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WET LAB MANUAL

GHANA BIOSECURITY, SURVEILLANCE AND OUTBREAK RESPONSE TRAINING OF TRAINERS (TOT)

4-15 AUGUST 2008

DISCLAIMER

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WET LAB

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Prepared by UC Davis Avian Flu School (funded by the Global Livestock CRSP). Illustrations for this project were created by Kathy West, 2006.

I. DONNING PPE

GUIDANCE FOR INSTRUCTORS

- Have a helper or table leader demonstrate slowly for the class. Allow the class to follow along at each stage.
- Do not wear PPE yourself because it will be too difficult for the class to hear your instructions.
- This part will be noisy, so talk loudly and clearly. Make sure you have everyone to the same stage of dress before starting the next stage.
- The table leaders will be immensely helpful for this exercise.
- After everyone is dressed, dust their hands and abdomens with fluorescent, black-light reactive powder. This will make an important lesson later about taking off PPE cleanly.

Key Points to Mention

- Talk about what to put in a decon area and how to set it up.
- Talk about when to wear PPE.
- Talk about common Do's and Don'ts (See handout)
- Place cell phone, GPS, wristwatch, or other personal items you may need to reference while in PPE in a plastic re-sealable bag so that you can use them without contaminating them.
- Make sure that their masks or respirators fit properly.
- Sleeves and hems of pants must have elastic and completely overlap with gloves and booties.
- If not, tape must be used to connect one with the other. Be sure that the tape is wrapped loose enough to allow easy removal of the PPE later.
- Make sure all hair is covered, including facial hair
- Talk about the concept of the clothes underneath being a clean space, and the outside of the PPE being a dirty space.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Objective: to wear clothing that will prevent contact with contaminated surfaces

PREPARING SITE

1. Select and demarcate site for decontamination.
2. You should determine how contaminated materials will be disposed of from this site.
3. Prepare decontamination site: hang trash bag, place Lysol or other spray disinfectant and two sealable plastic bags (Ziploc) at site.
4. Cut duct tape into 12 inch strips (3 per person) to use for securing pocket on PPE and for sealing sleeves to gloves.

NOTE: Illustrated directions are on the following pages.

DON PPE BEFORE ENTERING CONTAMINATED SITE

1. Place cell phone in bag, seal, and set aside.
2. Collect virus sampling materials (make sure tubes are properly labeled), and place into a sealed bag.
3. Put on inner pair of gloves (if two sets of gloves are provided).
4. Put coveralls on and zip up.
5. Put shoe coverings on.
6. Attach pocket to coveralls with tape .
7. Fill pocket with cell phone and sampling materials (labeled tubes, syringes, tube racks, swabs).
8. Put on mask or respirator .
9. Put on goggles.
10. Put on bonnet.
11. Put on outer pair of gloves.

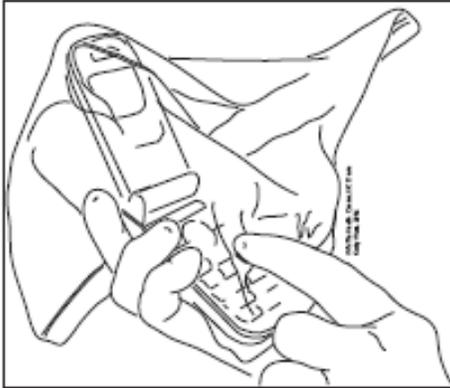
DOFF PPE AT DECONTAMINATION SITE

1. Loosen shoe coverings, but do not remove.
2. Remove outer gloves and place in trash bag.
3. Remove all items from pocket (samples, cell phone), spray the outside of plastic bags with Lysol or other disinfectant, set aside.
4. Open disposal bag included in kit.
5. Remove coveralls and shoe coverings touching only the outside and place in disposal bag.
6. Remove bonnet, place in disposal bag.
7. Remove goggles, place in plastic bag, seal and spray outside of bag with Lysol.
8. Remove mask, and place in disposal bag.
9. Remove inner gloves, and place in disposal bag.
10. Remove cell phone from plastic bag and put in pocket.
11. Seal disposal bag and spray outside with disinfectant.
12. Dispose of trash bag at designated disposal site.
13. Wash hands (or use alcohol hand sanitizer)

DONNING PPE

1. Phone and collection materials

Place mobile phone into a sealable bag, seal, and set aside. The phone can then be operated without being contaminated. Collect virus sampling materials (make sure tubes are properly labeled), place in sealed bag and set aside.



2. Coverall suit

Put on the protective suit. Make sure to completely close the zipper.



3. Gloves

If two pairs of gloves come with kit, put on the inner pair.

DONNING PPE

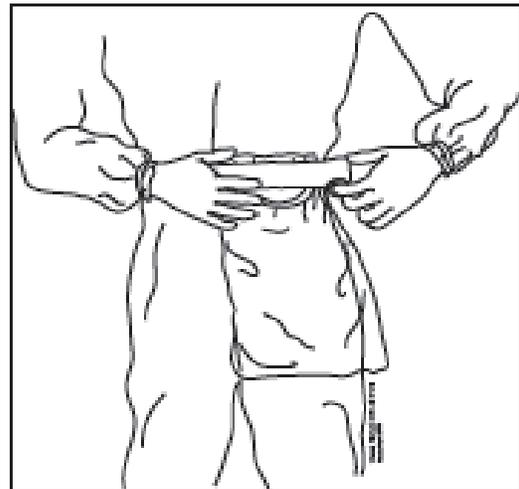
4. Shoe covers

Pull disposable boots on over shoes. Remember that thin plastic boots can quickly get holes in them if you are walking on cement. In some situations, it is better to use protective footwear that is already on site.



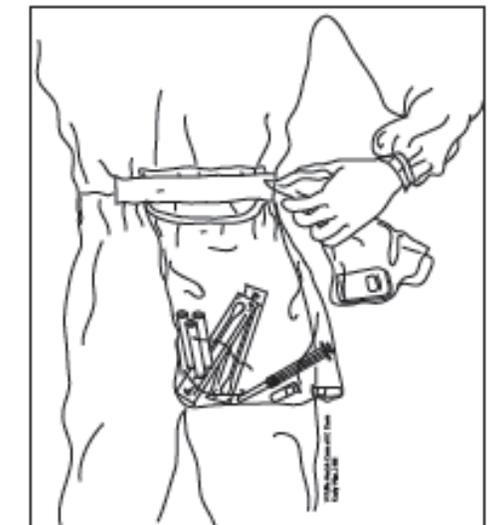
5. Side-pouch

This bag is for holding supplies and phone. The preferred procedure is **not** to take cell phones into the contaminated site. However, if a supervisor must carry a cell phone, they should put it in a plastic bag as indicated.



6. Side-pouch, cont.

Tape only one edge of bag, leaving the bag open as a pouch.



DONNING PPE

7. Mask

Put on mask, placing one band around the neck and the second one over the head. Put on the goggles and pull up the hood, being sure to cover all the hair.



8. Put on apron.

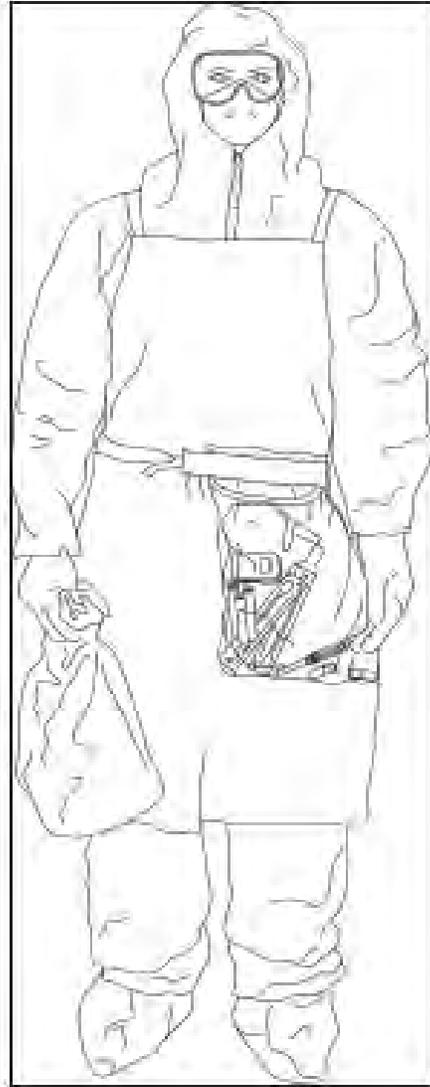


DONNING PPE

9. Put on the inner and outer gloves.



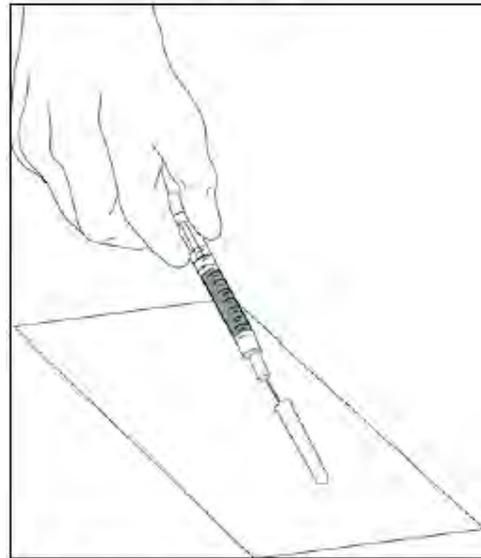
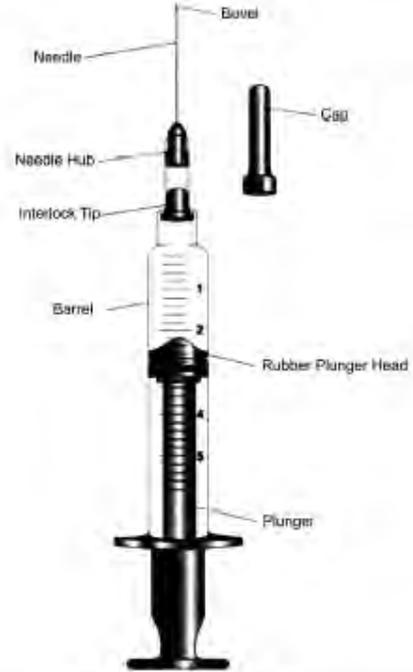
10. Ready to go!



