination-Inhibition Tests for Avian Paramyxovirus (APMV) Identification
- **NVSL-SOP-0013**, Preparation of Antibiotics to Treat Inoculums for Virus Isolation in Embryonated Eggs
- **NVSL-SOP-0022**, Candling and Harvesting Embryonated Eggs for Isolation and Identification of Avian Pathogens
- **NVSL-SOP-0068**, Real-time RT-PCR Detection of Influenza A Virus and Avian Paramyxovirus Type-1
- **NVSL-WI-0023**, Avian Sample Collection for Influenza A and Newcastle Disease
- **NVSL-WI-1207**, Biological Risk Assessments for Diagnostic Virology

7 Revision History

- **NVSL-SOP-0018.05** supersedes SOP-AV-0018.04 due to a new numbering system. Referenced document numbers were updated.
- **SOP-AV-0018.04** March 2021, updated references removed WI-SA-0005, WI-SA-0006, WI-SA-0010, and replaced with WI-SA-0014 and updated formatting.
- **SOP-AV-0018.03** November 2016 added option to use 8 or 12-day-old when necessary to Section 4.2. Changed “embryonating” to “embryonated” throughout

the document except in the title for SOP-AV-0013 since the title has not been updated. Changed AIV to IAV throughout document. Added wording for performing a second passage on AAF from samples testing positive by rRT-PCR. Added rRT-PCR and LFD as alternative test methods to HA/HI. Removed duplicate Associated NVSL Quality Documents/Reference from page 1. Added REF-AV-0008 American Association of Avian Pathologists Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens to Section 6. Added WI-AV-0020, REF-AV-0009, REF-AV-0010, and SOP-AV-0068 references to Section 6. Removed information from Section 1 that is available in WI-AV-0020. Added note for working with select agents to Section 3.

8 Appendices

8.1 Pooling of Tissues for Processing

Tissues may be pooled according to following organ systems.

Respiratory
Lung
Trachea
Air sacs

Cardiovascular
Heart

Digestive
Liver
Pancreas
Small Intestine (duodenum, jejunum, ileum)
Cecum (at the junction of ileum and colon)
Proventriculus
Ventriculus (Gizzard)
Large intestine

Lymphoreticular system
Spleen
Bursa of Fabricius

Urinary
Kidney

Reproductive
Oviduct
Ovaries

Nervous
Brain

- Heart can be pooled with spleen and Bursa of Fabricius
- Respiratory and cardiovascular organs can be pooled
- Spleen and lung can be pooled
- Liver and kidney can be pooled
- Pool organ systems separately with the exception of the pools listed above
- Don’t pool tissues from more than one bird
- Don’t pool the brain with any other tissue
- Don’t pool digestive organ tissues with any other organ system

Signature Manifest

Document Number: NVSL-SOP-0018 **Revision:** 05
Title: Isolation of Avian Influenza and Avian Paramyxoviruses Viruses in Chicken Embryos from Avian Species
Effective Date: 26 May 2022

All dates and times are in Central Standard Time.

NVSL Set #1 Renumbering Project (SOPs)

Step 1 Collaboration

Name/Signature	Title	Date	Meaning/Reason
LINDA HOFER (LHOFER)	Quality Mgmt Assistant, DO QA	29 Oct 2021, 04:17:00 PM	Complete & Quit
NICHOLE HINES BERGESON (NHINES)	Supvy Micro, MOLECULAR	22 Nov 2021, 01:35:21 PM	Complete & Quit
COLE WILSON (COLE.WILSON)	DMACC, Intern	14 Dec 2021, 01:58:17 PM	Complete

Step 2 Approval

Name/Signature	Title	Date	Meaning/Reason
SHAWNA MIDDLETON (SLMIDDLETON)	Quality Coordinator, NVSL	15 Dec 2021, 02:17:44 PM	Approved

Quick Approval

Approve Now

Name/Signature	Title	Date	Meaning/Reason
JAMIE GRIMES (APJAMIERGRIMES)	QA Specialist, DO	26 May 2022, 09:41:58 AM	Approved

Review: NVSL-SOP-0018 05 Isolation of Avian Influenza and Avian Paramyxoviruses Viruses in Chicken Embryos from Avian Species

