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HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) OUTBREAKS

WORKBOOK For UPAZILLA AND DISTRICT OFFICERS

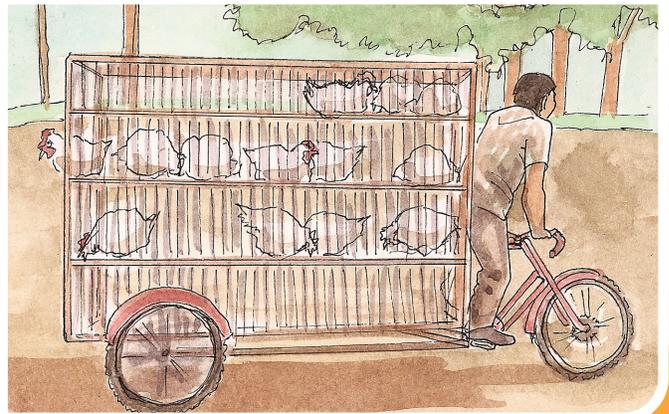
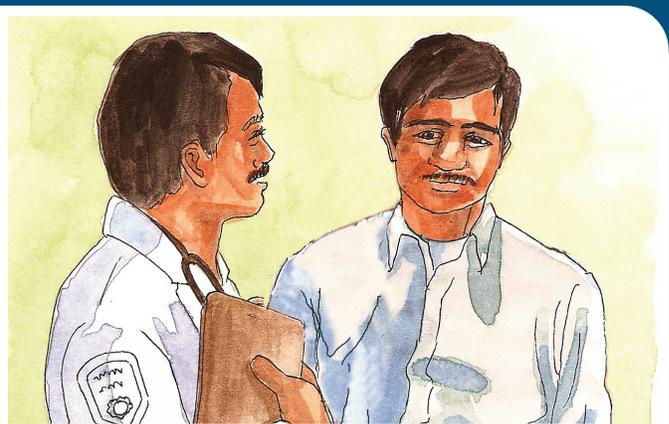
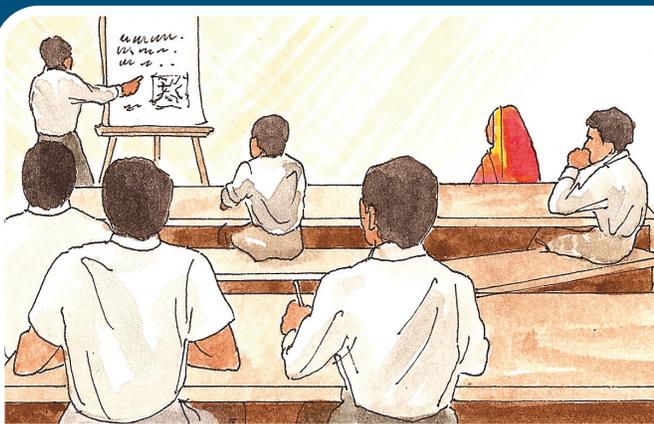


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A top banner with a light orange background featuring several stylized, sketch-like illustrations of chickens in various poses, some facing left and some right, scattered across the top. The word "FOREWORD" is printed in large, bold, black, sans-serif capital letters over the left side of this illustration.

FOREWORD



ACKNOWLEDGEMENTS



INTRODUCTION TO ADDRESSING AVIAN INFLUENZA IN BANGLADESH

This manual highlights the knowledge, skills, and tools necessary to support the development of local action against avian influenza. Utilizing and sharing this information will allow District Officers and Upazilla Officers-- including livestock officers, veterinary surgeons, veterinary field assistants, and livestock assistants—to strengthen the capacity of their communities to confront avian influenza (bird flu) and promote livestock and public health at the local, national, and international level.

Avian influenza can be prevented through the preservation and promotion of both animal and human health. Unfortunately, the interaction between poultry/birds and humans increases the risk of an avian influenza outbreak. Such an outbreak would greatly affect the well being of individuals, families, communities and countries.

There are a multitude of costs association with an avian influenza outbreak. **Figure 1**, below, displays just a few examples of the human and economic costs associated with an outbreak.

The impact of avian influenza on animal health is great. One half of all chicken flocks are on family farms. In addition, Bangladesh has a large duck population with 35 million ducks raised on family farms. Both small and commercial poultry farmers depend on the country's poultry industry to survive. Millions of Bangladeshi households also rely on poultry for income and for nutrition. Though avian influenza outbreaks have been reported in only some of the districts in Bangladesh, it is imperative that Officers at all levels do what ever they can to prevent further spread of avian influenza to other districts.



FIGURE 1: COSTS OF AN AVIAN INFLUENZA OUTBREAK

<p>Costs to the public health</p>	<ul style="list-style-type: none"> • People get sick and die, including children • Increased burden and strain on health care systems and their capacity to deliver care
<p>Costs to family-owned and small businesses</p>	<ul style="list-style-type: none"> • Family farms and poultry-related businesses are hit hard when flocks must be destroyed • Families lose an important source of income when they can't sell poultry meat or eggs
<p>Nutritional costs</p>	<ul style="list-style-type: none"> • Families lose an important source of food and protein when they can't produce or buy meat and eggs
<p>Nutritional costs</p>	<ul style="list-style-type: none"> • Families lose an important source of food and protein when they can't produce or buy meat and eggs

Many countries regions, and villages are confronting the staggering costs of avian influenza outbreaks, including Bangladesh. Fortunately, the government of Bangladesh is taking steps to strengthen it's response to prevent and control avian influenza outbreaks. To support this effort, the USAID mission in Bangladesh is working with the government of Bangladesh to conduct training programs for the District and Upazilla level officials on prevention and control.



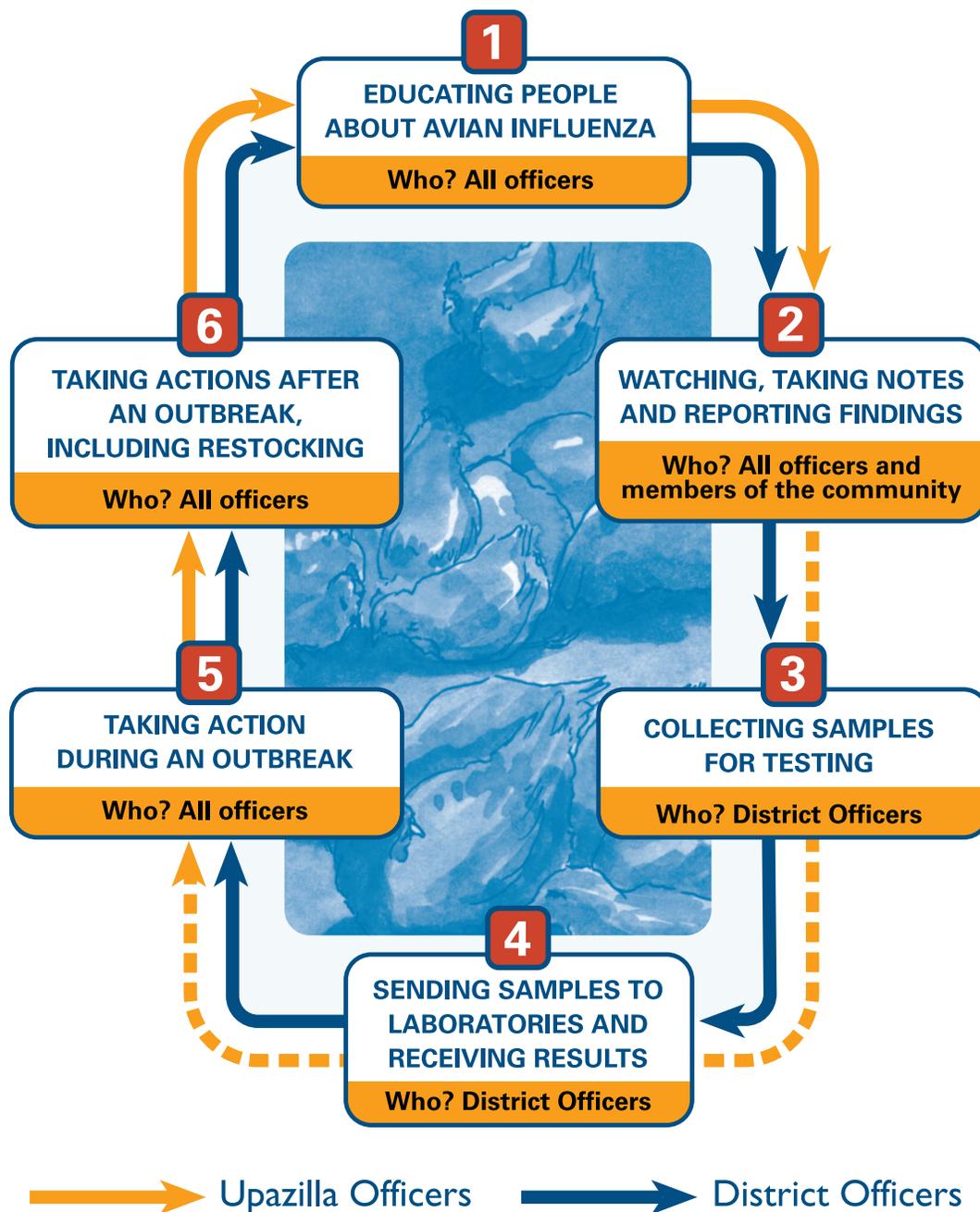
The Department of Livestock Services (DLS) initially selected six districts where outbreaks have occurred to train livestock officers and veterinary surgeons to conduct local/village level trainings on preventing and containing avian influenza outbreak. An earlier version of the workbook that you see here today was part of those first training sessions. The workbook was developed based on the *Avian Influenza Operation Manual*, which was written by the Department of Livestock Services, Ministry of Fisheries and Livestock in Bangladesh, with technical assistance by the Food and Agriculture Organization of the United Nations (FAO). In the first training sessions, participants from the 6 districts reviewed this workbook and made important comments and changes to the content based on their own experience in the field. Under this initiative a new and improved workbook and training materials, for both the District and the Upazilla level personnel, have been produced so that AI teams at any level can work to prevent, combat and contain avian influenza.

The foundation of the workbook and the training sessions are based on a Job Cycle, seen here in **Figure 2**. The job cycle helps to spell out, in simple terms, which kind of officer is responsible for which activities and exactly why those activities will help to control avian influenza.

THE JOB CYCLE

Figure 2

The Job Cycle for District and Upazilla Officers





Capacity for prevention and control at the local level is vital as local officers offer the first line of defense against any epidemic. District and Upazilla officers are in a unique and important position to educate co-workers, community members, friends, and families about how avian influenza can be prevented.

This manual offers guidelines in the form of six steps organized into a Job Cycle. This cycle includes risk reduction, data collection, bio-security, and appropriate outbreak response strategies including restocking of poultry. The six steps in the job cycle include:

1. Educating People about Avian Influenza
2. Watching, Taking Notes, and Reporting Findings
3. Collecting Samples for Testing
4. Sending Samples to Laboratories and Receiving Results
5. Taking Action during an Outbreak
6. Taking Actions after an Outbreak, Including Restocking

The Job Cycle illustrates that the six steps flow, one to the other. Everyone has their own roles and responsibilities to play in preventing and controlling avian influenza (bird flu). The work of District, and Upazilla Officers before, during, and after an avian influenza outbreak is critical to the health and economy of Bangladesh. The next 6 chapters of this workbook describe each job cycle in more detail.

REVIEW OF HOW AVIAN INFLUENZA IS SPREAD

In order to understand how avian influenza can be prevented, it's important to review the most common ways that it can be spread. Avian influenza can be spread by both animals and humans. Avian influenza can make animals and humans sick.

HOW PEOPLE CAN BECOME INFECTED WITH AVIAN INFLUENZA:

- Cleaning a yard where there is poultry feces or litter without covering your nose or mouth. If AI exists in your country it can be found in poultry feces and poultry litter. The virus can get picked up by dust through sweeping or cleaning. If you breathe in the dust that has the virus in it, you can get very sick.



- Culling chickens or other poultry without covering your nose or mouth. The virus can get picked up by dust when catching and culling chickens. If you breathe in the dust that has the virus in it, you can get very sick.



- Eating unhealthy poultry, like chicken or ducks. Do not kill, cook and eat sick poultry. Do not cook or eat poultry that has died suddenly.
- Undercooking poultry meat, eggs or poultry blood before eating them.
- Eating poultry meat that is undercooked and pink in the middle.
- Eating eggs that are runny and undercooked.

This workbook and training materials will cover the many ways that you can protect yourself if you are cleaning a poultry yard or catching and culling poultry. It also covers the best methods for cooking poultry and eggs.