2. LABORATORY SKILLS AND SAFETY REVIEW

Here are some basic topics that may need to be talked about at the start of the laboratory session, depending on the skill level of the participants.

1. No food or drink where the practical session is held.
2. Uncapping Syringe needles.
 - Pull the cap straight off to remove it and expose the needle.
 - Never leave an uncapped needle lying around.
3. Removing syringe needles.
 - Making sure not to remove the cap, twist the entire needle to take it off the syringe.
4. Recapping syringe needles.
 - If needles are used on humans, they should never be recapped. However, when working with animals and in the field, it may be necessary to carefully recap a needle to avoid accidental sticks.
 - To recap a needle:
 - Lay the cap on a table or on a flat surface.
 - Hold the syringe by the plunger end.
 - Insert the needle on the syringe into the cap.
 - Tilt the plunger end of the syringe up, so that the needle inside the cap is point down onto the surface.
 - Apply enough pressure to set the cap onto the needle.
 - Always put the cap back on the needle before trying to twist off the needle. However, **BE VERY CAREFUL WHEN PUTTING THE CAP BACK ON A NEEDLE.**
 - Report any needle sticks to a medical professional.



Place needle cover on a flat surface and thread the needle into it. Once the needle is encased in the cover, it is safe to pick up both ends and snap the cover into place.

5. How to use syringes

- The goal is to both hold and draw fluid into the syringe using only one hand. We recommend this technique for drawing blood. Other techniques can be used for other situations.
- Make sure the needle in its cap is tightly screwed on to the syringe.
- Make a fist around the body of the syringe with the needle coming out the same side of your fist as your thumb.
- Use your little finger to move the plunger of the syringe up and down. Do this before trying to draw blood so that you know the syringe is working properly.
- Remove the cap by pulling straight off.
- Turn the body of the syringe in your fist so that the hole at the end of the needle is facing up.
- Now you are ready to draw blood.
- Leave blood samples in the syringe. Put the cap back on the needle. **BE VERY CAREFUL WHEN PUTTING THE CAP BACK ON A NEEDLE** and follow the correct procedure outlined above.

6. Be sure to follow the proper needle disposal methods of your country. If there are no specifications in place, we recommend the following:

- Place all used needles and syringes into designated "Sharps" containers, which are sturdy, puncture resistant containers that have locking lids.
- Proper disposal of sharps is covered in the Clean-up section below.

7. Placing swabs into sample vials.

- Be sure to only use sterile swabs with plastic handles and polyester or dacron swab ends, as wood handles and cotton swab ends can interfere with some diagnostic tests.
- Put the swab end into the collection tube so that it is not touching the bottom of the tube.
- Break off the swab end by bending the stick over the brim of the tube.
- The swab end should fall into the tube, leaving enough space to cap the tube.

8. Vaccines: demonstrate both how to draw up vaccine into a syringe and the correct method for giving subcutaneous and intramuscular vaccines. Use Handout L.

9. Labels: Prepare and apply labels to all collection tubes and containers prior to collecting samples.

10. Prepare all sample logs or other paperwork prior to dressing in PPE.

3. CHICKEN HANDLING

1. The best method for holding a live chicken or duck is to have the bird's body resting on your arm, tucked in close to your body, while holding the legs just above the feet with the fingers of the same arm. The bird can be facing either towards the front or towards the back.
2. You can also hold the bird by placing one hand palm down on the back of the bird and holding on to the wings where they join the body of the bird with your fingers. But, this method is not recommended if you are going to be holding the bird for a long time because it is stressful, particularly to heavy bodied birds like ducks.
3. Do not hold the chicken up-side-down by its legs for a long time because it is stressful to the bird. Never hold a duck, goose, or other heavy bird up-side-down by the legs.
4. Do not squeeze the chicken too tightly, as this will keep it from breathing.
5. Even when held correctly, chickens can become overheated by close contact with a human body. If a chicken starts to breathe with its mouth open, put it in a box or carrier until it has a chance to cool down.
6. Chickens can be laid down on a table if their legs are tied together at the ankles and a cloth placed over their head so they cannot see. By setting the chicken down calmly like this, it is possible to make it stay on the table for an extended period of time.

4. CLOACAL AND ORAL CAVITY SWABBING TECHNIQUE

GUIDANCE FOR INSTRUCTORS

- Now that everyone is wearing PPE, it is time to get out the birds.
- Make sure that everyone has their own bird (or however many you have assigned).
- Check to make sure that the participants are properly handling the birds.
- One person and two person sampling techniques should be demonstrated and tried by all participants.

Key Points to Mention

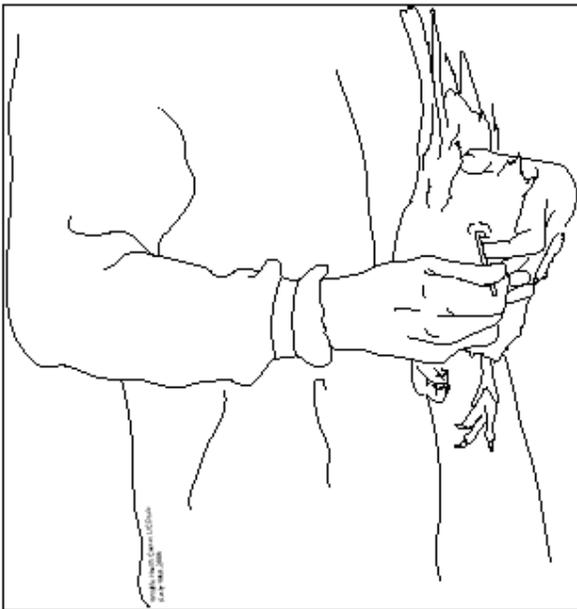
- Where the choana is (the cleft in the roof of the mouth).
- How insert the cloacal swab so that it samples from the digestive tract and not the reproductive tract.
- Proper technique for placing swabs in collection tubes:
 - Place the swab end in the collection tube so that it is not touching the bottom of the tube.
 - Break off the cotton end by bending the stick over the brim of the tube.
 - The swab end should fall into the tube, leaving enough space to cap the tube.

COLLECTING FECAL AND ORAL CAVITY SAMPLES

Objective: to obtain samples for diagnostic purposes while minimizing virus exposure. Persons sampling birds should wear PPE, including gloves.

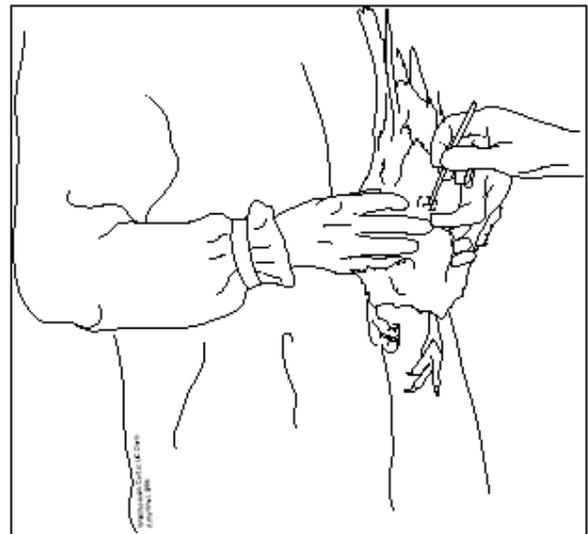
1. Cloacal swab: one person

Hold the bird with its head under your elbow. Pull up the bird's tail to expose the cloaca and gently insert the swab. Rotate gently 360°. Remove the swab and place the swab into the testing media.