Review

Name/Signature	Title	Date	Meaning/Reason
NICHOLE HINES BERGESON (NHINES)	Micro (SUPV), DVL Molecular	20 May 2024, 11:19:33 AM	Reviewed
KYM HEFLER (KYM.HEFLER)	Program Assistant, DO QA	29 May 2024, 10:55:43 AM	Reviewed

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Intravenous Pathogenicity Assessments for Avian Influenza Virus in Chickens

Document Number: **NVSL-SOP-0014** **Revision:** **07**

Previous Number: SOP-AV-0014.06

Author: NVSL-Supervisor-DVL-MOLECULAR Section Head

Section/Area: Laboratory Activities

Release Date: 29 Oct 2021

Notes:

Intravenous Pathogenicity Assessments for Avian Influenza Virus in Chickens

Table of Contents

1 Purpose/Scope

Avian influenza (AI) is caused by influenza A viruses (IAV) of the family *Orthomyxoviridae* and is distributed worldwide. Avian influenza viruses have been isolated from a number of domestic and wild avian species. Infections in domestic poultry with AI virus have been associated with a variety of diseases, ranging from subclinical, to an acute, generalized fatal disease. All newly isolated avian influenza viruses, or representative isolates of a specific subtype from each submission must be inoculated into specific pathogen-free (SPF) chickens according to one of the procedures described in this document to assess the pathogenicity of the virus in chickens. There are two procedures for determining *in vivo* pathogenicity [highly pathogenic AI (HPAI) or low pathogenic AI] (**Table 1**) and both are described by the World Organisation for Animal Health (OIE) in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals:

A. **Chicken lethality pathogenicity** assessment**,** and the
B. **Intravenous pathogenicity index (IVPI)** assessment

Both the chicken lethality assessment and the IVPI are complete at the end of 10 days or once all birds have died.

Table 1. Summary criteria for Chicken Lethality vs IVPI procedures:

	Lethality	**IVPI**
Hemagglutinating virus when tested by HA assay	Yes	Yes
Minimum HA titer required	No	>16 HAU
Number of birds required	8 birds	10 birds
Age of bird required	4-6 weeks	6 weeks
Dilution of inoculum	10T blanks	Sterile PBS
Amount of inoculum per bird	0.2 ml	0.1 ml
Record of daily observation and scoring required	No	Yes
Duration of test	10 days	10 days
Interpretation of Results	% survival	Index Value
Criteria for HPAI	≥75% mortality = HPAI	IVPI index > 1.2 = HPAI

* Refer to page 825-826 of https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.04_AI.pdf

2 Definitions

- ABSL Agricultural biosafety level
- BSL Biosafety level
- BSC Biological safety cabinet
- HPAI Highly pathogenic avian influenza is defined by the OIE as an influenza A virus of avian origin that causes mortality in 75% or more of inoculated chickens within 10 days, has an intravenous pathogenicity index (IVPI) of 1.2 or higher, or has an amino acid sequence at the hemagglutinin cleavage site that is compatible with other HPAI viruses
- IAV Influenza A virus
- LPAI Low pathogenic avian influenza refers specifically to infection with H5/H7 subtypes of influenza A virus found to be of low virulence in poultry; the virulence of the virus is assessed in domestic poultry and has been defined as a type A influenza virus of avian origin that causes mortality in less than 75% of inoculated chickens within 10 days, has an IVPI of less than 1.2, and does not have an amino acid sequence at the hemagglutinin cleavage site that is compatible with other highly pathogenic influenza viruses

3 Special Precautions

- **Personnel Requirements**
  - Personnel will have read, understood, and agreed to abide by the contents of this protocol and its associated documents (including equipment).
  - Personnel performing the protocol will be aware of the potential hazards associated with the procedures performed. Specific hazards include biological hazard, chemical hazards, and the ergonomic hazard associated with repetitive motions.
  - Personnel will have completed safety training on biological safety cabinet (BSC) operation, autoclave safety, and must be familiar with any Material Safety Data Sheets (MSDS) associated with chemicals required to perform this SOP.
  - Personnel will observe standard laboratory safety procedures, including using appropriate personal protective equipment (PPE): disposable lab coat, safety glasses, and disposable gloves.