HERE'S HOW ANIMALS GET SICK:

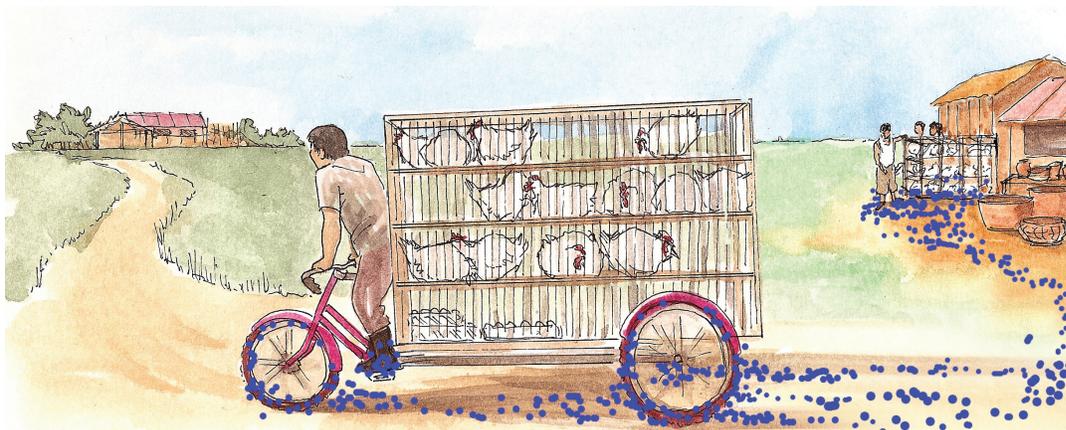
- Purchasing, trading or carrying any kind of poultry, chicks or eggs, from the family farm to another farm or to the market. If your poultry or eggs are infected with AI taking them to another farm or market can infect poultry or eggs there.



The avian influenza virus, though invisible, can be carried on tires and shoes.



- Purchasing, trading or carrying any kind of poultry, chicks or eggs from another farm or from the market back to your family farm. If the poultry or eggs that you buy, trade or carry are infected with AI bringing them back to your family farm may infect the poultry/eggs already on your farm.



The avian influenza virus, though invisible, can be carried on tires and shoes.

- Purchasing wet litter from the market or a farm and then transporting it to another market or farm. If the litter happens to be from infected birds, then taking the infected litter to another market or farm can infect poultry or eggs there.
- Allowing your poultry to get too close to wild birds or the water where they drink. Local and migrating water birds that have the virus can then carry it when they fly from watering place to watering place.

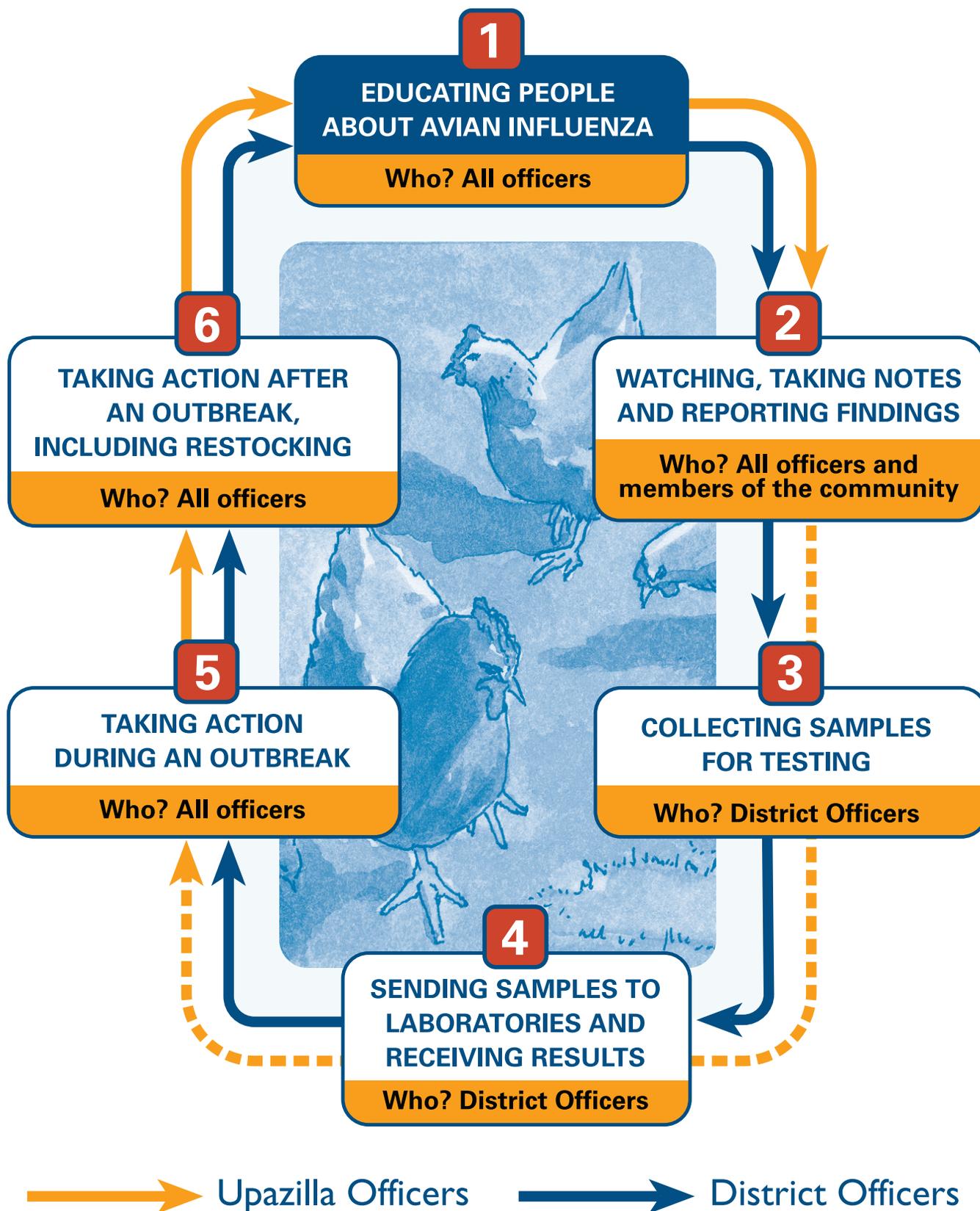




- Humans can spread the avian influenza virus on their shoes, tools, rickshaw vans, bicycles, autos or other vehicles. Bangladesh has many people and poultry living in the same areas. This makes it easy for humans to spread the virus not only on their shoes and tires, but also on used handkerchiefs, used tissue paper or other things that have touched the feces, blood, mucous, saliva or other parts of an infected bird.



The Job Cycle for District and Upazilla Officers



JOB CYCLE-STEP 1

EDUCATING PEOPLE TO PREVENT AVIAN INFLUENZA

Now that we know more about how AI is spread, let's take a look at Step 1 in the Job Cycle. You'll see in the diagram that ALL officers are responsible for carrying out Step 1 in the job cycle. That includes District officers, Upazilla officers and livestock officers, veterinary surgeons, veterinary field assistant, and livestock assistants. Step 1 includes ***educating people to prevent avian influenza***.

So who should the District and Upazilla officer reach out to in order to educate them about preventing AI? Every one they can! Including farmers, coworkers, family, friends, teachers and professors, members of union parisad and NGO workers.

Step 1 doesn't happen one time. It involves promoting behaviors that make prevention an ***ongoing task***. Educating people to prevent avian influenza involves promoting specific behaviors in specific settings. The list below identifies those settings.

1. Promoting ***farmyard*** behaviors that help prevent and control avian influenza for persons involved with raising poultry/eggs.
2. Promoting selling/trading or ***market*** behaviors that help prevent and control avian influenza for persons involved with selling/trading poultry/eggs.
3. Promoting ***household*** behaviors that help prevent and control avian influenza for persons preparing chicken/eggs for human consumption.
4. Promoting bio-security measures on the ***farm***, and in the ***market***.
5. Promoting food safety and good hygiene in the home.

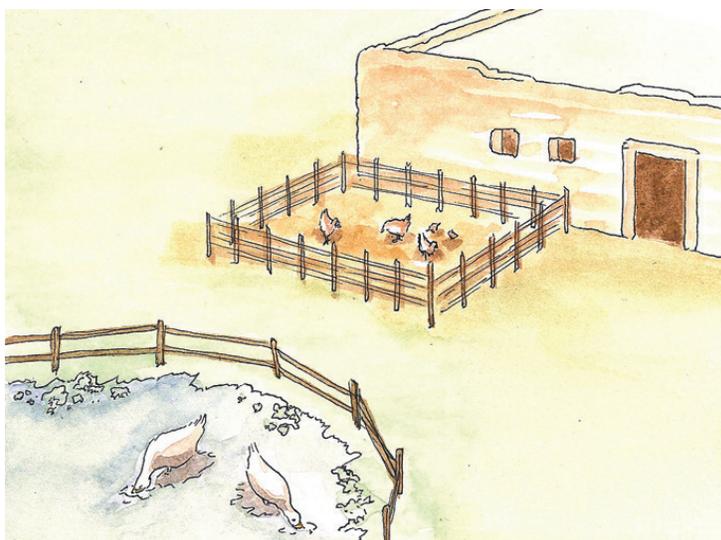
The specific behaviors that Upazilla officers should be promoting and encouraging are described below.

1. **Promote good farmyard behaviors** that help prevent and control avian influenza for persons involved with raising poultry/eggs including:

- Wearing a cloth or mask over the nose and mouth to avoid breathing in the virus from infected bird droppings. If you do not have a mask, tie a cloth (handkerchief) over your nose and mouth when cleaning the farm yard.



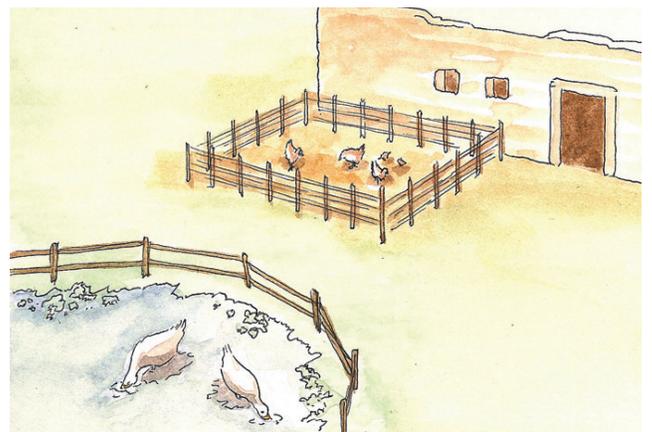
- Keeping all poultry in a fenced area or separate building so poultry does not roam free.



- Giving poultry fresh feed and clean water every day.
- Cleaning the farmyard every day. Remember to wear a mask or cloth over your nose and mouth when cleaning the farmyard.



- Keeping chickens separate from ducks. Ducks can be infected with avian influenza and spread disease, but not show any signs of being sick.



Chickens and ducks often live together but it is better to keep them in separate pens.

- Keeping poultry and ducks separate from pigs because pigs can also spread diseases or get sick from being around infected poultry.
- Keeping new birds in a fenced area or cage, away from the rest of your flock for at least 14 days.
- Keeping poultry separate for at least 14 days if you have taken your birds to the market but did not sell them. This quarantine would be helpful in preventing spread to your flock in case the returning birds came in contact with the virus at the market.
- Discourage anyone who feeds poultry waste like organs or blood to dogs, cats, scavenging birds, pigs etc.
- Discourage farmers to keep their poultry away from waste like feathers, organs, or blood.
- Burning or burying poultry waste such as feathers, organs and blood. Be sure to bury the waste deep enough (and with slaked lime) so that pigs, foxes, dogs, or cats do not dig it up.



- Not throwing poultry waste or droppings into water.



Never throw dead chickens into bodies of water.

- Discourage the use of fresh poultry litter as food for fish.
- Discourage the use of bird droppings as fertilizer unless you have composted it first. Composting should be done in an enclosed area like a shed. Make sure that birds, cats, dogs, foxes, pigs or other animals cannot get to the compost heap.
- Keep poultry out of your house.
- Keep children away from the poultry drinker, the feeding trough or feeding pan.

- Keep children from playing with live or dead poultry or birds.



Never let children play with chickens.

- Keeping poultry away from other animals, especially wild and migrating birds.
- Keeping poultry away from any source of water that could have been infected by wild & migrating birds—including rivers, ponds, standing puddles.

2. Promote good *trading/selling and market behaviors* that help prevent and control avian influenza by

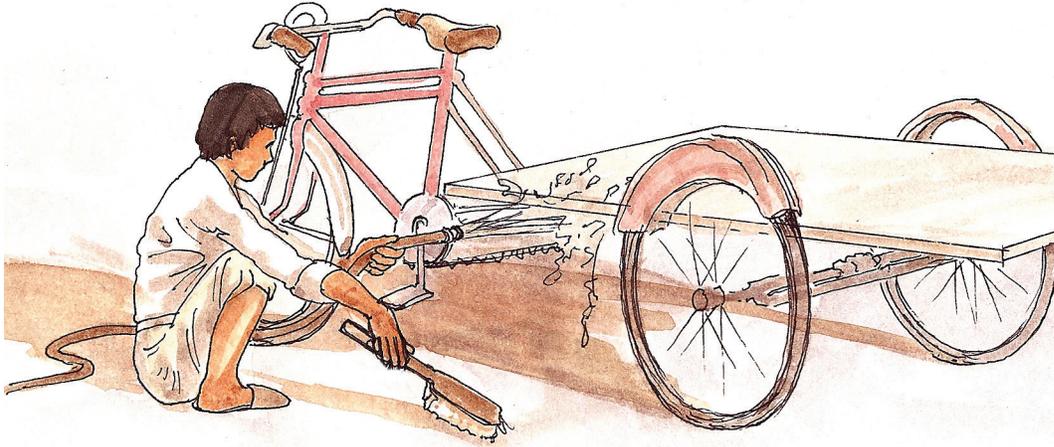
- Making sure that anybody who enters or exits the farmyard, washes their shoes, especially the bottom of the shoes, with soapy water (or disinfectant if you have it). If washing is not possible give visitors clean shoes at the farm gate.
- Making sure that everyone brushes off and washes with soap and water *anything that comes to their farmyard* that may

have touched poultry feces from another place—such as another farm or live-bird market. This is so no one accidentally brings the virus to their home or someone else’s home on vehicle tires, clothing, shoes, tools, and cages. Encourage the use of disinfectant if farmers have it. Remember, people can carry the virus that causes avian flu on their shoes, vehicle tires, egg crates, broiler carrying nests, poultry cages or on farm equipment. This is how the virus can spread from one farm to another or from one district to another.