2. Cloacal swab: two persons.

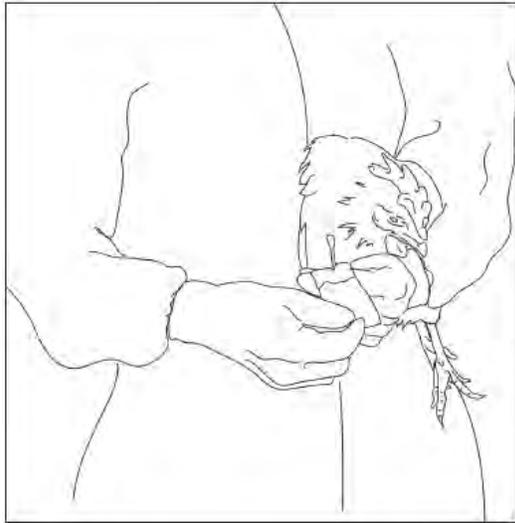
One person holds the bird, securing the head under her arm and holding the feet with her other hand. The swabber picks up the bird's tail and places the swab gently into its cloaca. Rotate gently 360°. Remove the swab and place the swab into the testing media. Note: For small, delicate birds or birds difficult to capture, fresh fecal samples may be taken.



COLLECTING ORAL CAVITY SAMPLES

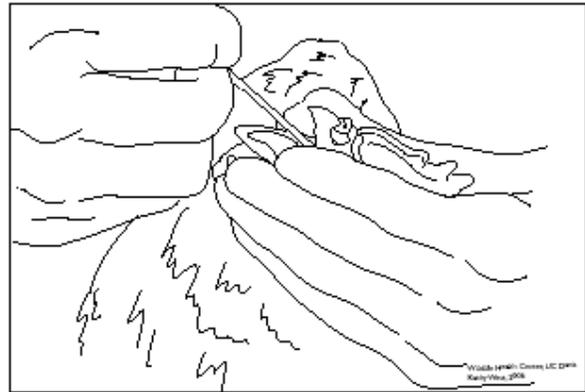
3. Oral cavity samples: one person

Hold the bird under your arm with its head facing outward. Grasp the comb with one hand and with the other hand, open the bird's bill with your fingers. To keep the bird's bill open, insert your finger at the corner of the bill. You should now have one hand free to hold a swab.



4. Oral cavity samples: one person

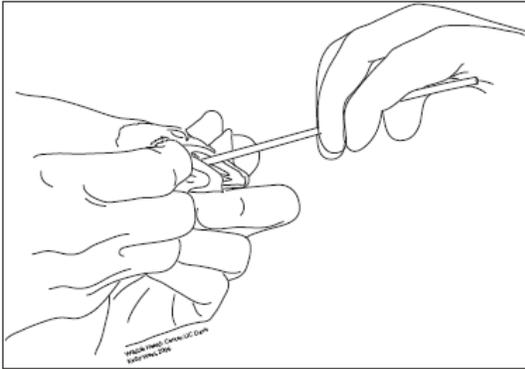
Insert the swab into the bird's bill and rapidly but firmly move the swab around the oral cavity. Remove. Insert the swab in a vial, break off swab stem and cap the vial.



COLLECTING ORAL CAVITY SAMPLES

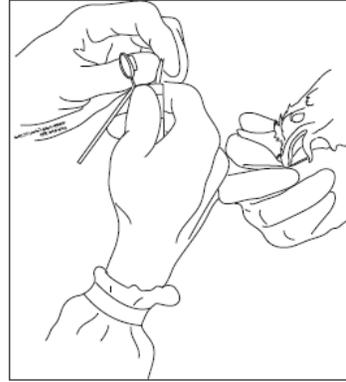
5. Oral cavity samples: two people

One person holds the bird under her elbow with its head forward and holds open the bird's bill. The second person inserts the swab and rapidly but firmly moves the swab around the oral cavity.



6. Oral cavity samples: two people

The second person then inserts the swab in a vial, breaks off the swab stem, and caps the vial.



Note: Vials containing swabs should be protected from UV light, stored on ice, in a freezer, or in liquid nitrogen shippers, and shipped or transported to a laboratory the next day. If it isn't practical to keep samples cool, the swabs should be placed in vials containing a medium that inactivates the virus like guanidinium thiocyanate or 100% ethanol.

PACKING AND SHIPPING DIAGNOSTIC SAMPLES

GUIDANCE FOR INSTRUCTORS

- Demonstrate proper packaging at the front of the whole class.
- Focus on demonstrating how to ship vials and bird body parts, as these are the most common sample types requested by diagnostic laboratories.

Key Points to Mention

- Emphasize the necessity of planning in advance and coordinating with diagnostic labs and shipping companies.
 - Does the lab have the ability and expertise to run the tests you need?
 - Can the lab get the tests done in a timely fashion?
 - What kind of samples will the lab need, and how do they want them preserved?
 - Can the shipping company get the samples to the lab?
 - Do you have the supplies on hand to box samples quickly and easily?
- Making the container leak proof
- Keeping the sample at the right temperature
- Pros and cons of using wet ice, dry ice, or liquid nitrogen
- Proper labeling for shipping

SHIPMENT OF DIAGNOSTIC SAMPLES AND VIRUSES

Samples that have been collected from possible cases of H5N1 highly pathogenic avian influenza (HPAI) should be shipped as diagnostic samples and not as a Category A infectious substance. However, if H5N1 HPAI has already been confirmed, then, the samples must be shipped as a Category A infectious substance. There are three main components you must carefully implement in order to ship diagnostic samples of avian influenza viruses.



MARKING & LABELING:

- The shipper is responsible for all marking and labeling on the package
- The marks and labels must contain: the contents of the shipment, the nature of the hazard, the volume of the samples
- Packaging must meet approved standards

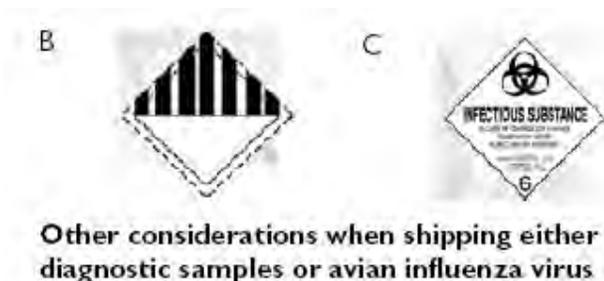
Shipping Diagnostic Samples:

- The quantity of sample should be included in grams or millilitres
- The name and address of the shipper
- The name and address of the person receiving the package
- The name and telephone number of the person responsible for the shipment
- A diamond shaped Diagnostic Sample label (see image A) that contains the UN number for diagnostic samples (UN 3373). You may use a label sticker, a photocopy of the label, or you may use a drawing of this label but it must be the same size as the printed label.
- A class 9 miscellaneous label (see image B) for dry ice. Include the weight of the ice in kilograms and the UN number for dry ice (UN 1845). You may use a label sticker or a photocopy of the label as long as it contains the UN number, the net weight of the ice, and is the same approximate size as a label sticker. Please note that some air carriers may accept packages marked with handdrawn dry ice labels. Please check with each carrier to be certain that drawings are acceptable.
- All packages must have an orientation label that shows the top of the package. This must be applied if it is not already printed on the box.
- Labels should not be placed on the corners of boxes.

SHIPPING AVIAN INFLUENZA VIRUS (CATEGORY A INFECTIOUS SUBSTANCE):

- The Shipping Category, which should be spelled correctly: "Infectious substance affecting humans"
- The UN number must always come before the shipping name. It is a 4-digit number assigned by the United Nations committee of experts. (UNCOE) to identify a substance or group of substances. The prefix UN is always required. Example: UN 2814 Infectious substance affecting humans or UN 2900 Infectious substance affecting animals

- The technical name for the contents should not appear on the package
- The quantity of sample should be included in grams or millilitres
- The correct and complete name and address of the shipper
- The correct and complete name and address of the person receiving the package
- The name and telephone number of the person responsible for the shipment (the shipper)
- If you are using dry ice, mark the package with the proper Shipping Name, the UN number and the net weight of the ice used in kilograms. You must also have a class 9 miscellaneous label (see image B). You may use label stickers or a photocopy of the label as long as it contains the UN number for dry ice (UN 1845), the net weight of the ice, and is the same approximate size as a label sticker. Please note that some air carriers may accept packages marked with hand-drawn dry ice labels. Please check with each carrier to be certain that drawings are acceptable.
- The diamond-shaped “Infectious Substance” class 6 label of the country of origin must be placed next to the proper Shipping Name and UN number. The following text should be present on the label: “In case of leakage, immediately notify the public health authority” followed by the name and telephone number of the health authority in the country to which you are shipping (see image B).