- **Disease agent-specific sample handling**
  - Influenza A virus (IAV) refer to **NVSL-WI-1207**, Biological Risk Assessments for Diagnostic Virology
  - Human-risk IAV[1]: Biosafety level 3 enhanced (BSL-3E) and Agricultural Biosafety level 3 enhanced (ABSL-3E) procedures outlined in **NVSL-WI-1207.**

[1] Emergent or re-emergent IAVs that are isolated from human clinical cases and have caused human morbidity or mortality and are considered a proximate threat to laboratory worker health or well-being,

- Non-human-risk IAV[2]: Biosafety level 3 (BSL-3) or biosafety level 3-Ag (BSL-3AG) procedures will be followed per **NVSL-WI-1207**.

- **Decontamination, Disinfection, and Waste Disposal**
  - Decontaminate surfaces and equipment and dispose of liquid and solid waste per **NVSL-MAN-0018**, Diagnostic Virology Laboratory Biosafety Security and Incident Response Plan

4 Equipment and Materials Required

- Approved Animal Care and Use Committee (ACUC) application
- Number of SPF chickens (**Table 1**):
- **Lethality assessment:** 8 chickens at 4-6 weeks of age
- **IVPI:** 10 chickens at 6-weeks of age
- Adequate cage space in BSL-3E [e.g. Building 20 vivarium with BSL-3E or ABSL-3 animal facility (e.g. Building 9, rooms 1195 and 1125)
- Syringe (3 ml) with needle (25 gauge 5/8-inch)
- Disposable latex/nitrile gloves
- 3x5" index cards (one to serve as a cage card and one for recording bird band and cage numbers)
- Small and medium plastic bags
- Sterile 12x75 mm snap-cap tubes containing 1.8 ml sterile tris-buffered tryptose broth (TBTB) **10T** media with antibiotics (NVSL Media # 10411 or see **NVSL-SOP-0013**, Preparation of Antibiotics to Treat Inoculums for Virus Isolation in Embryonated Eggs)
- IVPI worksheet if conducting **IVPI assessment** (**NVSL-FM-0067,** Intravenous Pathogenicity Index (IVPI) Worksheet)
- Sterile 0.01 M phosphate buffered saline (PBS) pH 7.2 (Media Preparation 30054)
- Beckman J-HC centrifuge with JS 4.2 rotor

5 Procedure

5.1 Ordering Birds

5.1.1. Order the birds by emailing the "ARS-MWA-NCAH-ARU-Chickens" email group as described in **NVSL-WI-0007,** Ordering 4-6 Week Old Chickens. Specify the age of birds in the email. For IVPI assessment remind animal resource personnel in the email that the birds need to be

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regardless of the pathogenicity in animal species (low pathogenicity non-H5/H7, LPAI H5/H7, HPAI, mouse, swine, or ferret models), are catergorized as "human-risk IAV," and include but are not limited to Asian H5N1, reconstructed 1918 influenza, and the 2013 Chinese lineage of H7N9; refer to NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES, Appendix G-II-C-5-a-(4): (accessed March 2014 http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines).

[2] All IAV that do not meet the definition of human-risk IAV.

observed daily (± 2.0 hrs of inoculation) and observations recorded on **FM-AV-0066**. A classified ad will need to be placed.

5.1.2. **6 week old birds** for the **IVPI assessment**

5.1.3. **4-8 week old birds** for the **Lethality assessment**

5.1.4. After receiving the e-mail that the classified ad is available, complete the ordering process in RAMS as described in **WI-AV-0004**.

5.2 Sample Preparation

5.2.1 Approximately 2.0 ml of inoculum is needed for either the Lethality or IVPI assessments

5.2.2 Pathotyping should be conducted on a single isolate; isolate must be free of bacterial contamination

5.2.3 Thaw the suspension containing the virus isolate(s) and centrifuge to clarify at 1,500 x g (2,500 rpm in a Beckman J-HC centrifuge with JS 4.2 rotor) for 10 min

5.2.4 **For the Lethality Assessment only:**

Dilute isolate(s) 1:10 in 10T media. Note: Manipulations of the live isolate(s) shall be performed in a class II biosafety cabinet.

5.2.5 **For the IVPI Assay only:**

5.2.5.1 Determine the hemagglutination titer (HAU) of the virus isolate by the HA assay, refer to NVSL-SOP-0009, Hemagglutination and Hemagglutination-Inhibition Test for Influenza A Viru. Note: Manipulations of live isolate(s) shall be performed in a class II biosafety cabinet.