Washing boots with soap and water.

- Keeping farm equipment and tools, vehicles, and bicycles clean by washing them with soap and water or disinfectant everyday.



3. **Promote good *household behaviors*** that help prevent and control avian influenza with persons in the house who prepare poultry and eggs by:

- Cleaning kitchen surfaces and other areas that come into contact with poultry with soap and water, or disinfectant if you have it.
- Eating only healthy poultry. Do not kill, cook and eat sick poultry.
- Do not cook or eat poultry that has died suddenly.
- Poultry meat, eggs or poultry blood should be cooked well before eating them (meat should not be pink in the middle, and eggs should not be runny or liquid like).

- Washing hands with soap and water before and after touching poultry or any eggs.



- Using gloves if possible, while plucking (defeathering) and cutting chicken into pieces for cooking.

4. Promoting bio-security measures

The Department of Livestock Services (DLS) will promote and enforce the following bio-security measures in poultry rearing with the support of local government and other stake holders.

- All commercial poultry holdings must have a documentation or recording of movement to and from the facility. The documentation will cover all live poultry, poultry products and by-products, feeds, farm personnel, visitors, transport and supplies and equipments.
- Poultry holding facilities are defined as commercial farms, hatcheries, breeding farms (parent stock or grand parent stock), processing plants, slaughterhouses, aviaries, and feed mills.
- Transportation/movement of commercial day-old chicks (DOC) will not be allowed without proper chalan/invoice.

- Strict implementation of ‘rest’ period (the time interval from the first disinfection to the loading of the next batch of poultry) and of the proper disinfection procedures of farmhouses and other structures in the farm will be enforced and monitored by local veterinary officials.
- The recommended minimum number of days of rest period (during non-outbreak period) shall be:

Poultry in an all-in-all-out set-up	14 days
Adult House	30 days
Caged poultry	14 days
Game fowls: growing houses	14 days

 These periods are at least 3 months (90 days) during active outbreak in Bangladesh.
- Bio-security control points (e.g. gates, shower rooms, footbaths, fumigation boxes) will be ensured as and where practical. At least a footbath, hand and feet washing facilities, changing room and protective clothing will be made mandatory for large and medium size farms. Small farmers will keep a container of water and soap for cleaning hands and feet at the entry and/or exit points of farm or poultry house.
- Poultry houses, feed storage and egg room must be inaccessible to stray animals, rodents, flies and free flying birds.
- All dead birds must be disposed off properly in a consolidated pit within farm premises or own backyard through burial, composting or incineration in accordance with facilities and capabilities available.
- Roaming of domestic ducks in wetlands will be restricted during the visit of migratory birds or there should be alternative method of preventing mixing domestic ducks and wild birds.
- Chickens, ducks and swine will be kept separately.
- Poultry litter/ droppings/ waste products must be disposed of properly.

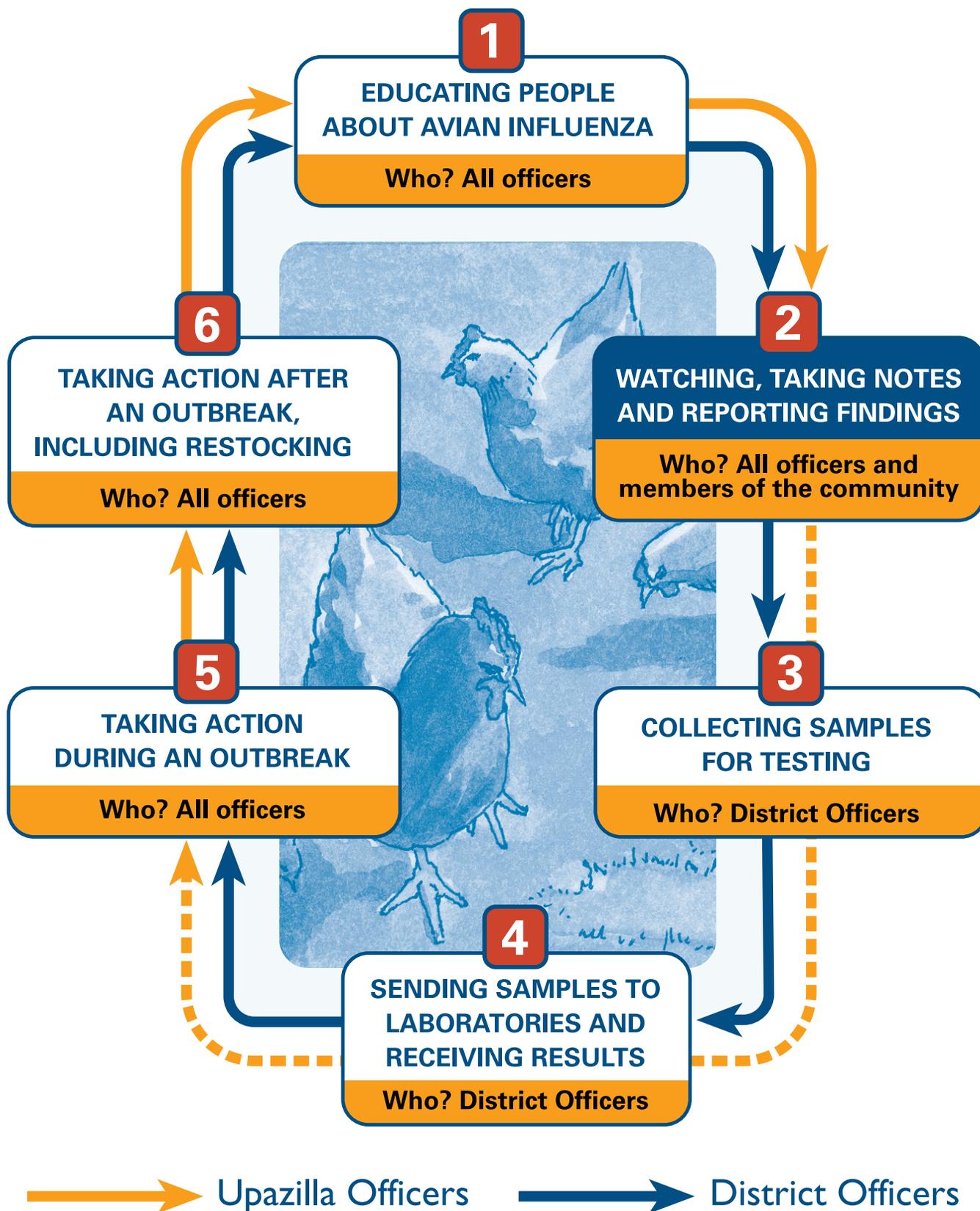
- Slat system of housing may be encouraged in backyard poultry rearing.
- Prevent mixing of different ages of poultry in the same house or shed.

As mentioned at the beginning of this discussion, prevention is an ongoing job. So is *Step 2 - Watching, Making Notes, and Reporting*. It is important that District and Upazilla officers tell people how to report any illness or death of chickens. Reports of sick or dead chickens would lead District and Upazilla officers into *Step 2 - Watching, Making Notes and Reporting*.

CHECKLIST REVIEW

- ✓ What do you know about bird flu?
- ✓ What do you know about how it is spread?
- ✓ What do you know about the threat of bird flu?
- ✓ What are some of the things you can do to prevent and control bird flu?
- ✓ What are some of the things that people in your community can do to prevent the spread of avian influenza?
- ✓ What specific information can you share with farmers? With coworkers? With family and friends and students teachers of schools, colleges and universities as well as chairman, members of union parisad and NGO workers?

The Job Cycle for District and Upazilla Officers



JOB CYCLE-STEP 2

WATCHING, TAKING NOTES AND REPORTING FINDINGS

Step 2 of the Job Cycle is about asking local people to watch for signs of avian influenza in poultry and wild birds, make notes about what is seen and then reporting findings to the appropriate officials—usually the Upazilla officer and his supervisor.

1. As an Upazilla officer you can ask your entire village to be part of the “AI Operation Team.” Ask **everyone** to observe or watch what is going on with the local poultry and wild birds. Enlist help from the following: farmers, farm workers, neighbors, villagers, bird watchers, fishermen, hunters, union council officials and members, government workers and NGO workers should all watch for signs of avian influenza.

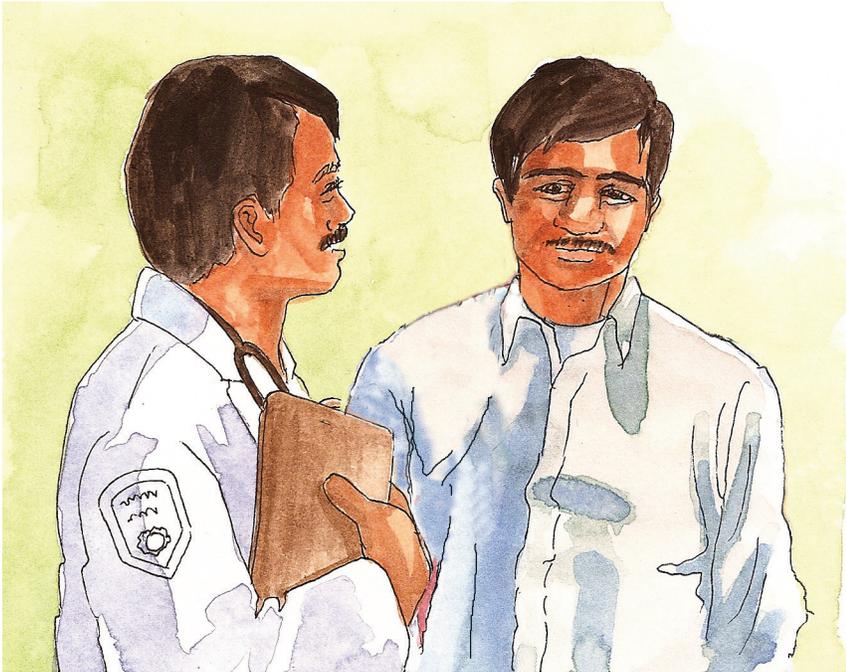
It is especially important that the Upazilla officers also enlist help from everyone involved with the local poultry industry including—feed, egg, and chick sellers/distributors; and broiler and layer sellers/distributors to watch for signs of an avian influenza outbreak.

2. Let the entire village know that they should contact you (the Upazilla Officer) immediately by telephone or in person if anyone observes poultry becoming sick or suddenly dying.

3. Request all private practitioners, company veterinarians, private or autonomous institutional laboratories, veterinary representatives and hatchery representatives to contact the Upazilla Livestock Office in case of suspected AI findings.

4. Ask the Department of Forest, any NGOs working with management or conservation of wetland water fowl and the Local Government to report any unusual wild bird mortality to the Upazilla Livestock Office.

5. Verify what has been reported to you and meet with the person (farmer, neighbor or villager, etc.) who has contacted you.



6. The Upazilla Officer (including veterinarian surgeons), should submit an investigative report to his supervisor. In case of strong AI suspicion, the report should be transmitted immediately to the Department of Livestock Office (DLO). The form is found here in Annex 1.

SO WHAT ARE WE WATCHING FOR?

Knowing when birds have avian influenza can be difficult. This is because a flock can sometimes die quickly with no visible signs of illness. Ducks and other water fowl—like geese—can be infected and infect other poultry, but not show any signs of being sick. Ducks, if they do die, may not die as quickly as chickens. That is why it is important for households and farmers to keep ducks and ducklings fenced or caged separately from chickens and chicks.

Though spotting signs of avian influenza may be difficult, there are some common signs to look for that might mean a single bird or an entire flock has been infected. Everyone should be on the lookout for:

- Wattles and combs that are cyanotic and oedematous. In other words—wattles and combs that are purple or swollen.
- Poultry with bowed heads, the birds grouping together, or appearing to be in a semi-comatose state.



Birds grouped together, with bowed heads is a sign that something is wrong.

- Breathing problems.
- Swelling in the head or eye lids.
- Bleeding on skin in areas where there are no feathers, especially on the feet.
- Poultry does not have energy or does not want to eat.
- Decrease in the number of eggs laid.
- Profuse, watery diarrhea.
- Coughing, sneezing, nasal discharge.
- Depression.
- Lack of coordination.

- Neurological signs like staggering gait, torticollis, and ataxia (mostly in broiler chickens).
- Poultry suddenly dies in large numbers with or without signs of illness.
- In post mortem examination, small spots or pinpoint hemorrhages are present under the skin.