OTHER CONSIDERATIONS WHEN SHIPPING EITHER DIAGNOSTIC SAMPLES OR AVIAN INFLUENZA VIRUS

- Special provisions
 - Provision A81 for body parts, organs or whole bodies — The quantity limits, which are 50mL/50g (passenger or cargo aircraft) and 4L/5Kg (cargo aircraft only), don't apply to body parts, organs or whole bodies. The proper Shipping Name must be supplemented with the technical name on the shipper's declaration. Technical names don't need to be shown on the package.
 - Provision A140 for unknown infectious substances suspected of being Category A - “Suspected Category A infectious substance” must appear in **parenthesis on the shippers declaration, but not on the package.**
 - When packing dry ice, the following information should be included in the label:
 - Proper shipping name – Dry Ice
 - Class or division number – class 9
 - UN number – UN 1845
 - Packing Group – III (minor danger)
 - Number of packages
 - Net weight of dry ice in the package (in kilograms)

- An overpack is a large package containing one or more smaller packages. Overpacks are a convenient way to transport more than one package in a single shipment. They are often used for chilled or frozen shipments. They are usually made of fibreboard (cardboard). The overpack should be marked with:
 - In addition to the requirements listed above, the term “OVERPACK” must appear on the outside of the package.
 - When completing the shipper’s declaration for dangerous goods, make sure you record “overpack used” in the quantity and type of packing box

PACKAGING

- If a shipper is purchased, follow manufacturer’s instructions for packaging. If not, then follow the instructions given by the laboratory to which you are sending the samples (see attached pages from OIE reference laboratories on shipping)
- Only use the shipping container as it was intended. Do not overfill it and make sure it is sealed as it was intended.
- Carrying packages aboard an aircraft by hand, even of compliant packages, is strictly prohibited for all passengers and crew.
- Packages may be reused for many shipments, but should be disinfected or sterilized between uses.
- When packing dry ice, several precautions must be taken:
 - Avoid direct contact of the dry ice with unprotected skin, since it causes irritation or skin damage. Always wear gloves.
 - Packages must be designed and constructed to permit the release of carbon dioxide gas (CO₂), which prevents the build-up of pressure that could rupture the package.
 - Arrangements must be made between the shipper and operator to ensure ventilation safety procedures are followed.
 - The shipping time should be as short as possible. Make sure that the shipment does not arrive after business hours on Friday, which may result in the sample sitting in the mailroom until Monday. Also, check for holidays, arrange prompt delivery by the courier and prompt pick-up by the person receiving the package.
- When packing overpacks, make sure that the inner packages don’t exceed the volumes on the list of dangerous goods.

SHIPPER’S DECLARATION

The shipper’s declaration is a legal document signed by the shipper, which creates a contract between the shipper and the carrier. This document must be accurate, legible, neat, and must contain no spelling errors.

- Only the shipper can complete the shipper’s declaration and the carrier must ensure that it has been properly completed.
- There should be three copies: one for the shipper’s records and one for the carrier and one to be included with the shipment.
- Changes or amendments on the declaration are only acceptable if signed by the shipper with the full signature that is used to sign the declaration.

- Whiteouts should never be used
- A declaration containing information that is not relevant to the particular dangerous goods being shipped, or the goods contained in the shipment, is not acceptable.
- If the information is not 100% correct, your shipment may be rejected.
- Information required on the shipper's declaration:
 - Full name of the shipper (telephone number is recommended)
 - Full name and address of the person receiving the package (telephone number is recommended)
 - Name and phone number of the person responsible for the shipment in case of an incident (this could be the shipper, the receiver or a third party)
 - The Air Waybill number (this information may also be entered by the carrier)
 - If known, the names of the airports of departure and the destination (this information is sometimes entered by the carrier) should be included
 - The class or division of the contents
 - The UN or ID number
 - The shipment quantities
 - Under "packing instructions," the packing instruction number should be included
 - UN 1845, packing group III and dry ice quantity (if used).
 - In the "additional handling information" area, a 24-hour emergency response number that is monitored 24 hours a day must be included
 - The box should be marked by the statement: "I declare that all of the applicable air transport requirements have been met".
 - The name and title of the signatory must be completed, as well as the date and place.
 - Shipper's signature

5. PERFORMING FLUDETECT TEST

GUIDANCE FOR INSTRUCTORS

- Allow each table to run a test sample from their own bird or, if the tests are in short supply, you can demonstrate for the class using your bird.
- The test can incubate while you teach vaccination techniques (next exercise)

Key Points to Mention

- This test only tells whether the disease problem is an influenza type A or not. It does not tell what kind of influenza it is.
- Useful for differentiating between Newcastle and Influenza.
- Further tests are needed if the test is positive in order to confirm the result, determine subtype of virus, and determine its pathogenicity.
- The test works well if virus concentrations are 10^3 - 10^4 EID₅₀ or greater.

FLUDETECT TEST PROCEDURE

GENERAL PRECAUTIONS

- Do not use kit components after expiration date
- Test kits should be stored between 2° and 30°C (35° to 86°F)

Personal protection required depends on the sample:

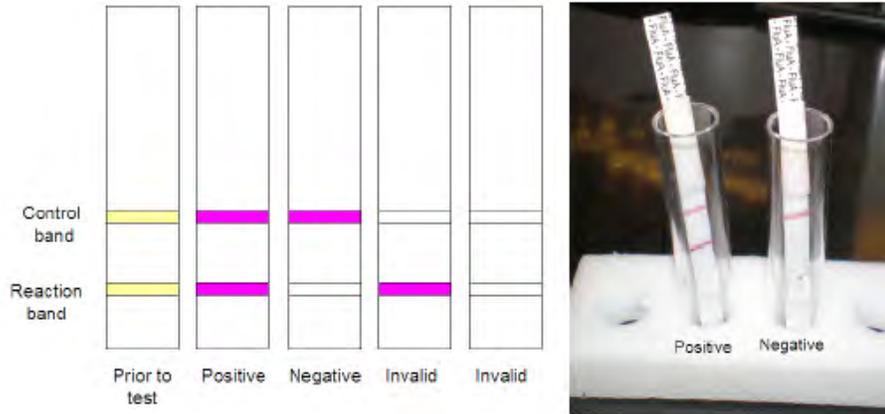
- If it is allantoic fluid → under Biosafety Level 2 (BSL2) conditions in the lab
- If it is a swab from a bird → wear Personal Protective Equipment (PPE) in the field or under BSL2 conditions in the lab



Straight from the bird (cloacal or oral):

1. Add 8 drops of extraction buffer to the test tube
2. Swab the bird's cloaca or oral cavity
 - a) Swabs with wooden handles or swabs containing calcium alginate must not be used
 - b) Swabs containing blood may obscure a weak positive
3. Put the swab into the test tube and swirl it
4. Press and turn the swab against the side of the test tube to get all the liquid from the swab
5. Discard the swab in an appropriate biohazard container
6. Place the test tube into the test tube rack
7. Swabs containing feces may interfere with the test. Allow feces to settle to the bottom of the tube then draw a sample of fluid from the top and place it in a new clean tube for testing.

8. Insert a test strip into the test tube so that the pink pad is submerged in the extracted sample
 - a) Handle the test strip on the labeled portion of the strip
 - b) Use the test strips within 10 minutes of removing from vial
 - c) Avoid contact with the surface of the test strip
9. Incubate the test strip in the sample for 15 minutes.



10. Read the test results. Observe the presence/absence of two pink-purple bands:
 - a) Very faint lines should be further investigated
 - b) The test can be considered to be complete if the test line on the lower part appears before the 15-minute incubation period is over
 - c) The test strips must incubate for full 15 minutes before the sample is interpreted as negative
 - d) If the test strip remains in the test tube for over 20 minutes, a false positive ghost band could appear in the place of the reaction band
11. Discard the test strip in an appropriate biohazard container

From Viral Transport Media (VTM):

Samples in VTM should be kept at 4°C for up to 48 hours or frozen (-70°C) for more prolonged storage. Usually the broken swab is still inside of the VTM tube.

1. Thaw the sample.
2. Pipette 200 µl of the VTM into the test tube
 - Allow samples to come to room temperature (15-30°C; 59-86°F) before testing
 - Do not centrifuge samples prior to use
3. Add three drops of extraction buffer to the test tube
4. Tap gently the side of the tube to mix
5. Continue from point 6 from the "Straight from the bird" protocol above