5.2.5.2 The minimum HAU titer that is acceptable for the IVPI assessment is 32 HAU or >16 HAU (OIE chapter 3.3.4). Isolates with HAU titers of $\leq$16 must be passed through embryos to increase the titer to $\geq$ 32.

5.2.5.3 Dilute the $\geq$ 32 HAU material by 1:10 in sterile PBS**.**

5.2.6 **For both the Lethality assessment and IVPI:**

5.2.6.1 Create a cage card using an index card and complete the information below for each isolator unit; number the cage card(s) sequentially in the order of inoculation

- ✓ Accession number
- ✓ RAMS requisition number
- ✓ Animal Care and Use Protocol (ACUP) number)

- ✓ Owners name
- ✓ Date of inoculation
- ✓ Initials of person performing the inoculation
- ✓ Type of assessment (lethality vs. IVPI)
- ✓ Number of days to hold birds (e.g. 10)
- ✓ Subtype of virus being inoculated

5.2.6.2 Create a second index card(s) with the name of the person performing the inoculation, type of assessment (lethality vs. IVPI), date of inoculation, and accession number of each isolate, leaving adequate space to record bird and cage numbers to return to the laboratory

5.2.6.3 Place the completed index card(s) and the prepared inoculum in a small plastic bag and secure with knot or twist tie. Alternatively, the inoculum may be loaded into syringe(s) in the laboratory and placed with the index card(s) into a plastic bag secured with knot or twist tie

5.2.6.4 For each isolate, place the bag containing the inoculum into a second medium size bag along with other needed materials (syringe and needle, index card and gloves (optional) for transportation to the animal facility

- Transfer of select agents from the laboratory to the appropriate animal facility is described in **NVSL-WI-0015,** Transport of Select Agents between the Diagnostic Virology Laboratory and animal/laboratory rooms in the vivarium in Building 20, or **NVSL-WI-0016,** Transport of Select Agents between the Diagnostic Virology Laboratory in Building 20 and animal or laboratory rooms in Building 9.

5.2.6.5 Enter the following information into the chicken inoculation records book:

- ✓ Inoculation date
- ✓ Owners name
- ✓ Accession number
- ✓ Subtype of avian influenza virus
- ✓ Sample number and harvest date
- ✓ Species from which virus was isolated
- ✓ RAMS requisition number
- ✓ Allow space to record chicken numbers and cage numbers between entries

5.3 Procedure for Entering Bld. 20 vivarium or Bld. 9 Animal Facilities

5.3.1 Enter and exit building 9 according to the Agricultural Research Service (ARS) guidelines for building 9 (NCAH Building 9 Standard Operating Policies and Procedures).

5.3.2 Enter and exit the vivarium according to SOP-ARU-0600, Vivarium Operations and NCAH Building 9 Standard Operating Policies and Procedures.

5.4 Bird Inoculation Procedure

5.4.1 Organize inoculation materials on a work area. Arrange isolates in the order of inoculation. Only 1 isolate bag should be opened at any time.

5.4.2 Record bird numbers on individual cage cards and the 3” x 5” index card(s) during the inoculation process and affix the cage card to the front of the appropriate cage door.

5.4.3 When filling syringe with inoculum take care not to create an aerosol.

5.4.4 Open cage door and remove all the birds, transferring them to a holding cage.

5.4.5 Remove the birds one at a time from the holding cage/dog carrier for inoculation.

5.4.6 Inoculate birds intravenously via the wing vein as follows:

- **Lethality assessment:** 0.2 ml of inoculum/bird for 8 birds
- IVPI: 0.1ml of inoculum/bird for 10 birds Place the inoculated chicken back into isolator cage and close door.

5.4.7 Remove contaminated gloves. Lock all closures tightly to seal cage door.

5.4.8 Discard materials as outlined in Vivarium (SOP-ARU-0600) and Building 9 specific Standard Operating Procedures

5.4.9 Additionally for the IVPI, attach the IVPI Worksheet (NVSL-FM-0067) to the isolator cage

5.5 Exit Procedures for Animal Room

5.5.1 Place the index card(s) on which bird numbers were recorded in a plastic bag and tie the bag shut. Surface disinfect the bag for removal from the room

5.5.2 . Disinfect and rinse all potentially contaminated surfaces and boots in the animal room and preparatory area.