Report what you see immediately to the Upazilla Officer. As the Upazilla Officer, you should ask everyone to report any of these signs to you or the nearest Upazilla Livestock Office, veterinary surgeon or veterinary field staff or veterinary hospital as soon as possible. Reporting can be done in person or by the telephone. Make sure that people in the village know how to contact you!

ONCE THE UPAZILLA OFFICER HAS BEEN CONTACTED WHAT SHOULD WE DO?

Farmers, neighbors and villagers should know what to do well in advance while they are waiting for the Upazilla Officer to arrive on the scene. The information below can be found here in **Annex 2**. As the Upazilla Officer you may want to make and distribute copies of it. Share the information below with farmers, neighbors and villagers well in advance, so that they know what to do and what NOT to do while they are waiting for the Upazilla Officer to arrive:

- Do not dispose of dead poultry. The Upazilla Officer or Veterinarian Surgeon will want to see it.
- Children should not be allowed to visit the farm where birds are sick or have died.
- Do not sell the sick or dead birds.
- Do not prepare the sick or dead birds to eat.
- Do not sell or eat eggs from the sick or dead birds.
- Protect the rest of your flock by taking the sick or dead birds out of the flock.

- When removing the sick or dead bird from the rest of the flock, make sure you cover your hands with gloves or a newspaper. The important thing here is to put a barrier between the dead birds and your hands. Cover your mouth and nose with a mask (N-95 if possible) or a cloth.



- If possible, do not pick up sick or dead birds with your bare hands. Use a shovel to pick up the sick or dead birds and take them to an area away from the rest of the flock. Then, place the dead bird(s) in a bag or other container away from other farm animals. Do not leave the dead birds in the farm yard or in an open field. They must be contained in a bag or other closed container. Do not dispose of the bag. The Upazilla Livestock Officer or Veterinarian Surgeon will want to study the contents.

- Do not throw dead birds or animals into a river, pond or any other body of water.



Never throw dead chickens into bodies of water.

- Clean the shovel and other tools used to move sick or dead birds with soap and water or disinfectant. Wash anything that came into contact with the sick or dead birds.
- Wash your hands with soap and water after removing the sick or dead birds from your flock.



- Wash off your shoes, especially the bottoms of shoes.
- If at all possible, restrict the contact of native and wild birds to the suspected farms. This may only be possible if farm poultry can be kept away in a shed or chicken coop.

WHO ELSE SHOULD THE UPAZILLA OFFICER TALK TO?

In addition to requesting help from local farmers, neighbors and friends the Upazilla Office and officer should also request all private practitioners, company veterinarians, private or autonomous institutional laboratories, veterinary representatives and hatchery representatives that they contact you in case of suspected AI findings.

- All private practitioners, company veterinarians, private or autonomous institutional laboratories, veterinary representatives and hatchery representatives shall be advised to report any suspected case of AI encountered during their diagnostic work to the nearest government Upazilla Livestock Officer or Veterinarian Surgeon for official investigation.
- Ask the Department of Forest, NGOs working with management or conservation of wetland water fowl and the Local Government to report any unusual wild bird mortality to the Upazilla Livestock Office or Veterinarian Officer for official investigation.

WHAT SHOULD YOU, THE UPAZILLA OFFICER, DO WHEN YOU RECEIVE A REPORT THAT CHICKENS ARE SICK OR HAVE DIED?

Your first task is to verify what has been reported to you and to contact your supervisor. Meet with the farmer, neighbor or villager who has contacted you. Work with the Upazilla Livestock Office or Veterinarian Surgeon to investigate the outbreak in person within 6 hours of receiving the report to:

- Verify the source of the information.
- Collect a copy of the vaccine schedule from the suspected farm.
- Determine if the poultry has been vaccinated with New Castle Disease vaccine. What other diseases has the poultry been vaccinated against? At what age? What are the dates of those vaccinations?
- Verify the kinds of drugs used on the farm.

Upazilla officers should prepare farmers, neighbors or anyone else making a report about avian influenza that they should report specific types of information. The specific information that needs to be collected is found in **Annex 1: The Government of the People’s Republic of Bangladesh, Department of Livestock Services, Disease Investigation Form (Poultry)**. The kind of information that should be gathered on the form includes:

- The *kinds of birds* that became sick or died. For example, chickens, ducks, quails, wild birds, etc.
- Flock size
- Age of flock.
- The *number of birds* that are sick or have died.
- The *signs of sickness* that made you think the birds were sick. For example, bruised combs, bruised feet, coughing or sneezing.
- Vaccination history.
- Any wild birds that you noticed in the area—for example, swans, geese, wild ducks. Did the wild birds look sick? Were they acting strange?

After taking information from farmers, neighbors or anyone else making a report about avian influenza, it is important for Upazilla officers to do the following:

- Report findings to supervisor and contact other officers or agencies that should know such as the Department of Livestock.
- Watch the other poultry in the area to make sure they are not showing signs of avian influenza.
- Advise other farmers to improve their bio-security and discourage them from visiting the suspected farm.

Once the Upazilla Livestock Officer or Veterinarian Surgeon has verified that birds are sick or dying, it is important for the District Officer to collect samples of the sick or dead birds as soon as possible. This is so that they can be tested in a laboratory to confirm that highly pathogenic avian influenza (hpaï) is causing the illness. Information for District Officers on collecting and shipping samples is found in the next two sections, *Step 3-Collecting Samples for Testing and Step 4—Sending Samples to the Laboratory and Receiving Results*. Though the Upazilla Officer may not be directly involved in sampling and shipping, he still has a very important job to do at the village level.

The last two steps in the Job Cycle, Steps 5 and 6 explains what **all** officers should do during and after an outbreak—including restocking poultry.

CHECKLIST REVIEW FOR THE UPAZILLA OFFICER

- ✓ What do sick birds look like?
- ✓ What type of information should you collect from farmers, neighbors or anyone else making a report about AI?
- ✓ What should you do when you receive a report of a possible outbreak of AI?
- ✓ What type of information should you give to farmers so they know what to do while they are waiting for you to arrive?
- ✓ What should you do when you receive a report that some chickens have died?
- ✓ Do you have copies of the Disease Investigation Form found here, in the Appendices? Do you know how to complete this form? Do you know where to send this form after it is completed?

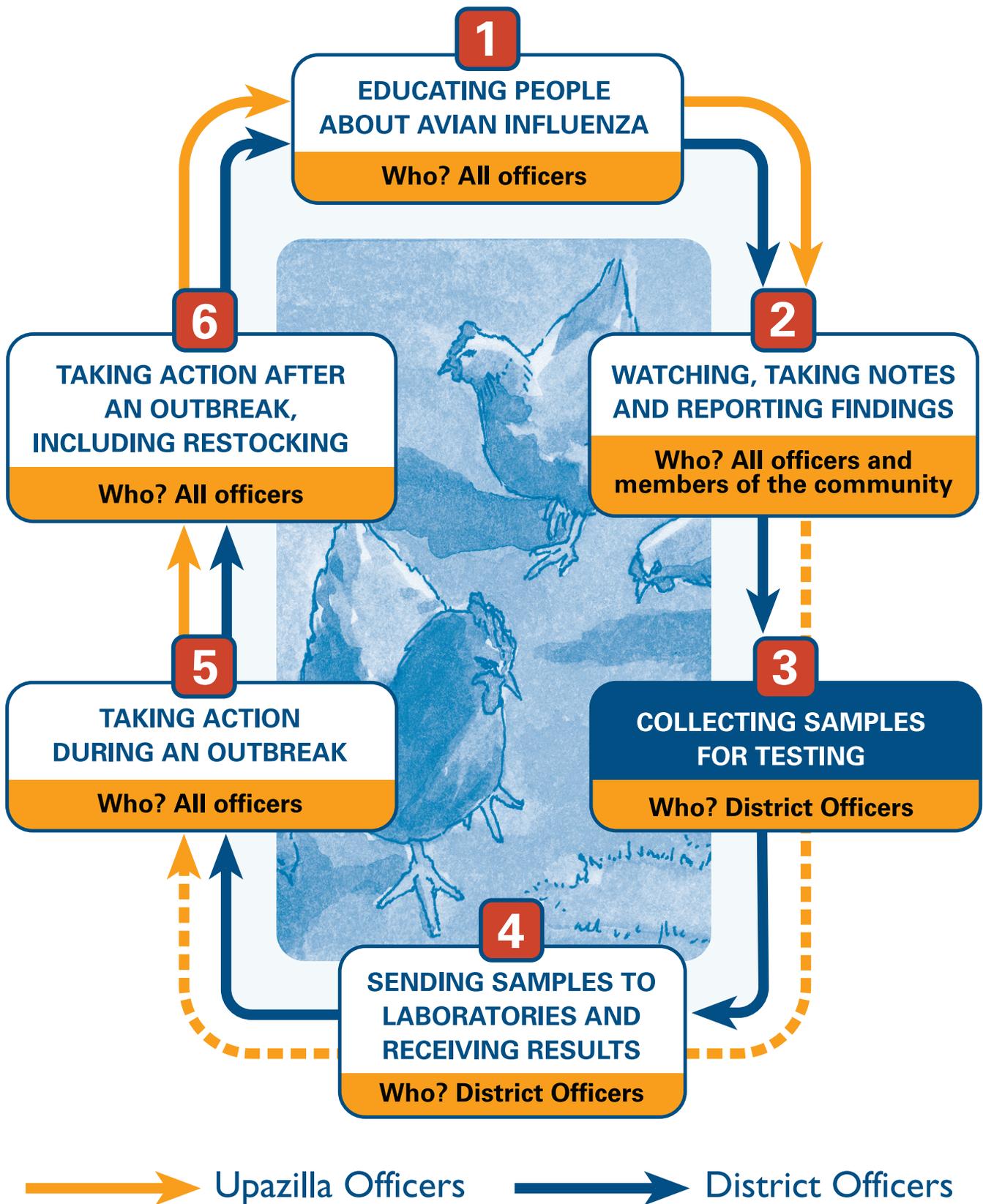
ANNEX I: DISEASE INVESTIGATION FORM

**Government of the People's Republic of Bangladesh
Department of Livestock Services**

Disease Investigation Form

Reference No.					Date:			
Name of the Farm:								
Name of the Farm Owner:								
Address:								
Contact telephone number:								
Type of Farm: (Tick)		GP Stock	Parent stock	Commercial layer	Broiler	Backyard		
Number of sheds in the farm:					Number of sheds affected:			
Type of shed: (Tick)		Environmentally controlled			Open sided			
Information about affected flock(s)								
Shed #	Species	Type of Birds	Breed	Source of Chicks (Hatchery)	Flock Size	Age (Wk's)	Litter/Cage/scavenging system	Date of onset of outbreak
Number of birds affected and died (date wise):								
Date	Shed #		Number affected			Number died		
Vaccination history of the affected flock:								
Vaccinated against		Age at vaccination/Date of vaccination				Name of vaccine		

The Job Cycle for District and Upazilla Officers



JOB CYCLE-STEP 3

COLLECTING SAMPLES FOR TESTING

THE IMPORTANCE OF SAMPLING

The only way to confirm that there is an avian influenza outbreak in your area is to properly collect samples and to make sure that the samples get sent to the correct location for testing. **Step 3: Collecting Samples for Testing** is a very important step in preventing and controlling the avian influenza virus in Bangladesh and around the world. **Step 3 is performed by District Officers and involves the following activities:**

1. **Using personal protective equipment (PPE)** such as respiratory masks, gloves, coveralls, etc. when collecting samples.



2. **Collecting samples** for testing once a confirmed report has been received.

3. **Collecting tracheal and cloacal swabs** from at least 5 sick or dead birds from each outbreak.
4. **Completing** the Disease Investigation Form and the Sample Submission Form, both found here in Annex 2 and Annex 3 of the Workbook.

PREPARING TO COLLECT SAMPLES FOR TESTING

How can you prepare for collecting virus samples from sick or dead birds?

- The types of Officers who can collect samples include District Officers, Veterinary Surgeons or Livestock Extension Workers (veterinary field assistants). Their experience and training will make it easier for them to take appropriate samples from sick or dead birds.
- The Officers needs to remember that they should only *use the rapid tests on sick or dead birds*. (See Step 2 of the Job Cycle in this manual for a list of avian influenza signs in birds).
- Officers should be prepared to use personal protective equipment (PPE) especially respiratory masks, like the N-95 respirator. It covers the nose and mouth so that dust containing the avian influenza virus does not get into the lungs. (See Annex 4 here in the Workbook for a complete list of PPE equipment.)



USING PERSONAL PROTECTIVE EQUIPMENT (PPE) WHEN COLLECTING SAMPLES

Note: Please see the handout on page 47 for more information.

1. The first step in preparing to put on PPE is to inspect your kit to be certain that it is complete. Each kit should contain:



1 N-95 face mask respirator—should be brand new. This is the most important piece of PPE because it covers your mouth and nose so that you don't breathe in dust that contains the avian influenza virus. Do not use the N-95 if it looks used or worn in any way.