6. VACCINATING A CHICKEN

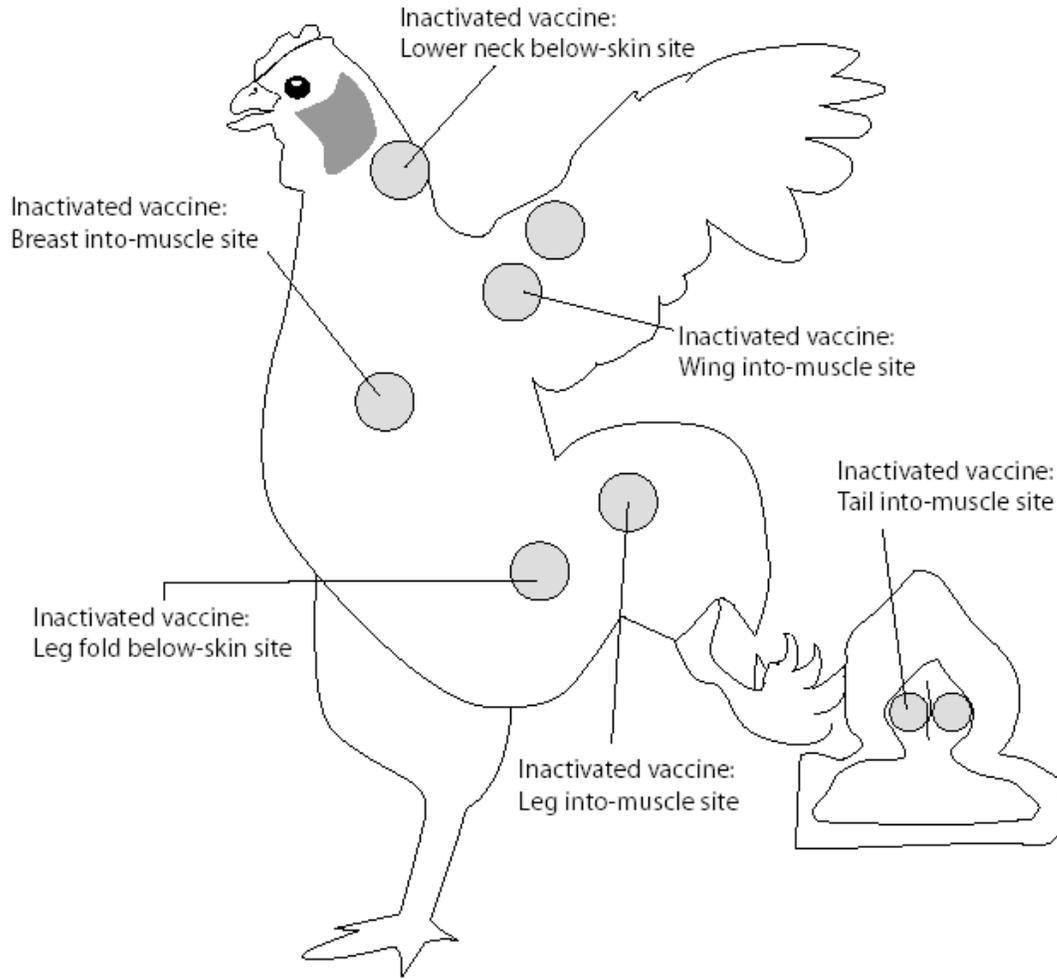
GUIDANCE FOR INSTRUCTORS

- In front of the class, point out the different locations where vaccines might be given and the different techniques and tools involved.
- With help from the table leaders, allow participants to try vaccinating their birds. Most avian influenza vaccines are given intramuscularly or subcutaneously. Handout L shows the sites that can be used
- At the end of this exercise, the FluDETECT tests should be ready to talk about.

Key Points to Mention

- What to do for needle sticks
- How to pick a vaccination site when the birds will be eaten later: withdrawal times (41 days for avian influenza), damage to muscle.

CHICKEN VACCINATION PROCEDURE



After Fort Dodge Australia Pty Limited

7. BLEEDING A CHICKEN

GUIDANCE FOR INSTRUCTORS

- At first, participants will work in teams of 2 people.
- Demonstrate how to make the chicken lay still on the table with a cloth over its head. One person from the team of two will do this to their bird so that they can both work on the remaining bird.
- Point out where the wing vein (brachial vein) is on the bird
- With a helper, demonstrate how one person restrains the bird while the other draws blood.
- Have both people from each team try drawing blood, one from each wing.
- Afterwards, demonstrate how one person can restrain the bird for blood draw.
- Participants are welcome to try the one-person technique.

Key Points to Mention

- Emphasize proper needle and syringe handling
- Pull out small feathers from over the vein so the vein can be seen properly
- Hold the syringe so that the hole at the end of the needle is facing up.
- Make sure that the participants orient to the landmarks on the wing as shown in the handout.
- Extend the wing.
- Hold off the vein where the wing leaves the body and pull the skin taut.
- Insert the tip of the needle into the vein near the elbow, where the vein dips over the bone.
- Draw 1-2 ml of blood.
- Release the vein and remove the needle. Hold the wing down to prevent flapping.

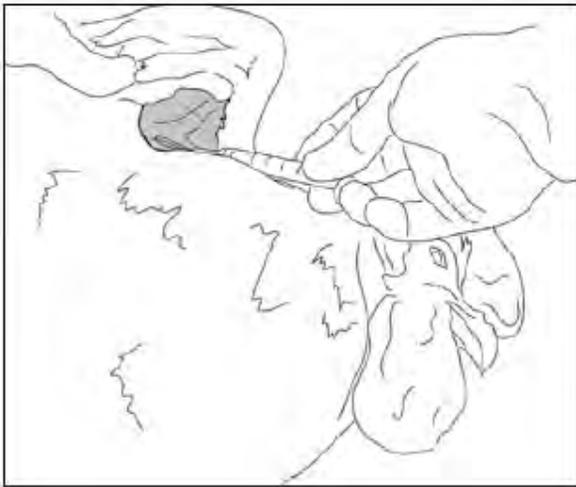
8. COLLECTING BLOOD

Objective: to collect blood for diagnostic purposes while minimizing virus exposure

1. Wing vein: one person bleeding.

All persons bleeding should wear Personal Protective Equipment (PPE).

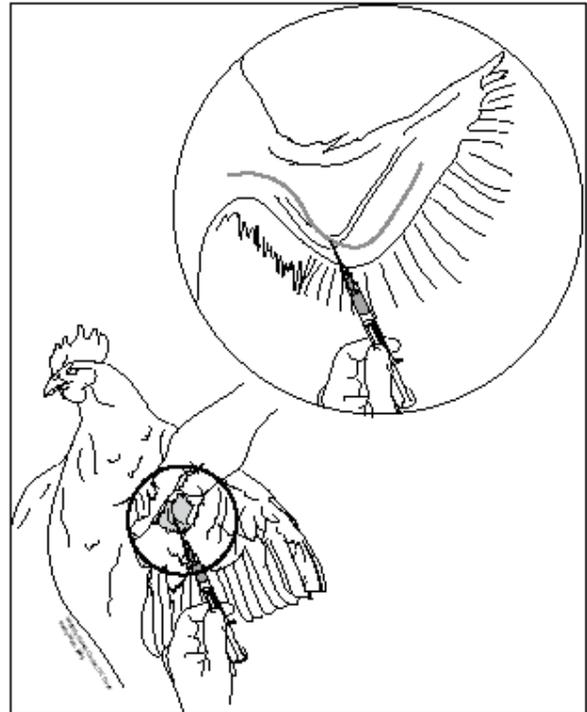
Hold the chicken's head under your left arm (if right-handed) securing it against your body. Hold the bird's feet with your left hand. Pull the wing out and align the needle with the brachial vein, pointing it away from the bird's heart. Hold off the vein where the wing leaves the body and pull the skin taut. Put the tip of the needle into the vein, close to the bird's elbow, just where the vein dips over the bone. Pull the plunger back until there is 1-2 ml of blood in the syringe. Release the vein where you are holding it off and pull out the needle. Put the bird's wing down to prevent flapping and put the bird back in its pen or cage.



2. Wing vein: two persons bleeding.

All persons bleeding should wear Personal Protective Equipment (PPE).

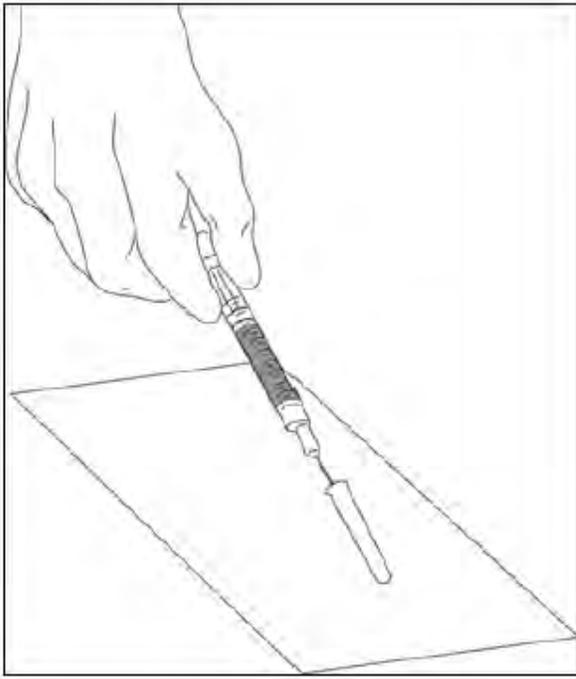
One person holds the chicken on its back, securely holding the legs and wings. The bleeder extends the wing toward himself and holds off the vein where the wing leaves the body, pulling the skin taut with the same hand. Align the needle with the now visible vein. Put the tip of the needle into the vein, close to the bird's elbow, just where the vein dips over the bone. Pull the plunger back until there is 1-2 ml of blood in the syringe. Release the vein where you are holding it off and pull out the needle. Put the bird's wing down to prevent flapping and put the bird back in its pen or cage.



COLLECTING BLOOD

3. Replacing needle cover

Place needle cover on a flat surface and thread the needle into it. Once the needle is encased in the cover, it is safe to pick up both ends and snap the cover into place.