5.5.3 Exit the room and building according to building specific SOPs.

5.6 Procedure for Daily Observation and Recording

5.6.1 Lethality and IVPI Assessments: Observe birds every 24 hr at approximately same hour the inoculation was conducted for ten days. Observation must be made ± 2.0 hrs of inoculation

5.6.2 IVPI Assessment (refer to NVSL-FM-0067): Record the number of birds in each category (normal, sick, severely sick, or dead) according to the descriptions below. Daily observations must be recorded for all 10 birds.

5.6.3 Observe birds for clinical signs including respiratory involvement, depression, diarrhea, cyanosis of exposed skin or wattles, edema of the face and/or head, nervous signs.

- **Normal** birds are alert and moving without incoordination
- **Sick** birds show one of the above clinical signs
- **Severely sick** birds show more than one of the above clinical signs

When birds are too sick to eat or drink, they should be humanely killed and scored as **dead** at the next observation time.

5.7 Interpretation of IVPI Values

The IVPI is the mean score per bird per observation over the ten-day period (refer to examples on NVSL-FM-0067). An index of 3.00 means that all birds died within 24 hours, and an index of 0.00 means that no bird showed any clinical signs during the ten-day observation period. Any isolate with an IVPI >1.2 is considered to be highly pathogenic avian influenza. Note: At a minimum, notification of select agent identification must be made to the Animal Resources Unit (ARU) Vivarium Veterinarian and appropriate Vivarium staff members and recorded on NVSL-FM-0073, Select Agent Identification Workflow Check-List.

5.8 Record Keeping

5.8.1 For the IVPI, record IVPI score on the front of the case worksheet as well as the chicken inoculation book and attach the IVPI worksheet to the

case submission form. Submit completed case to the Avian Viruses Section Head, veterinary medical officer, or microbiologists for reporting

5.8.2 **For the** Lethality assessment**, record in the chicken inoculation book the bird number and date for all dead birds and the number of healthy birds at the end of the 10 day observation period.**

5.9 Recording Bird and Cage Numbers

Record bird and cage numbers in the chicken inoculation records book under the appropriate accession number and in the room specific vivarium AV tracking excel sheet when applicable.

6 Associated NVSL Quality Documents/References

- **NVSL-FM-0067,** Intravenous Pathogenicity Index (IVPI) Worksheet
- **NVSL-FM-0073**, Select Agent Identification Workflow Check-List
- **NVSL-MAN-0018**, Diagnostic Virology Laboratory Biosafety Security and Incident Response Plan
- OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018; (accessed February 2021 https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.04_AI.pdf)
- **NVSL-REF-0945**, Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition (https://www.cdc.gov/labs/BMBL.html)
- **SOP-ARU-0600,** Vivarium Operations and NCAH Building 9 Standard Operating Policies and Procedures (2015 version available on Sharepoint)
- **NVSL-SOP-0009,** Hemagglutination and Hemagglutination-Inhibition Test for Influenza A Virus
- **NVSL-SOP-0013,** Preparation of Antibiotics to Treat Inoculums for Virus Isolation in Embryonating Eggs
- **NVSL-WI-0007,** Ordering 4-6 Week Old Chickens
- **NVSL-WI-0015,** Transport of Select Agents between the Diagnostic Virology Laboratory and animal/laboratory rooms in the vivarium in Building 20.
- **NVSL-WI-0016,** Transport of Select Agents between the Diagnostic Virology Laboratory in Building 20 and animal or laboratory rooms in Building 9
- **NVSL-WI-1207**, Biological Risk Assessments for Diagnostic Virology

7 Revision History

- NVSL-SOP-0014.07 supersedes SOP-AV-0014.06; NVSL documents have undergone renumbering from section specific numbering to generic numbering to accommodate any future NVSL reorganizations. No technical changes were made to this document.

- SOP-AV-0014.06 February 2021: updated BMBL, OIE, and Biological Risk Assessment references; and updated formatting.

- SOP-AV-0014.05 January 2017: Addition of WI-SA-0005 and WI-SA-0010 to Section 3 for PPE requirements. Updated document numbers for WI-DVL-0024 to WI-SA-0005 and WI-DVL-0026 to WI-SA-0010. SOP-AV-0023 “Policies and

Procedures for Rooms 1125 and 1195 in Building 9" was obsoleted, removed reference from document. Addition of vivarium AV tracking excel sheet to Section 5.9.