1 red plastic disposal bag marked with the universal bio-hazard symbol



1 plastic apron



1 pair of white vinyl gloves



1 pair of blue nitrile gloves



1 pair of boot covers



1 set of white plastic coveralls (gown with hood)



1 set goggles



4 Alcohol wipes for hands



2 germicides wipes for surfaces



You may also want to add a roll of duct tape to your PPE kit.

2. Before putting on PPE, make sure your hands are clean. Proper hand hygiene means washing hands for 30 seconds with soap and water. If no water is available, the alcohol-based packet of hand sanitizer supplied in the kit is an effective alternative for cleaning hands.
3. Put on a primary layer of clothing (next to the body) including undergarments, socks, medical scrubs, suits or coveralls followed by rubber boots which can be easily cleaned. The pant legs of the primary clothing must go inside the boots.

Once you have inspected your PPE, washed your hands and put on a primary layer of personal clothing it's time to put on the PPE. The step by step method for putting on PPE is as follows:

1. Put on the first set of white vinyl gloves.
2. Put on the white coveralls from the kit but leave the hood down for now. Pull up the front zipper and put the legs on the outside of your rubber boots.
3. Put on the boot covers and wrap duct tape at the ankles. If the boot covers become torn, bio-security may not be breached if the rubber boots underneath are leak proof and easily disinfected.
4. Follow these 7 steps to put on the single-use N-95 face mask respirator:
 - If you wear glasses, temporarily remove them.
 - Pre-stretch top and bottom straps.
 - Cup the respirator in your hand with the nose piece at your fingertips and allow the headbands to hang freely down below your hand.
 - Put the respirator under your chin with the nose piece up. Pull the top strap over your head allowing the strap to rest high on the top back part of your head. Pull the bottom strap over your head and position it around your neck below yours ears.
 - Place your fingertips from both hands at the top of the metal nosepiece. Use two hands and mold the nosepiece area to the shape of your nose by pushing inward while moving your fingertips down both sides of the nose piece. Do not pinch the nosepiece using one hand as this will result in an improper fit.
 - Perform a user seal check before you begin work. To check the seal on the face, place both hands completely over the respirator and exhale. Be careful not to move the respirator. If air leaks around the nosepiece re-adjust by repeating the above step. If air leaks at the respirator edges work the straps back along the sides of your head to tighten them.

- If you can not get a proper seal, don not enter the contaminated area. Ask for help.
5. Next, you'll put on your goggles covering your eyes (and glasses). Use both hands to pull the strap over the head and adjust the strap for a tight but not uncomfortable fit. [If necessary, clean with mild soap and warm water, rinse clean then remove excess water with a soft cloth. Do not use ammonia or alkaline cleaners, solvents or abrasives.]
 6. Then pull the coverall hood over your head. The elastic should hold it in place.
 7. Pull on the nitrile (blue) gloves being sure there is no exposed skin. Use duct tape to seal sleeves if necessary then tie on the white vinyl apron.

Now you are ready to enter the contaminated site. For safety reasons it is best to work with a partner. If you feel ill, cannot breathe easily, or have damaged gloves or respirator mask, signal to your partner that you are leaving the site and then do so immediately.

Remember when you exit a contaminated work place you must go directly to a designated "dirty" site. It is recommended that there be a portable sprayer along with a disinfectant such as Virkon available at this site.

TAKING OFF PPE

1. Remove the apron, boot covers, blue nitrile gloves (only),
2. Push the hood back
3. Remove goggles, glasses and N95 respirator.
4. For removal of the respirator pull the bottom strap over your head and keeping the respirator in place- pull the top strap over your head and remove. Replace your glasses with your right hand. The N95 respirator is not reusable and must be discarded and replaced.
5. Remove disposable white coveralls and boots covers.
6. Use packets of germicide (supplied) to clean rubber boots if no other disinfectant is available.

7. Remove latex (white) gloves, repeat hand washing steps as above, then change from your primary clothing into clean street clothing.
8. Then, place all contaminated equipment into the red bio-hazard bag provided in the kit and seal it tightly.

Note: If you have been culling, you run the risk of being contaminated and so must take a shower and shampoo before putting on clean street clothing and leaving the site. Handouts on and taking off PPE is found at the end of this chapter and in Annex 4

COLLECTING SAMPLES FOR TESTING ONCE A CONFIRMED REPORT HAS BEEN RECEIVED

HOW TO COLLECT SAMPLES AND PERFORM RAPID DIAGNOSTIC TESTS ON BIRDS

District Officers are responsible for performing diagnostic tests. In order to protect yourself, it is important to wear the Personal Protective Equipment (PPE) in the Laboratory Kit before collecting the samples from sick or dead birds.

THE RAPID TEST

The rapid test is called a rapid AI detection test because you get results in 15 minutes. Other things to know about the test are:

- It should be performed by the District Officer.
- It is easy to learn to use.
- It can be used in the laboratory and on the farm.
- It can detect even small amounts of virus.
- It detects all subtypes of Type A influenza virus, which includes the type of influenza that has caused all of the recent avian influenza outbreaks.

- It can be used to test all types of birds, including chickens, turkeys, ducks or wild birds.

The name of the test is Flu Detect Test and was manufactured by Synbiotics Corporation. It has been used around the world, including by the USAID (which supplies the PPE kits discussed here), and by the United Nations Food and Agriculture Organization (FAO) in infected and high-risk countries, including Bangladesh.

The Flu Detect Kit was designed to test tracheal samples, but can be used with both tracheal and cloacal samples. The first item you will need from your test kit is the swab that you will use to take a sample from the bird.



Flu Detect Test Kit for Influenza A Diagnosis (for animals)



COLLECTING TRACHEAL AND CLOACAL SWABS FROM AT LEAST 5 SICK OR DEAD BIRDS FROM EACH OUTBREAK

FLU DETECT TEST - STEPS TO TESTING A TRACHEAL SAMPLE

1. To take a sample from a live bird, you insert the swab into the trachea and gently swab the wall of the trachea.



2. The USAID Laboratory Kit should have something called “transport media” included. This is a liquid in a tube that keeps the sample clean and safe while it is being sent to the laboratory for further testing. After you swab the trachea of a bird, you place the swab into the transport media that is provided.

3. If the kit does not include transport media, you can make your own using alternative media such as isotonic phosphate buffer saline (PBS), PH 7.0-7.4 containing antibiotics such as 100 g/ml gentamycin sulfate, 2 g/ml amphotericin B or using guidelines that have been published by the OIE (the World Animal Health Organization or see GOB/DLS/FAO Manual, Annex-3, page-26).

These guidelines can also be found on the Internet at:

<http://www.who.int/csr/resources/publications/surveillance/Annex8.pdf>

Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection: Guide for field operations, October 2006

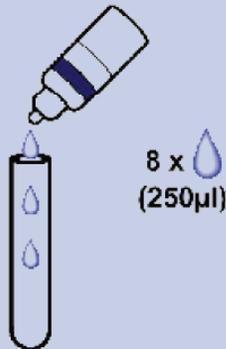
The swab is then placed in transport medium. If you are going to test one tracheal sample *on site*, before packaging the rest of the *samples for shipping, then follow this process:*

1. All samples should be at normal “room temperature” before running the test.
2. Place 8 drops (approximately 250 µl) of Extraction Buffer in a clean test tube.
3. Place the swab containing the sample in the tube and rotate the swab 5 to 10 times in the Extraction Buffer.
4. When removing the swab from the tube, press the swab against the side of the tube repeatedly until no more liquid comes from the swab.
5. Discard the swab in a biohazard container (such as the red bio hazard bag that comes in the Laboratory Kit).
6. If the samples will not be tested immediately, cap the tube with the provided cap and store the sample in the cooler provided in your kit.)
7. Insert the test strip into the tube up-to the mark.
8. Allow the test strip to sit inside the tube for 15 minutes.
9. After 15 minutes, remove the test strip from the tube and read the test strip results.

Using the Flu Detect Test Strip (for animals)

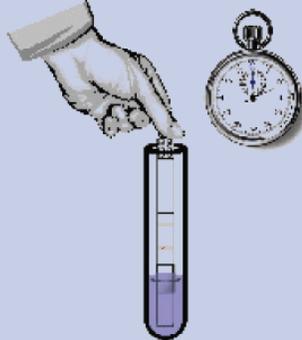
Step 1

8 drops (~250µl) extraction buffer into test tube



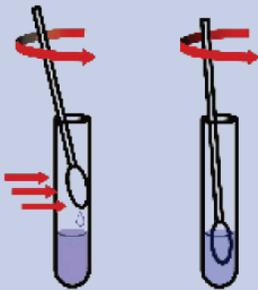
Step 3

Insert test strip, labeled side up, so that pink pad is just submerged into extracted sample. Incubate for 15 minutes. Remove strip and read results.



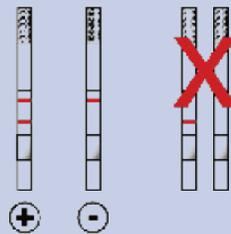
Step 2

Insert swab, rotate. Press swab against side of tube to extract liquid. Dispose of swab.



Step 4

Reading results and validation: Control line at top, closest to handle. Absence of control line indicates invalid test.



FLU DETECT TEST - STEPS TO TESTING A CLOACAL SAMPLE

1. Cloacal samples should be taken from within the cloacal area of the bird, avoiding excess solid fecal material or visible blood.
2. To take a cloacal swab from a live bird, you insert a swab deeply into the vent and vigorously swab the wall. The swab should be deeply stained with fecal material. Then you place the swab in the transport medium.



3. If you are collecting samples from fecal droppings from the cages of sick poultry in bird markets or from wild birds in the field, the fecal droppings should be recent (wet). Make sure that the swab is heavily covered with feces. The swab is then placed in transport medium.

If you are testing one cloacal sample on site, before packaging the rest of the samples for shipping, then follow this process:

1. All samples should be at typical “room temperature” before running the test
2. Place 8 drops of BHI or Viral Transport Media into a clean test tube
3. Dip 1 cloacal swab into Brain Heart Infusion (BHI) or Viral Transport Media.
4. Place the swab containing the sample in the tube and rotate the swab 5 to 10 times in the Extraction Buffer.
5. When removing the swab from the tube, press the swab against the side of the tube repeatedly until no more liquid comes from the swab
6. Discard the swab in a biohazard container (such as the red bio hazard bags that come in the Laboratory Kit).

7. Drop 200ul of extracted sample into a second test tube
8. Add 3 drops of Extraction Buffer into the tube and mix it thoroughly
9. If the samples will not be tested immediately, cap the tube with the provided cap and store the sample in the cooler provided in your kit.
10. Insert the test strip into the tube up-to the mark.
11. Allow the test strip to sit inside the tube for 15 minutes.
12. After 15 minutes, remove the test strip from the tube and read test strip results.

COLLECTION OF BLOOD SAMPLE:

- Site: Wing vein/Heart
- Volume: 2-3 ml
- After collection of blood by disposable plastic syringe, blood may be allowed to clot within the syringe. The syringe should be placed at 45 degree angel for better serum separation at room temperature or at 37°C. After 30 minutes serum comes out.
- Serum is to be collected in Eppendorf tube for sending to the Lab.
- Each tube to be labeled with code number corresponding to that in the sample submission form

Serum Collection



Blood Collection



COMPLETING THE DISEASE INVESTIGATION FORM AND THE SAMPLE SUBMISSION FORM

Once you have taken these samples, the Upazilla officer must properly complete two forms that accompany the samples. These two forms are the *Disease Investigation Form and the Sample Submission*. Both are found here in the Workbook at the end of this chapter and under Annex 2 and Annex 3.

After completing the forms, it is important to accurately prepare the samples for shipping to the nearest FDIL/CDIL. *Step 4 in the Job Cycle: Sending Samples to the Laboratory* covers these next activities to be taken by the Upazilla officer.

CHECKLIST REVIEW

- ✓ How should you prepare for collecting samples from sick or dead birds?
- ✓ What do you need to know about collecting samples from sick or dead birds?
- ✓ How do you collect sample and perform rapid diagnostic tests on sick or dead birds?
- ✓ How do you test a tracheal sample?
- ✓ How do you test a cloacal sample?
- ✓ How do you properly put on and take off personal protective equipment?
- ✓ Do you have the Disease Investigation Form found at the end of this chapter?
 - Do you know how to complete this form?
 - Do you know where to send this form after completed?
- ✓ Do you have a Sample Submission Form found at the end of this chapter?
 - Do you know how to complete this form?
 - Do you know where to send this form after completed?