4. Properly handling the collected blood: Vacutainer

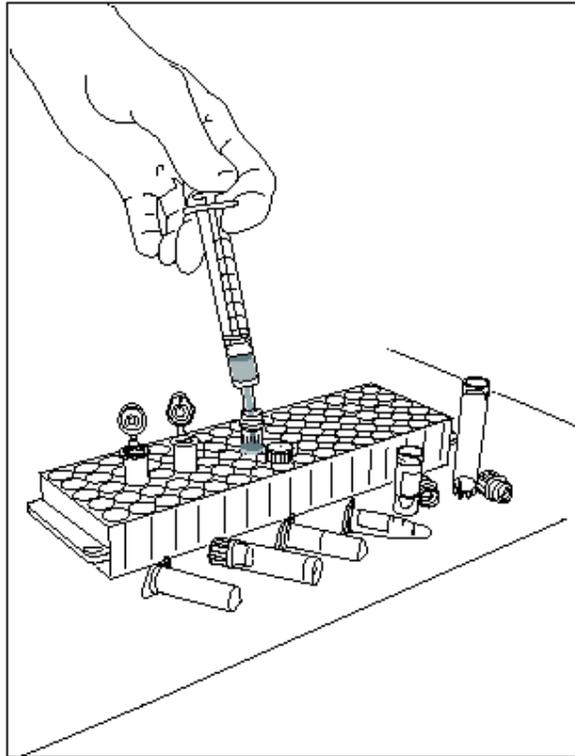
With the vacutainer in a rack, insert the needle into the rubber stopper and allow the vacuum to pull out the blood.



COLLECTING BLOOD

5. Tube

Tube. Many types of tubes can be used for blood. For most tubes, the needle should be removed from the syringe, before the blood is expelled into the tube.



9. HUMANE EUTHANASIA OF INDIVIDUAL BIRDS

GUIDANCE FOR INSTRUCTOR

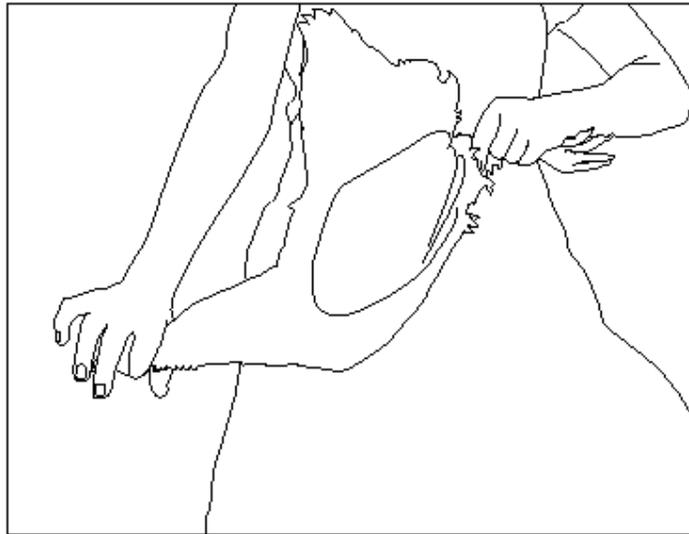
- Have the participants put on their aprons to protect their clothes
- What qualifies as humane euthanasia may change depending on the country and local customs. Be sure to check in advance.
- Demonstrate with one or two birds
- Be prepared for some participants finding this section highly distasteful and refuse to participate.

Key Points to Mention

- Death needs to be as instantaneous as possible.
- These techniques are only advised for small numbers of birds.

How to Perform Cervical Dislocation in Poultry

- Hold the chicken by the legs with one hand
- Grasp the head immediately behind the skull with the other hand
- Stretch the head so that the neck is fully extended.
- Pull downward sharply and quickly. Abruptly turn the head as you pull downward
- The head should come away from the neck without tearing the skin
- Slit the chicken's throat and drain out the blood.



10. NECROPSY OF BIRDS

GUIDANCE FOR INSTRUCTORS

- About half of the euthanized birds should be necropsied.
- The remaining euthanized birds should be saved for the Safe Slaughter and Cleaning of a Chicken exercise later.
- Help the participants carefully necropsy their birds

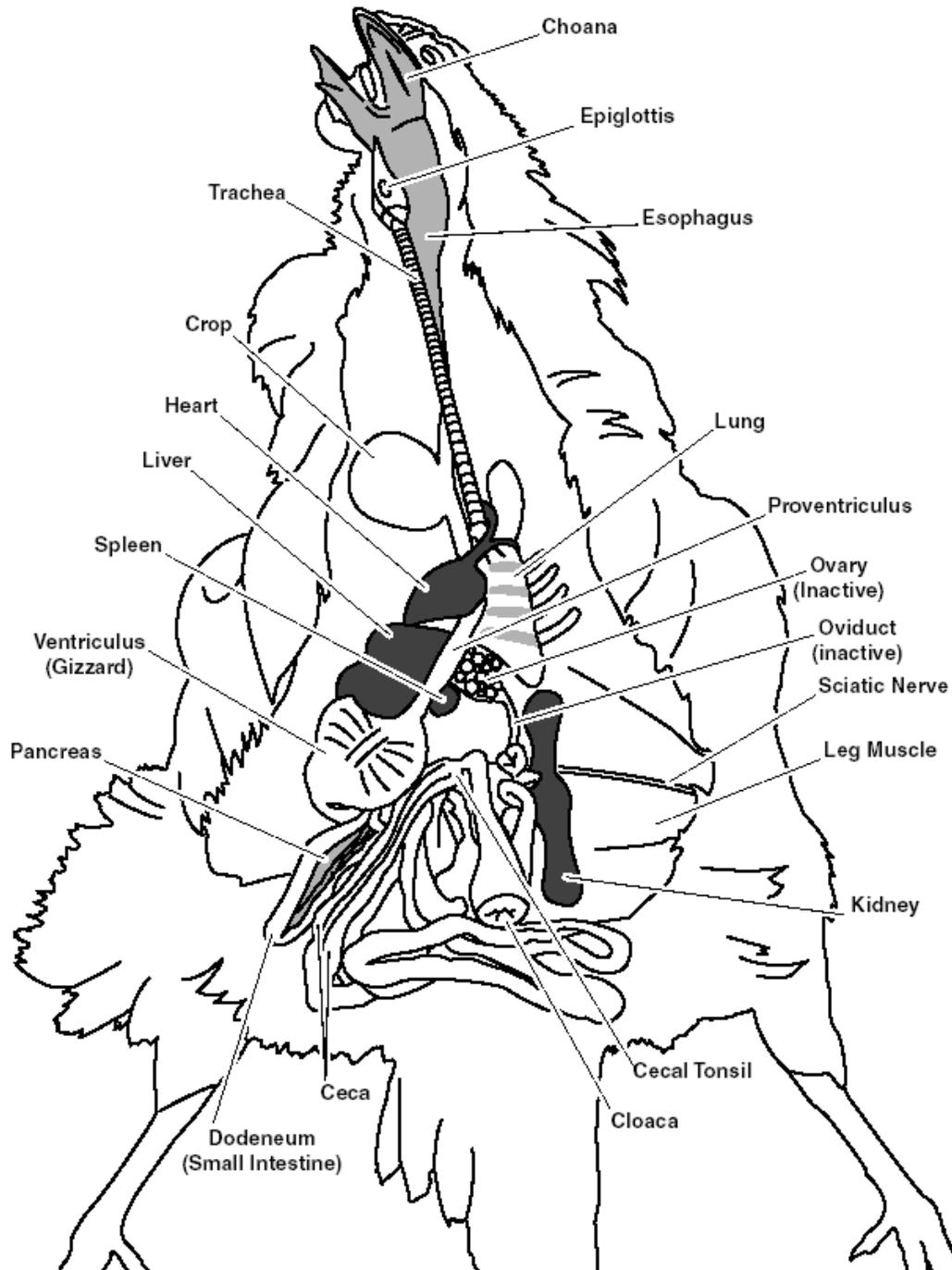
Key Points to Mention

- Necropsies should be performed while wearing PPE.
- Necropsies should be done carefully, with great attention to detail, to insure that nothing is missed.