8 Appendix

N/A

Signature Manifest

Document Number: NVSL-SOP-0014 **Revision:** 07

Title: Intravenous Pathogenicity Assessments for Avian Influenza Virus in Chickens

Effective Date: 29 Oct 2021

All dates and times are in Central Standard Time.

APHIS Project - Set #1

Step 1 Collaboration

Name/Signature	Title	Date	Meaning/Reason
LINDA HOFER (LHOFER)	Quality Mgmt Assistant, DO QA	25 Oct 2021, 10:12:57 AM	Complete & Quit
SHAWNA MIDDLETON (SLMIDDLETON)	Quality Coordinator, NVSL	29 Oct 2021, 12:13:12 PM	Complete

Step 2 Approval

Name/Signature	Title	Date	Meaning/Reason
SHAWNA MIDDLETON (SLMIDDLETON)	Quality Coordinator, NVSL	29 Oct 2021, 12:16:26 PM	Approved

Gmail **Christine, an unincorporated woman <cmssyc@gmail.com>**

USDA APHIS Freedom Of Information Act Request #2024-APHIS-06103-F

Christine, an unincorporated woman <cmssyc@gmail.com> Sun, May 25, 2025 at 9:05 PM
To: "Kuralt, Wallace - MRP-APHIS" <wallace.h.kuralt@usda.gov>, FOIA.Officer@usda.gov, "Ajua, David - MRP-APHIS" <David.Ajua@usda.gov>, FOIA.MRP.Appeals@usda.gov, tonya.g.woods@aphis.usda.gov

Greetings Wallace, David and Tonya,

Thank you for Tonya's letter dated March 17, 2025.

Contrary to what is indicated in Tonya's letter, I did provide a date range along with my order. Also, no grounds were provided for her insinuation that I ought to have provided "*specificity as to the program personnel required for this search"* or ought to have cited *"specific program areas to search."* What are the grounds?

Also, the 2 documents provided are a *protocol* and a *procedure* document, not *studies.* They only provide evidence of pseudoscientific methodologies, not of a virus, contagion or even a "genome". They are not responsive to the order.

The evidence sought clearly does not exist, based on the literature and the FOI responses below. Like all other virus/contagion narratives, avian influenza is a deadly, horrific hoax.

Life in prison.

My notarized affidavit is attached.

May 10, 2023:
The “experts” at Canadian Food Inspection Agency confessed to having zero scientific proof of the existence of any alleged “virus” that they claim has ever affected livestock in Canada.
Response, pgs 15/16:
“We have been assured by responsible officials of the Agency that no documents exist concerning the requested information.”
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/06/Canadian-Food-Inspection-Agency-PACKAGE-redacted.pdf
Excel file of 655 unscientific “virus” studies:
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/06/2023-05-11-excel-Papers-NCFAD_and_ADRI-Lehtbridge-ATIP_request.xlsx

June 10, 2024:
"H5N1": Canadian Food Inspection Agency still can't find valid evidence that it exists:
https://christinemasseyfois.substack.com/p/h5n1-canadian-food-inspection-agency

July 11, 2024:
'bird flu' hoax update: 🍁 FOI response from Canadian Food Inspection Agency shows that they have no scientific evidence of 'H5N1'.... not even a "genome"... and no scientific evidence of contagion:
https://christinemasseyfois.substack.com/p/bird-flu-hoax-update-lead-agency

April 9, 2025:
Agriculture and Agri-Food Canada confesses re bird flu hoax: we have zero evidence of a virus or contagion… not even a "genome" to be found:
https://christinemasseyfois.substack.com/p/agriculture-and-agri-food-canada

May 20, 2022:
Public Health Agency of Canada confirmed that they have no record of any alleged “avian influenza virus” having been found in and purified from the bodily fluid (which includes milk) or tissue or excrement of any diseased “host” on the planet (in order for “it” to be sequenced, characterized and studied with valid controlled experiments) by anyone, anywhere, ever.