DISEASE INVESTIGATION FORM

**Government of the People's Republic of Bangladesh
Department of Livestock Services**

Disease Investigation Form

Reference No.						Date:		
Name of the Farm:								
Name of the Farm Owner:								
Address:								
Contact telephone number:								
Type of Farm: (Tick)		GP Stock	Parent stock	Commercial layer	Broiler	Backyard		
Number of sheds in the farm:				Number of sheds affected:				
Type of shed: (Tick)		Environmentally controlled			Open sided			
Information about affected flock(s)								
Shed #	Species	Type of Birds	Breed	Source of Chicks (Hatchery)	Flock Size	Age (Wk's)	Litter/Cage/scavenging system	Date of onset of outbreak
Number of birds affected and died (date wise):								
Date	Shed #		Number affected			Number died		
Vaccination history of the affected flock:								
Vaccinated against			Age at vaccination/Date of vaccination			Name of vaccine		

SAMPLE SUBMISSION FORM

Government of the People's Republic of Bangladesh
Department of Livestock Services

Sample Submission Form

Collection date:		Submission date:			
Submitting veterinarian:					
Designation:					
Address:					
Contact telephone number:					
Farm/Owner:					
Address:					
Species of bird	Type	Breed	Age		
No. of birds in affected flocks	No. affected	No. died	No. of birds sampled		
			Sick	Dead	
History of Outbreak:					
Clinical signs:					
Necropsy findings (if done)*:					
Preliminary diagnosis:					
Details of samples submitted:					
Specimen type	Specimen ID#	Tests requested	Specimen type	Specimen ID#	Tests requested
Date:			Signature:		

*Necropsy should be done only at designated places. Special protection must be taken if AI is suspected

**Information on preservatives, antibiotics, pooling of samples, etc.

If commercial feed supplied, specify brand:								
If custom formulated feed supplied, give information:								
If any supplement is given in feed or water, give details:								
If any treatment already given in the present outbreak, give details:								
History of any previous outbreak in the farm:								
Shed #	Flock size	Age at outbreak	Disease diagnosed	Duration of outbreak	No. of birds affected	No. of birds died	Treatment given	Vet consulted

CLINICAL OBSERVATIONS

Clinical signs:					
Necropsy findings (if any)*:					
Samples collected:					
Specimen type	Number of specimens	Laboratory referred to	Date of Shipment	Date of Shipment **	Test requested for:
Investigating veterinarian's name:					
Date:			Signature:		

***Necropsy should be done only at designated places. Special protection must be taken if AI is suspected**
****Must accompany Sample Submission Form**

HANDOUT

PERSONAL PROTECTIVE EQUIPMENT (PPE)

SEVEN-STEP PROCESS FOR PUTTING ON PPE

Before you begin putting on your PPE, it is important to designate a clean location to put on the equipment, preferably away from anything that could be contaminated with avian influenza or other viruses.

Also try to wash your hands with soap and water, if it is available, before you begin, and remove watches and other non-smooth jewelry like bracelets.

I. COVERALLS

Put on coveralls first. Step into the “feet” of the coveralls first, and pull them up. Zip up the front of the coveralls. You should keep your regular clothing and shoes on under the coveralls.



2. SHOE COVERS

Put on shoe covers second. They should fit over your coverall feet, giving you another layer of protection to protect your shoes from contamination.



3. RESPIRATORS

Put the respirator under your chin with the nosepiece up. Pull the bottom strap over your head, and place it around your neck below the ears. Then pull the top strap over your head and rest it high at the top back of your head. Place your fingertips from both hands at the top of the metal nosepiece. Using two hands mold the nose area to the shape of your nose by pushing inward while moving your fingertips down both sides of the nosepiece.





Then pull coverall hood over the head, the elastic should hold it in place



5. APRON

Aprons are provided to fit over the coveralls. They are in a small packet that you will open up, place the apron over



6. INNER GLOVES

Put on the inner pair of gloves first (usually white or clear).



7. OUTER GLOVES

Will usually be a different color than the inner gloves and may be thicker. Pull them over the inner gloves. Pull the edge of the gloves over the cuff of your coveralls or gown, if possible.



NOW READY TO ENTER THE CONTAMINATED AREA.



HANDOUT

TEN-STEP PROCESS FOR REMOVING AND DISPOSING OF PPE

Before you begin the process of removing your PPE, you should find an area that is not contaminated with blood, soil, feces or other waste.

I. OPEN THE POUCH WITH THE GERMICIDAL WIPE



And use it first on your outer gloves



Then on your outer boots



Place it in the red biohazard bag when done.



2. REMOVE AND DISPOSE OF YOUR APRON
3. REMOVE AND DISPOSE OF YOUR OUTER SHOE COVERS IN THE RED BIOHAZARD BAG.



3. REMOVE AND DISPOSE OF YOUR OUTER SHOE COVERS IN THE RED
BIOHAZARD BAG.



4. REMOVE AND DISPOSE OF YOUR OUTER GLOVES IN THE RED BIOHAZARD BAG.



5. UNZIP AND ROLL DOWN YOUR COVERALL UNTIL IT IS INSIDE-OUT, AND THEN STEP OUT OF IT. PLACE THE USED COVERALLS INTO THE RED BIOHAZARD BAG.



6. REMOVE YOUR GOGGLES BY PULLING THEM UP OVER YOUR HEAD. YOU SHOULD HANDLE THEM BY THE HEAD BAND OR EAR PIECES. PLACE THEM IN THE RED BIOHAZARD BAG.



7. REMOVE YOUR RESPIRATOR BY GRABBING THE TOP AND THEN THE BOTTOM ELASTIC BANDS AND PULLING THEM UP OVER YOUR HEAD. PLACE THE RESPIRATOR AND GOGGLES IN THE RED BIOHAZARD BAG.



8. REMOVE YOUR INSIDE GLOVES. BEGIN WITH ONE HAND, ROLLING DOWN THE GLOVE (WITH YOUR OTHER HAND), STARTING AT THE WRIST UNTIL THE GLOVE IS INSIDE OUT. HOLD THE REMOVED GLOVE IN YOUR HAND IN A LITTLE BALL, AND THEN ROLL DOWN THE OTHER GLOVE – STARTING AT THE WRIST – WITH YOUR FIRST HAND. PLACE THEM IN THE RED BIOHAZARD BAG.

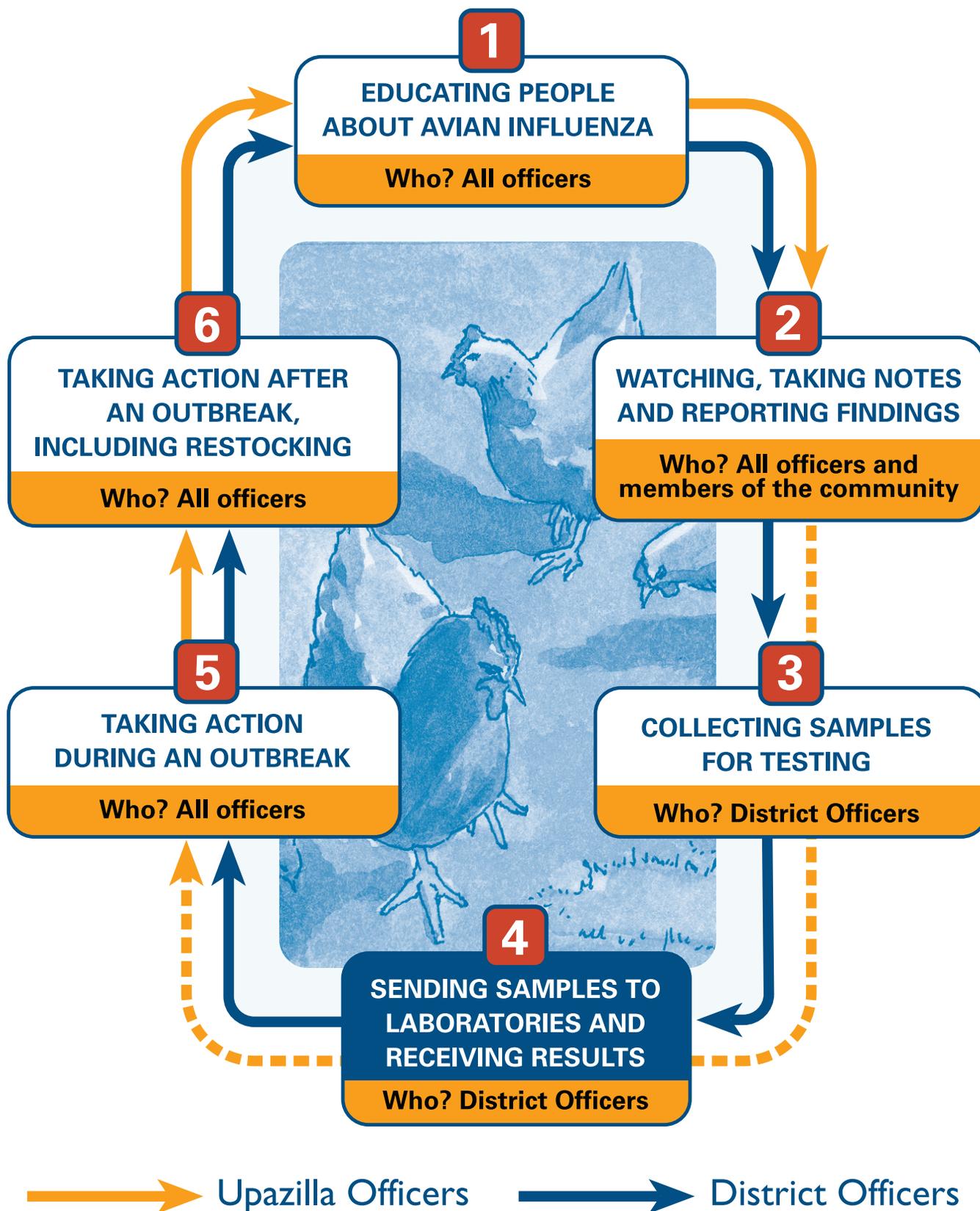


9. CLOSE THE RED BIOHAZARD BAG BY TYING A KNOT AT THE TOP OR OTHERWISE TYING IT SHUT. THE RED BIOHAZARD BAG SHOULD BE PLACED AT A DESIGNATED LOCATION SO THAT IT CAN BE COLLECTED AND BURNED OR BURIED.

10. WASH YOUR HANDS, FOREARMS AND FACE WITH SOAP AND WATER.



The Job Cycle for District and Upazilla Officers



JOB CYCLE-STEP 4

SENDING SAMPLES TO THE LABORATORIES AND RECEIVING RESULTS

Step 4 in the Job Cycle: ***Sending Samples to the Laboratories and Receiving Results***, informs District officers on how to be involved in the following activities:

1. Preserving any samples (if there is a delay in sending samples).
2. Providing necessary documents and logistical support for packaging, labeling and shipping samples to the Field Disease Investigation Laboratory and the Central Disease Investigation Laboratory.
3. Preparing for transport of samples for laboratory investigation.
4. Classifying, packaging, labeling and shipping samples to overseas laboratory (only for Central Disease Investigation Laboratory or Bangladesh Livestock Research Institute).

Receiving results from Field Disease Investigation Laboratory, Central Disease Investigation Laboratory or Bangladesh Livestock Research Institute Laboratory.

1. Preserving any samples (if delayed for sending)

- All samples should be sent immediately, if not possible the samples should be refrigerated at 4°C for a short period (up to 48 hrs).
- Avoid repeated freezing and thawing which can cause loss of infectious properties

2. Providing necessary documents and logistic support

for packaging, labeling and shipping samples to Field Disease Investigation Laboratory and Central Disease Investigation Laboratory

- Sample Submission and Disease Investigation Form.
- Sample collection kit.
- Separate refrigerator for AI suspected samples.
- Gloves, N-95 Respirator, eye protection, like goggles.
- Plastic bags, cool box with ice packs or double chambered thermo flask with wet ice.
- Two layered plastic bags, cotton threads.
- Permanent marker, adhesive tape.
- Special messenger.

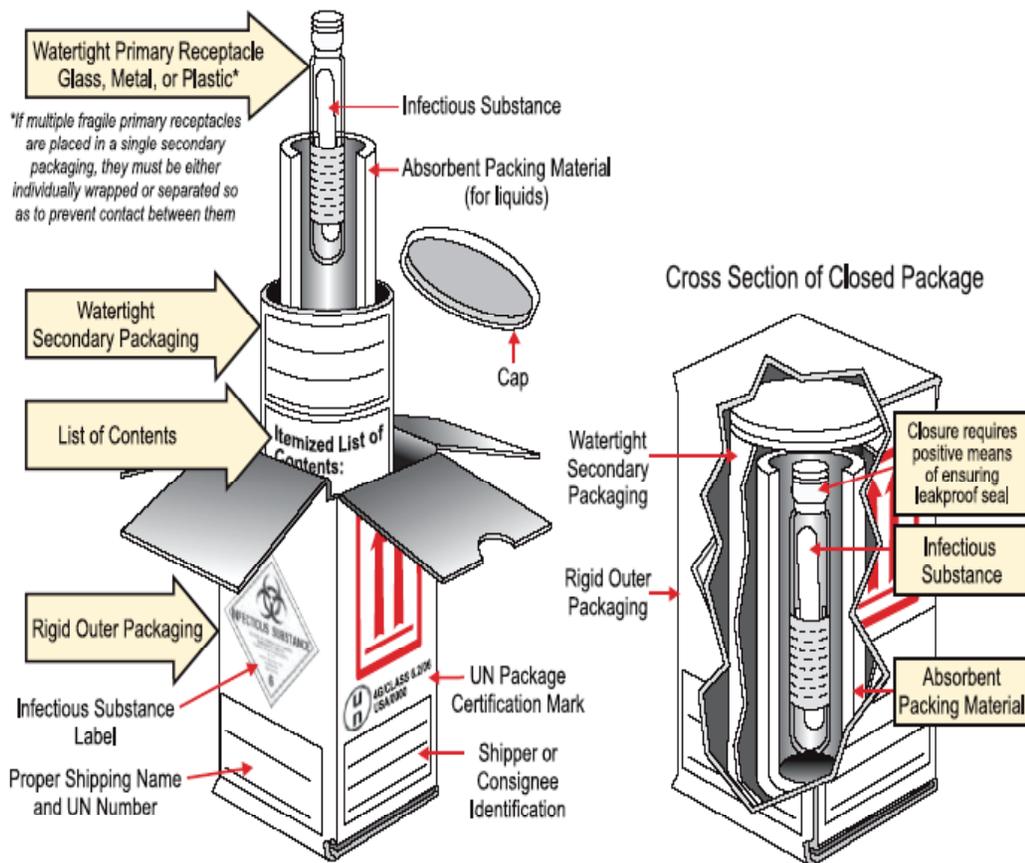
3. Preparing to transport the samples for laboratory investigation

- Dispense 1.0 – 2.0 ml (8 drops) of transport media to the sterile vials (plastic screw cap). Clinical specimens for viral isolation or detection should be placed in plastic bags in a cool box with ice packs or in double chambered thermo flask with wet ice and transported to the nearest Field Disease Investigation Laboratory/Central Disease Investigation Laboratory. If necessary the whole carcass should be sent to the nearest Field Disease Investigation Laboratory/Central Disease Investigation Laboratory wrapped in two layers of plastic bags in a chilled condition in a leak-proof container. Number the vials to correspond with those on the *Sample Submission Form*.