NECROPSY OF BIRDS

1. Review the clinical history and consider what the likely diagnosis is.
2. Examine the outside of the bird. Observe how the bird acts if the bird is still alive. Check for external parasites.
3. Humanely euthanize the bird (See Handout Y: "Humane Euthanasia of Individual Birds")
4. Moisten the feathers with water that contains a small amount of soap.
5. With scissors, cut through one corner of the mouth so that the oral cavity can be examined.
6. Continue the cut down the neck of the bird from the mouth to the chest, through the skin only. Examine the thymus, if present.
7. Make an incision down the esophagus from the mouth to the crop. Examine.
8. Make an incision down the trachea from the mouth to the chest. Examine.
9. With heavy scissors, cut across the beak just in front of the eyes. Examine the nasal cavities.
10. Using a scalpel or one side of a small scissors, cut into each infraorbital sinus, just below the eye. Examine the color and look for any extensive mucous or other material.
11. Pull the leg bone out of the hip joint. Bend the legs backwards, towards the back of the bird.
12. Cut the skin on the inside of each thigh from the hip to the stifle joint. Pull the skin back so you can see the muscles.
13. Make another cut through the skin of the abdomen that connects the two cuts on the thighs. Pull the breast skin up and the abdominal skin down so that the midsection of the bird is exposed.
14. Using scissors, make a cut in the abdominal body wall that follows the bottom edge of the rib cage. Be careful not to puncture the intestines.
15. Continue this cut up through the ribs on either side. Cut through the bones without damaging the organs underneath. Be sure to cut through the strong coracoid bones at the top of the rib cage.
16. Now that the ribs have been cut through, remove the rib cage and breast muscles as one piece. Observe the air sacs as you do this, because they will be disrupted as the rib cage comes off.
17. All the organs should now be exposed. Look at them without moving them first.
18. Examine the pancreas.
19. Cut across the esophagus just above the proventriculus. Pull downward so that the digestive tract comes away from the bird and can be examined in detail. If desired, remove the digestive tract from the bird entirely by cutting down near the cloaca.

20. Using scissors, cut length-wise down the digestive tract to examine the inside. You may need heavy scissors or a scalpel to get through the tough muscle of the ventriculus. Examine for wounds or parasites.
21. Remove and examine the liver and spleen
22. Examine the reproductive organs. In the female, remove the ovary and oviduct. Cut the oviduct length-wise to look at the inside.
23. Remove and examine the heart.
24. Examine the lungs. Remove them by freeing them from their attachment to the ribs.
25. Look at the sciatic nerves in each thigh. You may need to move the leg muscles to find it.
26. With a sharp blade, open each knee (tibiotarsal) joint and examine.
27. With a sharp blade, split one leg bone length-wise to expose the bone marrow.
28. To examine the brain, remove the head from the body. Skin the head. With strong scissors, carefully chip and peel off the top of the skull to expose the brain. Be careful not damage the brain.



I I. DOFFING PPE

GUIDANCE FOR INSTRUCTOR

- Have participants put their birds back into their boxes or lay them down on the table with a cloth over their heads.
- Demonstrate how to take of the PPE while only touching dirty parts to dirty parts
- Have table leaders help
- Place all dirty PPE into trash bags and seal.
- Disinfect the outside of the bags
- Don't forget to check for black-light reactive stains at the end.

Key Points to Mention

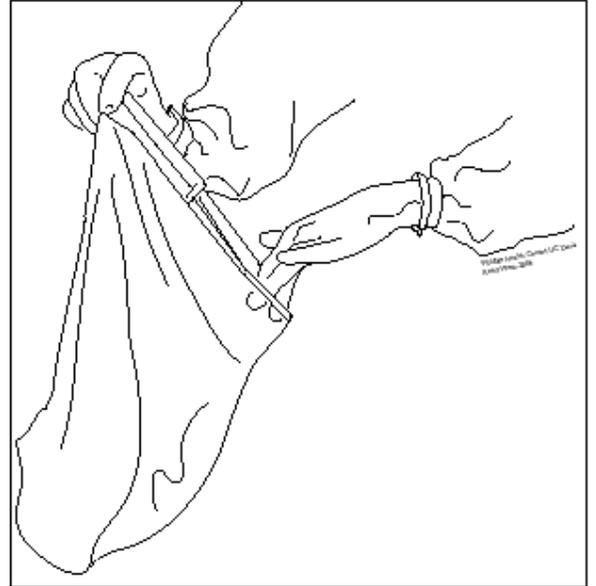
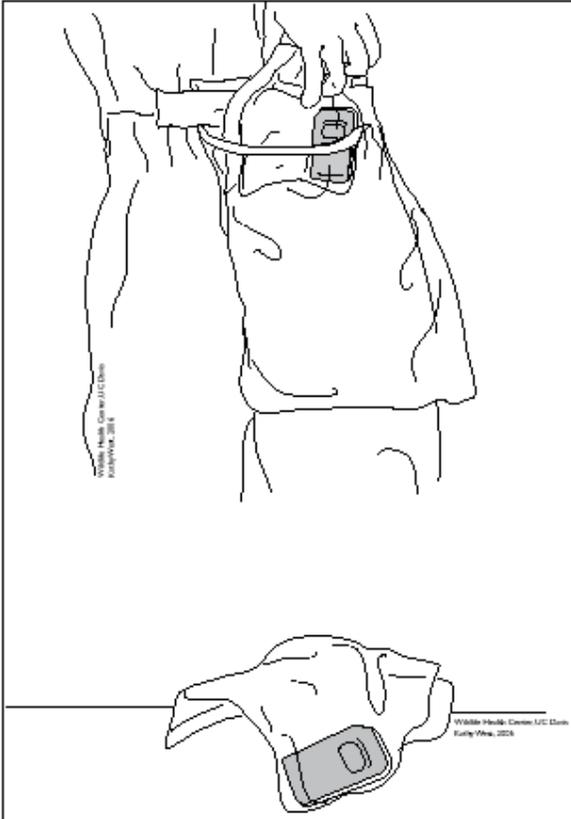
- Only touch the dirty parts of the PPE with other dirty parts. Do not touch dirty parts to clean parts
- Disinfect the outside of all bags with spray disinfectant
- Wash hands afterwards using the method below or the alcohol gel provided.
 - Using a good soap, suds your hands for at least 20 seconds.
 - Rise hands with clear running water
 - Wash forearms and face with soap and water as well.

DOFFING PPE

Objective: to remove protective clothing while avoiding contact with contaminated surfaces

1. Loosen shoe coverings, but do not remove.
2. Remove outer gloves
3. Remove plastic bag containing phone from side pouch attached to coverall. Spray outside of plastic bag with disinfectant and set aside.

4. Open disposal bag



DOFFING PPE

5. Remove coverall and shoe coverings touching only the outside and place in the disposal bag.

6. Take off the bonnet and place in disposal bag.

7. Remove goggles and place in small plastic bag for disinfection.

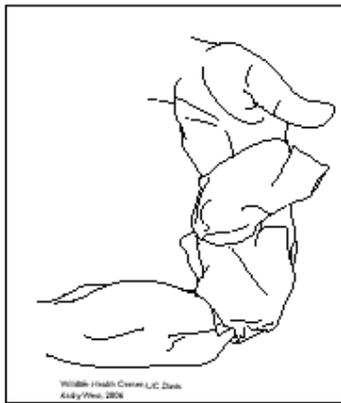
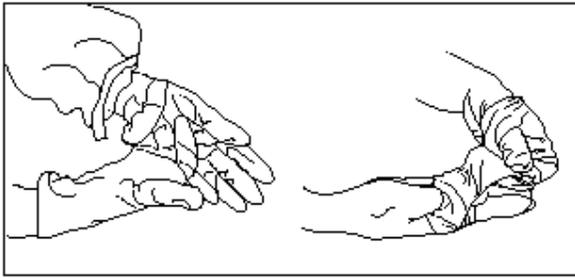
8. Remove mask and place in disposal bag, mask and head covering and place them into the disposal bag.



DOFFING PPE

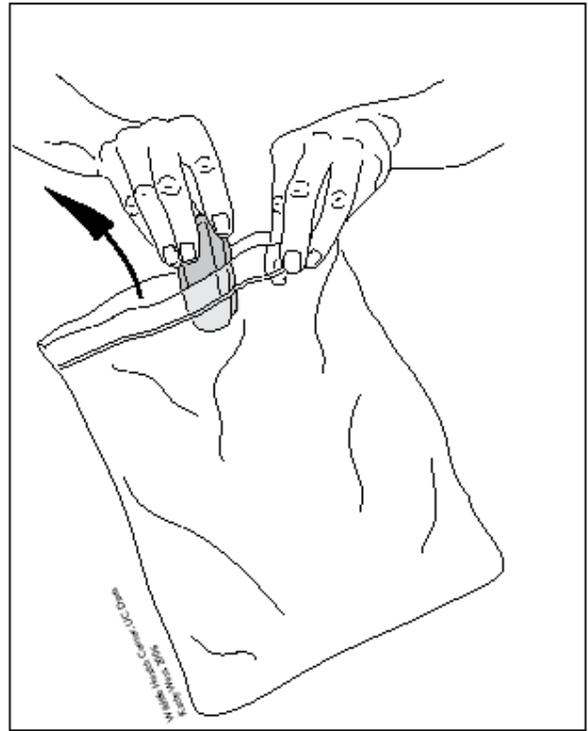
9. Removing undergloves

Remember, it is only safe to touch the inside of the gloves with your bare hands and only safe to touch the outside of the glove with your gloved hands. Fold the cuffs of both gloves up. Touching only the outside of the glove, pull the first glove off turning it inside out over your other hand (the one still with the glove). Pull the second glove off by pulling it off with the inverted glove. Place the gloves into the disposal bag.