Insanely, they insisted that:
1) “viruses” are in so-called “hosts” despite their utter inability to find them there,
2) it’s necessary to “grow viruses” in non-host cells (as if “they” would “grow” better there than they allegedly “grew” in the dis-eased host LOL), and
3) they pretended that mixing complex substances together = purification.

https://www.fluoridefreepeel.ca/wp-content/uploads/2022/05/PHAC-avian-influenza-PACKAGE-redacted.pdf

December 20, 2021:
Public Health Agency of Canada (PHAC) confirmed that they have no record of any alleged “virus” having been purified from a sample taken from any diseased human on Earth, by anyone, ever, period.

PHAC then gave a red herring excuse, implying that my request had ruled out studies wherein any other medium was used to achieve purification, when it had only ruled out addition of genetic material.

PHAC then claimed that “isolation in cell culture” (an oxymoron) is the gold standard for determining the presence of “intact virus”, and applied circular reasoning by asserting that stressed cells breaking down are evidence of a “virus” since a “virus” would cause cells to break down – which is on par with asserting that finding presents under a Christmas tree is the gold standard evidence for the presence of Santa Claus.
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/01/PHAC-ANY-virus-PACKAGE-redacted.pdf

November 1, 2021:
U.S. Centers for Disease Control and Prevention confirmed that they have no record describing purification of any “influenza virus” from a patient sample by any method, by anyone, anywhere on the planet:
https://www.fluoridefreepeel.ca/wp-content/uploads/2021/11/CDC-Nov-1-2021-influenza-PACKAGE-redacted.pdf

May 5, 2022:
The geniuses at U.S. CDC and Agency for Toxic Substances and Disease Registry confirmed that a search of their records failed to find any that describe anyone on Earth finding any alleged “avian influenza virus” in the bodily fluids (which includes milk) or tissue of any diseased “host” (animal or human) and purifying “it”

… which would be necessary for valid sequencing, characterization and for scientifically studying the purported particle with valid controlled experiments in order to see if it fits the definition of a "virus":
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/05/CDC-avian-influenza-PACKAGE-redacted.pdf

Japan’s National Institute of Infectious Diseases has no record of any “influenza virus” being found in and purified from anyone, ever:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/04/Japan-Natl-Inst-of-Infec-Dis.pdf

The “experts” at Japan’s National Institute of Infectious Diseases (NIID) came up empty and admitted that they have no records when asked for confirmation of pathogenicity using purified “influenza virus” or “avian influenza virus” particles:
https://www.fluoridefreepeel.ca/japan-natl-inst-of-infectious-diseases-has-no-scientific-evidence-of-viruses/

https://www.fluoridefreepeel.ca/wp-content/uploads/2024/04/Japan-National-Institute-of-Infectious-Diseases-avian-influenza-virus-PACKAGE.pdf

April 21, 2023:
UK Animal and Plant Health Agency (APHA) cannot show the existence of any "H5N1 virus" in birds
An anonymous man/woman with the Animal and Plant Health Agency (APHA), UK confessed to James Henderson that no one there has any record of anyone on Earth finding and purifying the alleged "H5N1 highly pathogenic avian influenza (HPAI) virus" from any dis-eased bird, ever, and thus they confessed to being unable to scientifically evidence "its" alleged existence since they know of no one obtaining a valid independent variable to even study.

According to the confession letter:

"APHA is an Executive Agency of the Department for Environment, Food and Rural Affairs and also works on behalf of the Scottish Government, Welsh Government and Food Standards Agency to safeguard animal and plant health for the benefit of people, the environment and the economy."
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/06/UK-Animal-and-Plant-Health-Agency-H5N1-PACKAGE.pdf

April 4, 2023:
European Centre for Disease Prevention and Control can’t prove the existence of “H5N1”
Vicky Lefevre, Head of Unit, Public Health Functions, ECDC, responding to my colleague, failed to provide or cite even 1 record of any alleged “H5N1 avian influenza virus” being found in and separated from the bodily fluid/tissue of any bird that supposedly died from “the virus” … in order for “it” to be sequenced, characterized and studied with valid controlled experiments, aka “science”:
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/04/ECDC-H5N1-avain-influenza-PACKAGE-redacted.pdf

July 29, 2024:
FDA confesses: zero scientific evidence of "avian influenza virus" or contagion... not even a "genome" found by anyone... anywhere