The submission form is found in the back of this chapter and in Annex 3.

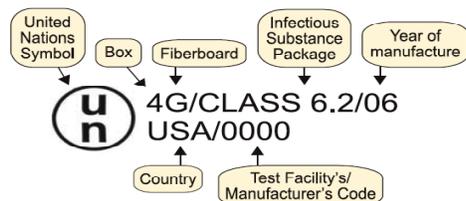
ONLY FOR CENTRAL DISEASE INVESTIGATION LABORATORY AND BANGLADESH LIVESTOCK RESEARCH INSTITUTE

1. Packing a possible infectious substance requires Triple Packaging to prevent breaking, puncturing or leaking
2. 3rd package must bear Specification Markings referred to as the “UN Package Certification Mark” or “UN Specification Mark”



- Note 1:** The smallest external dimension of the outer packaging must not be less than 100 mm (3.9 inches)
Note 2: The primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa
Note 3: Follow package manufacturer’s closure instructions

SAMPLE OF UN PACKAGE CERTIFICATION MARK



3. Classifying, packaging, labeling and sending samples to reference laboratory

Note: the following protocols are based on international regulations. District officers need to follow national regulations adapted from international guidelines.

Classifying

Infectious substances affecting humans or animals are classified as Class A and need to be assigned the following shipping UN number:
UN 2814

Packaging

Packing a possible infectious substance requires Triple Packaging to prevent breaking, puncturing or leaking (see picture 1):

1st Package – A watertight receptacle with the sample

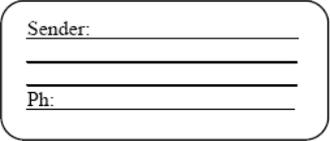
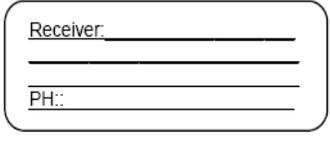
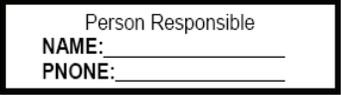
2nd Package – A watertight inner package (secondary packaging or container)

3rd Package – Rigid outer packing of adequate strength with at least one surface having a minimum dimension of 100 mm x 100 mm

3rd package must bear Specification Markings referred to as the “UN Package Certification Mark” or “UN Specification Mark”

ONLY FOR CENTRAL DISEASE INVESTIGATION LABORATORY AND BANGLADESH LIVESTOCK RESEARCH INSTITUTE

Labeling: the name and telephone number of the person responsible for the shipment must be on the outside of the box

LABEL	DESCRIPTION	POSITION
	The full name, address and phone number of the sender	Place in upper left hand quadrant of box.
	The full name, address and phone number of the receiver	Place below sender label in upper left hand quadrant of box.
	Emergency contact – the name and phone number of the emergency contact must be available 24 hours a day until the shipment is received	Place below receiver label in upper left hand quadrant of box.
	Class 6 “Infectious Substance” Label	Place in upper right quadrant of box. Label must be 4” square.

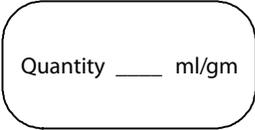
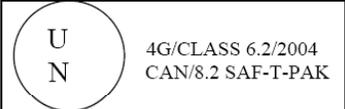
Labeling: Class A Infectious Substances

The outside of every box must be labeled/marked with the following:

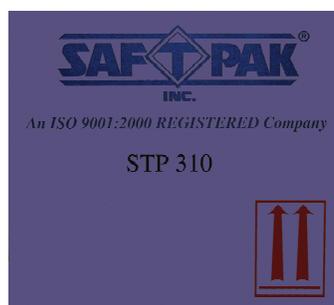
LABEL	DESCRIPTION	POSITION
	UN 2814, Infectious Substance, affecting human	Place adjacent (left) to Class 6 Infectious Substance label.

Labeling: Class A Infectious Substances

The outside of every box must be labeled/marked with the following:

LABEL	DESCRIPTION	POSITION
	The total quantity of infectious substance shipped in the box	Place adjacent (left) to Class 6 label.
	The box must have the UN compliant packaging certification mark	Not a label but marking on the box printed by the box manufacturer

Labeling on outside of package should be placed as shown.



LABEL	DESCRIPTION	POSITION
SAF-T-PAK INC. An ISO 9001: 2000 REGISTERED Company	Manufacturer of the box and packing material	
STP 310	Box Part Number	
	Two (2) up arrows on opposite sides of the box	Place on opposite sides of the box

Pack samples with freezer gels or cold packs.

Consignee

Address (in detail)

Consigner

Address (in detail)

Documenting

Documentation must follow national procedures and the filling out of national regulation forms.

RECEIVING RESULTS FROM FIELD DISEASE INVESTIGATION LABORATORY (FDIL), CENTRAL DISEASE INVESTIGATION LABORATORY (CDIL) AND BANGLADESH LIVESTOCK RESEARCH INSTITUTE LABORATORIES (BLRI).

- If Rapid Antigen Test yields a positive result and National AI Reference Laboratory confirms positive test result, all officers will begin to implement *Step 5 of the Job Cycle: Taking Action During an Outbreak* according to the Government of Bangladesh, Department of Livestock Services and the Food and Agriculture Organization manual.
- If Rapid Antigen Test yields a negative result, and if the death in a flock still continues all officers must refocus on Step 1: Educating People to Prevent Avian Influenza, and Step 2: Watching, Taking Notes and Reporting Findings. The District Officers will refocus on Step 3: Collecting Samples for Testing.

CHECKLIST REVIEW

- ✓ What do you do with any leftover samples?
- ✓ What are the four procedures required in sending samples to laboratories?
- ✓ How do you properly pack a possible infectious substance for the Central Disease Investigation Laboratory and Bangladesh Livestock Research Institute?
- ✓ What are the steps to follow after receiving results of tests?

SAMPLE SUBMISSION FORM

**Government of the People's Republic of Bangladesh
Department of Livestock Services**

Disease Investigation Form

Reference No.						Date:		
Name of the Farm:								
Name of the Farm Owner:								
Address:								
Contact telephone number:								
Type of Farm: (Tick)		GP Stock	Parent stock	Commercial layer	Broiler	Backyard		
Number of sheds in the farm:				Number of sheds affected:				
Type of shed: (Tick)		Environmentally controlled			Open sided			
Information about affected flock(s)								
Shed #	Species	Type of Birds	Breed	Source of Chicks (Hatchery)	Flock Size	Age (Wk's)	Litter/Cage/scavenging system	Date of onset of outbreak
Number of birds affected and died (date wise):								
Date	Shed #		Number affected			Number died		
Vaccination history of the affected flock:								
Vaccinated against			Age at vaccination/Date of vaccination			Name of vaccine		

If commercial feed supplied, specify brand:								
If custom formulated feed supplied, give information:								
If any supplement is given in feed or water, give details:								
If any treatment already given in the present outbreak, give details:								
History of any previous outbreak in the farm:								
Shed #	Flock size	Age at outbreak	Disease diagnosed	Duration of outbreak	No. of birds affected	No. of birds died	Treatment given	Vet consulted

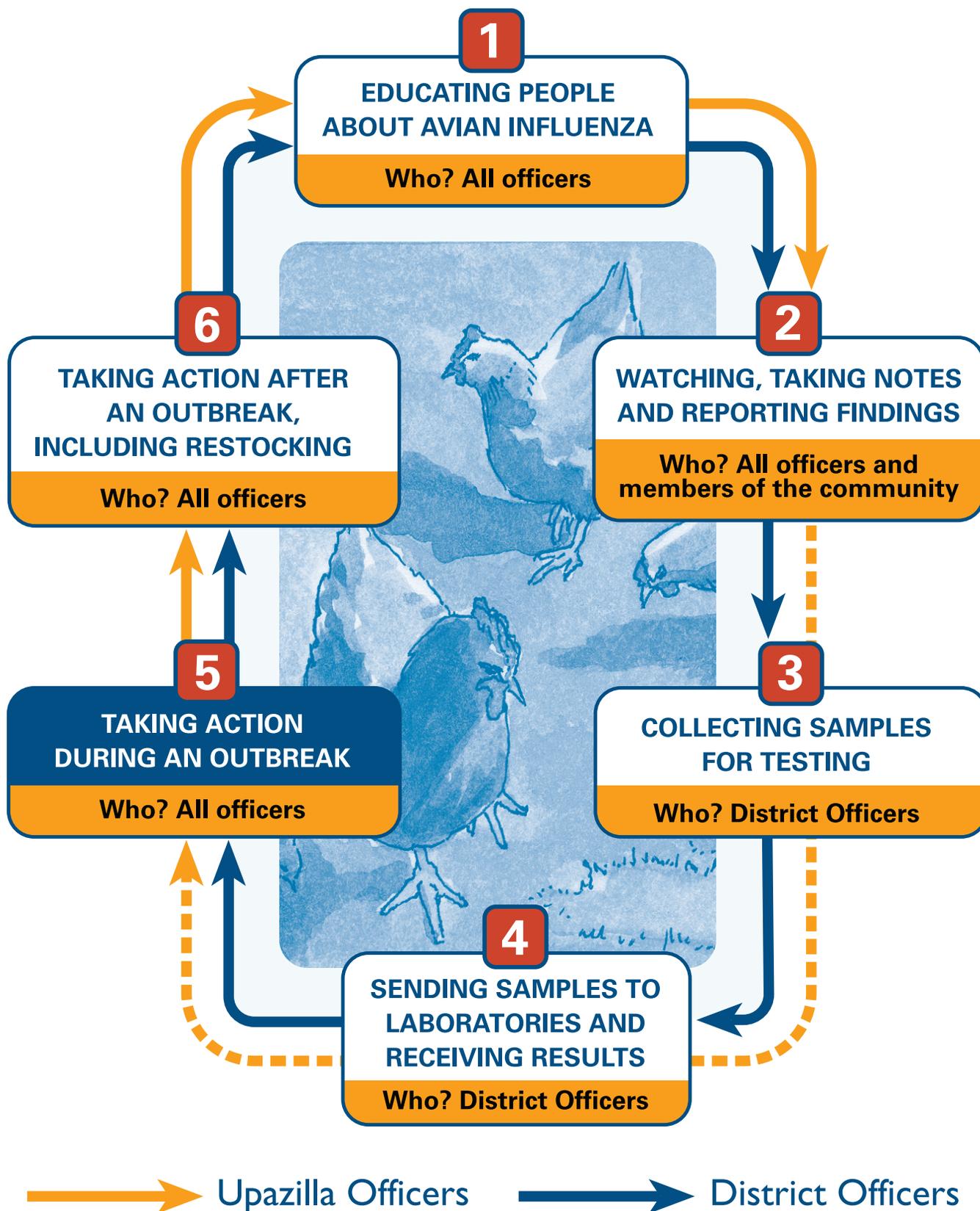
CLINICAL OBSERVATIONS

Clinical signs:					
Necropsy findings (if any)*:					
Samples collected:					
Specimen type	Number of specimens	Laboratory referred to	Date of Shipment	Date of Shipment **	Test requested for:
Investigating veterinarian's name:					
Date:			Signature:		

***Necropsy should be done only at designated places. Special protection must be taken if AI is suspected**

****Must accompany Sample Submission Form**

The Job Cycle for District and Upazilla Officers



JOB CYCLE-STEP 5

TAKING ACTION DURING AN OUTBREAK

In Step 4 samples were collected and sent to the nearest Field Disease Investigation Laboratory (FDIL) and Central Disease Investigation Laboratory (CDIL). The FDIL and CDIL performed the Rapid Antigen Test for AI. If the test results are positive Step 5 begins. The FDIL and CDIL immediately submits a report to the AI Focal Point. The AI Focal Point is a person who acts as the Central Outbreak Manager. The *Central Outbreak Manager* is assisted by a communication officer, a planning officer and an operation officer. These persons are identified well before an outbreak from existing Department of Livestock mid level officers and are trained in responding to an outbreak. The Central Outbreak Manager along with the Field Outbreak manager maintains a log of day to day activities as they respond to an outbreak.¹

Many people are responsible for activities during **Step 5**. For example the **Director General** and **District Livestock Officers** have several responsibilities including:

- Treating the farm(s) having rapid antigen test positive cases as the “suspected premises” until further notice.
- Alerting the National and Field Out Break Managers for probable outbreak management operations.
- The Director General and District Livestock Officers will declare the Infected Zone and the Control Zone. This declaration will be published by a Government Gazette (newspaper) notification.
- Instructing the Upazilla livestock officer or veterinary surgeon to impose movement control and enhanced bio-security measures in the “suspected premises.”