10. Phone

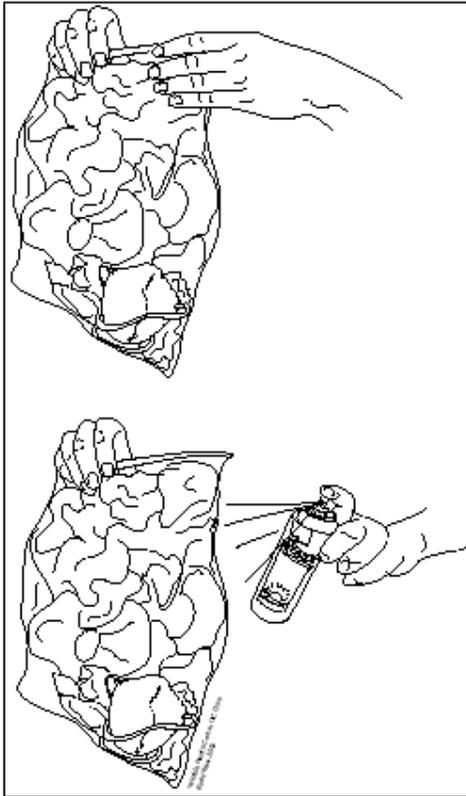
Remove your phone from the bag and place it in your pocket.



DOFFING PPE

11. Disposal bag

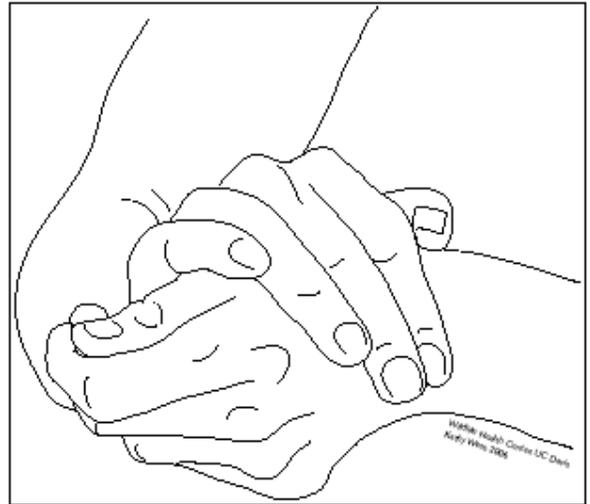
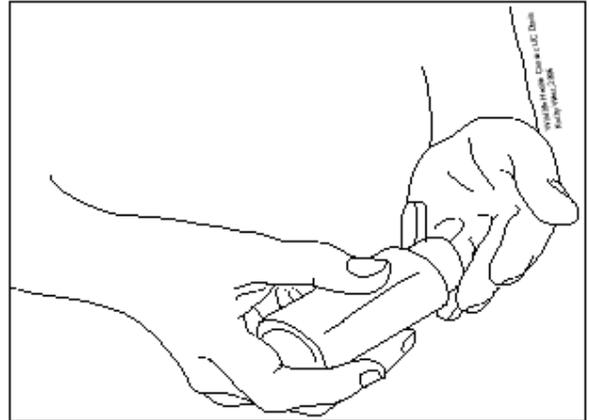
Once you've completely removed the protective suit, boots, mask, and gloves, put them into the disposal bag and close it securely. Spray outside with disinfectant.



12. Dispose of trash bag at designated disposal site.

13. Hand sanitizer

Once you've removed all protective clothing and placed it into the disposal bag, use an alcohol-based hand sanitizer to clean your hands or wash hands thoroughly with soap and water.



ADDITIONAL INFORMATION

SMALL SCALE DEPOPULATION KIT: CULLING BAGS



SUMMARY

The small scale depopulation kit is based on a system of culling birds in special bags using carbon dioxide in the form of dry ice. It is a low cost, easily transported system for culling poultry in remote areas where there is little access to heavy equipment or other infrastructure.

Only a small team of trained people is used, including one veterinarian, one team leader and a further one person per hundred birds that need to be culled. A single bag can be used to cull up to 500 birds per hour, but by setting up a group of 4 or 5 bags this rate can be increased proportionally.

The team set up all the facilities themselves, cull the birds and carry out the preliminary cleansing and disinfection before departure. The carcasses should be sealed in two plastic bags and the exterior disinfected if they are to be transported off site for disposal. Alternatively they can simply be carried to a nearby site for burial, if bio-degradable bags are used they can be buried directly, otherwise they should be emptied into the pit and the used bags burned.

PREPARATION

The veterinary inspector who confirms the disease should direct the owner to:

- Wear personal protection equipment
- Set up disinfection footbaths at entrance to the property or to all sheds or yards
- Close all nest boxes (if applicable)
- Stop egg collection (if applicable)
- Keep the affected birds in an enclosed, segregated area

TEAM

- 1 veterinarian
- 1 team leader
- 1 catcher/culler per 100 birds

EQUIPMENT

Foot dip container	
Disinfectant	Approved locally for AI
Water	Enough to fill footbath, sprayer and for handwashing.
Sprayer	Knapsack sprayer or other similar equipment for distributing disinfectant.
Barrier tape	
Wooden stakes	
Netting	
Cable ties (plastic) or string	
Outer bags and support	Number of bags at each culling point will depend on the total number of birds at that place and the time and people available for the operation
Inner liner bags	Allow one inner liner bag per 125 birds (@ approx 2kg) Each bag should can safely hold 250 kg of poultry. If carcasses are to be moved away from the culling site in the plastic liners, the bags should be doubled up for additional bio-security
CO2 as dry ice	Allow 4 kg per 250 kg (125) birds. 1kg of dry ice vaporises to approximately 0.5 m ³ of gas. Follow all health and safety precautions when handling dry ice.
CO2 monitor	OIE standards require the monitoring of gas concentration. Alternatively a residual oxygen monitor can be used to indicate a maximum O ₂ level of 6.3% which equates to 70% CO ₂ in atmospheric air, in a sealed container.
Minimum 2 sets PPE per person	

ROUTINE

1. Park at entrance outside infected site
2. Put on PPE
3. Set up foot dip and sprayer
4. ONLY Veterinarian enters site to discuss the depopulation plan with owner, examine culling site, verify the number for birds to be killed, their health status, the details of the owner and any additional paperwork required by the veterinary authority particularly in respect of compensation.
5. Team clean and disinfect 10 m area around entrance outside of site.
6. Team should only bring a vehicle on to the site if it is absolutely necessary and then only when instructed to do so by the veterinarian.
7. Veterinarian should verify and prepare disposal arrangements before any culling takes place.
8. The team should mark out the culling area and designate it as “High Risk” or the “RED” zone. A separate area between the red zone and the entrance to the property should be marked out for the personal decontamination area. If this is not directly at the gate, a clean corridor should be marked out between the decontamination area and the gate, where an additional foot dip and hand washing facility should be provided.
9. Team briefing on culling and disposal procedure.
10. Build protective barrier by entrance to shed or yard to prevent birds escaping and to restrict access.

<p>a) Set up outer bag(s) by inserting 4 sheets of cardboard in to the side pockets</p>		
<p>b) Insert plastic liner into the outer bag and fold over the sides.</p>		

<p>c) Add 2 kg of dry ice pellets to the bag and allow to vaporise. The addition of a litre of hot water can initially speed this up in cold weather</p>		
<p>d) Measure the concentration CO₂. It should be 70 % (or residual oxygen level of 6.3%) within 20cm of top of bag.</p>		

Culling procedure

- a. Start by placing some used litter, hay or straw in the base of the bag to cover the solid dry ice. This prevents any risk of the birds coming into contact with it and will not affect the vaporisation of the gas.
- b. Once the correct concentration of CO₂ is achieved, birds can be caught and placed in the bag at a rate of no more than 10 per minute to prevent injury and smothering.
- c. The concentration of CO₂ should be periodically checked. If it falls below 70% then additional dry ice should be added and allowed to vaporise.
- d. Maximum of 250 kg of birds to be placed in the bag.

<p>e. Once full, the top of bag should be sealed securely with string or preferably a plastic cable tie.</p>	
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f. Bottom of outer bag can be opened and inner bags will drop out to be stored safely for collection or buried in an appropriate manner.



g. New inner bag is inserted into the outer bag and procedure repeated.

11. Once all birds are culled, any eggs should be collected, destroyed and disposed of.

12. Preliminary cleansing and disinfection

- a. Spray approved disinfectant to the indicated concentration inside buildings or sheds
- b. Close and seal buildings or sheds
- c. Remove all visible contamination in immediate area by sweeping, collecting and bagging any loose material.
- d. Culling site to be sprayed with disinfectant
- e. Wash down concrete and other hard surfaces with detergent and rinse.
- f. Spray again with approved disinfectant (to be left for 24 hours)
- g. All equipment to be washed with detergent, rinsed and disinfected before removal from site.
- h. Any equipment which it is not possible to clean should either be destroyed on site or be sealed in two plastic bags and the exterior sprayed with disinfectant. These bags can be removed to a secure specialist decontamination unit.

13. Secondary cleansing and disinfection team to clear sheds and clean all remaining buildings and equipment left on farm. Procedure to be agreed with veterinary C&D officer.

14. Set pest control system