August 22, 2024, updated January 30, 2025:
US Department of Agriculture failed my official legal challenge to provide or cite scientific evidence of "avian influenza virus" existence and/or illness contagion, or even a “genome”… because it doesn't exist:
https://christinemasseyfois.substack.com/p/us-dept-of-agriculture-were-still

September 11, 2024, updated January 18, 2025:
Nathaniel Erskine-Smith and a group of 'senators' have completely ignored my challenges posed to them in September 2024, re valid evidence showing the existence of any virus or contagion or even the 'genome' of an alleged virus, and as far as I'm concerned their tacit agreement has been established that none exists and virology is pseudoscience:
https://christinemasseyfois.substack.com/p/a-challenge-for-nathaniel-erskine

January 27, 2025:
The “Scottish Government” confessed to having no scientific evidence of any bird flu virus: "we cannot provide information which we do not hold":
https://christinemasseyfois.substack.com/p/scottish-government-confesses-to

2022 - 2025:
Finland’s National Institute for Health and Welfare (THL) can't cite scientific evidence of "H5N1"... or any virus:
https://christinemasseyfois.substack.com/p/finlands-national-institute-for-health

Below are more official ‘avian influenza virus’ confessions and failures from local/municipal institutions.

March 16, 2022:
Peterborough Public Health and Thomas Piggott, the man who acts as Medical Officer of Health, have no record describing anyone on Earth finding and purifying any alleged SARS, H5N1, “swine flu virus” aka H1N1, MERS, Ebola, or SARS-COV-2 “virus” from the bodily fluids of any diseased human, ever… or any study that in Thomas’ opinion proves the existence of any of those alleged viruses:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/04/Peterborough-PH-SARS-H5N1-H1N1-MERS-Ebola-SCV2-PACKAGE-redacted.pdf

April 11, 2022:
Thomas Piggott, the man who acts as Medical Officer of Health at Peterborough Public Health (Ontario, Canada) has no scientific studies to show that the alleged “avian flu virus” even exists. The health unit was unable to cite even 1 record of “it” ever having been purified from any alleged host anywhere on Earth (which would be a necessary step in proving that an alleged virus does exist)… and doesn’t even have a copy of the testing protocol that was implemented to “confirm cases” in Peterborough.
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/04/Peterborough-Public-Health-avian-flu-PACKAGE-redacted.pdf

The following local institutions in England also failed when challenged to provide or cite records of any “influenza virus” being found in and purified from anyone, ever:

Brighton and Hove City Council:
https://www.fluoridefreepeel.ca/wp-content/uploads/2021/11/Brighton-Influenza-PACKAGE-redacted.pdf

Nottingham City Council/Public Health:
https://www.fluoridefreepeel.ca/wp-content/uploads/2021/11/Nottingham-County-Council-PACKAGE-redacted.pdf

Public Health at Rotherham Metropolitan Borough Council:
https://www.fluoridefreepeel.ca/wp-content/uploads/2021/12/PH-at-Rotherham-Metropolitan-Borough-Council-PACKAGE-redacted.pdf

Leicestershire County Council in the UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/01/Leicestershire-many-PACKAGE-redacted.pdf

Derby City Council in the UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2021/12/Derby-City-Council-many-PACKAGE-redacted.pdf

Hertfordshire County Council in the UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2021/12/Hertfordshire-County-Council-many-and-sc2-PACKAGE-redacted.pdf

Rutland County Council in the UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/01/Rutland-many-no-sv2-response-only.pdf

London Borough of Bromley:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/01/BOROUGH-OF-BROMLEY-many-PACKAGE-redacted.pdf

Derbyshire County Council in the UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/05/Derbyshire-flu.pdf

Kirklees Council, UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/07/Kirklees-many-PACKAGE-redacted.pdf

Cheshire West and Chester Council, UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/08/Cheshire-West-and-Chester-Council-many-PACKAGE-redacted.pdf

London Borough of Lambeth:
https://www.fluoridefreepeel.ca/wp-content/uploads/2022/09/Lambeth-many-PACKAGE-redacted.pdf

West Sussex, UK:
https://www.fluoridefreepeel.ca/wp-content/uploads/2023/11/West-Sussex-many-PACKAGE-redacted.pdf

Christine
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2024 08 12 virology and FOIs affidavit NOTARIZED.pdf
806K