¹ Procedures of the Central Outbreak manager taken from the Avian Influenza Operation Manual, Department of Livestock Services, Ministry of Fisheries and Livestock, Bangladesh, January 2007, pp. 8-9.

- Instructing the Upazilla Livestock Office to impose movement control and enhanced biosecurity measures in the Suspect Premises. The suspect premises are those farms having rapid antigen test positive cases.

The Upazilla Livestock Officers (ULO) and the Veterinarian Surgeons (VS) also have many responsibilities including:

Informing the farmer(s) of the “suspect premises” about the suspicion of outbreak and advise all farm personnel not to visit other farms and not to allow anybody to visit suspect premises. Stopping all movement of birds into and out of infected site.

Informing the Local Forest Authority in the case of a wild bird habitat affected by AI type-A. Advising all people in surrounding areas not to visit the affected area.

Stopping the movement of crews, carcasses, manure, and contaminated vehicles.

Stopping all movement of birds, stray animals, (dogs, cats) large animals etc. into and out of infected site

Stopping the sale of birds or bird products, egg, equipment, supplies, feeds, etc out of the suspect premises.

Imposing restriction on the movement or sale of poultry, poultry products, equipment, supplies, feeds, manure, etc. into and out of the suspect premises until further notice.

Stopping the sale of birds or bird products, egg, equipment, supplies, feeds, etc out of the suspect premises

Ensuring and supervising the spraying of veridical agent inside and outside poultry houses or around the suspected premises at least 3 times a day.

Depopulating birds by culling, disposal, and decontamination on infected premises/zone. Veterinarians will act as the supervisor of all depopulation activities. Workers should wear personal protective equipment (PPE), dispose of bird carcasses using an

environmentally acceptable manner and use decontamination commodities in an environmentally acceptable manner.²



CULLING, DISPOSING OF CARCASSES AND DECONTAMINATION PLANNING FOR CULLING ACTIVITIES

One of the most efficient ways to cull, decontaminate and destroy carcasses is to consider what you will need before hand.

Consider the following questions and answers before you cull–

Where should the poultry be culled?

To minimize handling of poultry and reduce the stress on poultry they should be culled on the affected farm as close to their housing as possible.

What are the species, numbers, age and size of the poultry to be culled?

Knowing this information helps ensure that the animals intended for culling are culled and disposed of.

What type of housing do the poultry live in?

Different culling approaches may be needed for cage, litter and free range systems.

² Culling, disposal and decontamination procedures are taken from the Avian Influenza Operation Manual, Department of Livestock Services, Ministry of Fisheries and Livestock, Bangladesh, FAO, pages 32-37.

What type of equipment is needed?

This includes tools like burddizos, PPE kit for each worker, CO2, disinfection kits, fuel, digging equipment, etc.

In what order should the poultry be culled?

Usually the infected birds are culled first, then the birds that had contacted with the infected birds, and finally the rest of the flock.

OTHER CULLING CONSIDERATIONS

Check to see if there are any facilities in the area that can assist with the culling process.

Decide how many cullers will be needed to get the job done.

Train the potential cullers on culling techniques and how to talk to the community about the culling process before the culling takes place.

Make sure a District Officer is present to oversee the Upazilla officers culling poultry.

The method chosen for culling must be safe, humane and efficient. Try to cull as close to where the birds are as possible.

Culling methods that shed blood should be avoided. Blood is a major source of avian influenza infection. Culling should be humane and avoid bloodshed. Avoid cutting the neck, beheading or any other method that creates a lot of blood.

PREPARING THE CULLING AREA

Clear the culling area of children and other bystanders. There should be no children or bystanders in the culling area. Limiting the number of people present to the cullers and their supervisors is a way to limit the number of people exposed to avian influenza virus from the air, blood, feathers, dust, animal parts, contaminated equipment and surfaces.

Anyone in the culling area, including workers and supervisors **MUST** wear personal protective equipment.

For small numbers of birds that are small to medium, the preferred method is dislocation of the neck using gloved hands.

You can also dislocate the neck by using burdizzos, bone cutters, pliers or forceps. Burdizzos are most useful when large numbers of poultry with strong necks—such as geese and ducks—are destroyed.

Note: Information on culling using CO2 can be found in the Annex.

DISCARDING PPE, ANIMAL CARCASSES AND OTHER ANIMAL PARTS

The first choice, by far, would be on-site burial. In a larger outbreak one common burial site should be considered. Considerations include the amount to bury, site availability, soil type, water table, nearby wells or ponds and digging equipment available.

The second choice is burning but this may be influenced by fire restrictions, prevailing winds, a small site and the availability of cremation fuel. Burning may be quicker, cheaper and a way to avoid a high water table.

BURIAL SITE SELECTION

Important considerations for burial site selection include:

- Access to the site – for both equipment to dig the burial pit and for the delivery of livestock, carcasses or other materials to be buried
- Environmental-distance to water sources, bores and wells; height of water-table; proximity to buildings, especially houses; proximity to neighbors or public lands including roads; slope of the land drainage to and from the pit; permeability of soil; sufficient space for temporary storage of overburden; and direction of prevailing wind
- Construction considerations-avoid rocky areas (slows digging and increases costs) but select soils with good stability capable of withstanding the weight of equipment used to construction

of diversion banks if required. Similar banks should be constructed to prevent any liquids escaping from the burial site. Fencing may be necessary to exclude animals until the site is safe for use.

EARTH MOVING EQUIPMENT

- Locally available earth moving equipment will be used.

BURIAL PIT CONSTRUCTION

- The dimensions of the burial pit will be dependent on the equipment used, site considerations and the volume of material to be buried. The preferred dimensions are for pits to be as deep as practically possible (Reach of machinery, soil type and water-table level being the usual constraints), with vertical sides.
- A pit of 2 meters wide, 2 meters deep and 2 meters long would accommodate 1800 birds. If the pit is made one meter deeper the capacity would increase up to 3000 birds.
- Make sure that no bird is still alive when dropped into the burial pit. If this happens, birds must be immediately caught and humanely killed.
- Carcasses should be covered by about 400 mm of soil then an unbroken layer of slaked lime {Ca(OH)₂}. If this lime is applied directly to carcasses the decomposition process will be significantly delayed.
- When closing the pit, surplus soil should be heaped over the pit as overfill. The weight of soil acts to stop carcasses rising out of the pit due to gas entrapment, prevents scavengers digging up carcasses, helps filter out odors and assists in absorbing the fluids of decomposition. After pit subsidence it will be necessary to replace any topsoil not utilized during pit closure.
- Disinfectants need to be sprayed on equipment used and on the pathway used to take carcasses to the pit. The way to

dispose of PPE, tools and animal carcasses and animal parts may be different in each situation or location. District and Upazilla Officials will decide on how best to dispose of dead birds and other items that have come in contact with blood or other animal parts.

- The burial hole should be located away from human and animal living areas and water—including wells, lakes, ponds or rivers.
- The burial hole should be large enough to hold all of the dead birds and at least 0.6 meters (2 feet) of soil on top of the carcasses.

IF YOU ARE BURNING CARCASSES OR USED PPE OR OTHER CONTAMINATED TOOLS KEEP THE FOLLOWING IN MIND:

- Carcasses may be burned on a stack with flammable liquid. Arrange fuel and carcasses so that enough air can enter the fire from below and achieve the hottest fire possible in the shortest period of time.
- After finishing piling the carcasses, pour fuel like kerosene (but not petrol/gas) on the fire bed and place rags soaked in kerosene every ten meters along the length of the fire bed.
- Start the fire by walking into the wind and lighting the rags along the way.
- Make sure that someone watches the fire at all times. To make sure that enough fuel is used and that any carcasses or animal parts that fall off the fire are replaced again.
- The ashes can be buried. See section on burial above.

DECONTAMINATION, CLEANING AND DISINFECTING

Decontamination Pre-activities. Wearing PPE and Preparing Disinfectant

- Before starting any decontamination procedures, everyone should put on their PPE to protect themselves.
- Virkon is the disinfectant usually used. It comes in powdered form that can be mixed with water.
- It is important to wear PPE when mixing and using disinfectants because it can irritate the skin and eyes.

After culling operations it is very important to properly clean and disinfect the culling area. Follow instructions from your local authority (District Officers) on how to disinfect cages, equipment, or other parts of the farmyard or to sweep up manure and other poultry waste. Make sure you are still wearing your PPE when cleaning and disinfecting the culling area! Because the avian influenza virus survives best in a moist and soiled environment, it is important to thoroughly clean and disinfect objects that have been soiled by blood, feathers, or any other poultry fluids, wastes or other animal parts. Avian influenza also survives well in water, so washing items with water only (and no soap or disinfectant) may spread the virus.

The first consideration would be to decontaminate contaminated areas. The following steps should be taken in order and under site supervisor direction.

PROPERTY ASSESSMENT

This should be detailed starting with making a map and marking in the location of electrical and water lines, drains, effluent run off, identification of a decontamination site as well as a clean unloading site, an estimate of the degree of contamination of each area, the amount of poultry manure and feed and especially heavy or light areas of expected contamination.

PRELIMINARY DISINFECTION

This is designed to quickly start and rapidly reduce the amount of virus present up to the completion of slaughter.

- Any area known or suspected to be contaminated is sprayed. This includes barn feeders, waters (after draining), and access roadways and around the outside of houses and poultry barns as soon as the birds are removed for disposal.
- Spraying should be repeated up to 5 times a day. For disinfection Virkon R S is treated as wonderful disinfectant but other locally available disinfectants are also effective against AIV. These include the use of soaps and detergents as well as phenols, Dettol and quaternary ammonia compounds used after proper cleaning.
- All contaminated materials and surfaces should be allowed to soak with disinfectants for at least 24 hours.

Be sure to disinfect any tools that will be used again and discard PPE by burning or burying it.

- Potassium permanganate is in wide use in the country especially for footbath disinfection. Literature review has revealed that this practice is of limited value against AIV and it is recommended that a commonly available product like Dettol (R) be used instead.

CLEAN-UP

The aim is to remove, without using water, all manure, debris, feed, etc, to expose surfaces for a second round disinfection. This is very important as organic material reduces any disinfectant effectiveness. These types of materials, such as feed and manure, should be buried with the birds. The next step is a wash down with a low pressure sprayer using a detergent or bleaching powder.

FULL SCALE DISINFECTION

- Disinfectant to be sprayed in the following order – roof, walls and finally the floor.
- Inspection is carried out to ensure that everything has been completed- repeat clean -up and disinfection if there is doubt.
- Another round of full disinfection 7 to 14 days later.
- Final disinfection before restocking.

DECONTAMINATION OF EQUIPMENT AND VEHICLES

- The other consideration is the decontamination of contaminated equipment. The primary concern would be for anything used during stamping out. This would include items like CO2 tanks, bins, excavators, back hoes, trucks and other vehicles. Use the decontamination site previously selected. Apply the same principles including cleaning first followed by a low pressure detergent spray, inspection then disinfection spray. Repeat the inspection and disinfectant spray.
- If any trucks, vehicles, motor cycles or rickshaw vans, egg tray are on the contaminated side they must be decontaminated before leaving the premise. Particular attention needs to be paid to mats under driver's feet. Vehicle interiors, including trunks, can be wiped down with disinfectants on cloths as required. All under parts and wheels of cars should be sprayed with water and disinfection.

DECONTAMINATION PROCEDURES OF VARIOUS OBJECTS

Disinfectant/chemical selections and procedures for avian influenza outbreak.

ITEM TO BE DECONTAMINATED	DISINFECTANT/CHEMICALS/PROCEDURE
Live Birds	Euthanasia
Carcasses	Bury or burn
Animal Housing/Equipment	Soap & Detergents, Sodium hypochlorite, Calcium hypochlorite, Virkon, Alkalis
Humans	Soap & Detergents
Electrical Equipments	Formaldehyde gas
Water Tanks	Drain to pasture where possible
Dams	Drain to pasture if practical
Feed	Bury
Effluent, manure	Bury or burn, Acids, Alkalis.
Human housing	Soap & Detergents, Sodium hypochlorite, Calcium hypochlorite, Virkon.
Machinery, vehicles	Soap & detergents, Alkalis.
Clothing	Soap & Detergents, Sodium hypochlorite, Calcium hypochlorite, Virkon, Alkalis
Aircrafts	Soap & Detergents, Virkon.

CHECKING YOUR HEALTH

If you have been culling, decontaminating or disposing of poultry always remember to monitor your health for at least 7 days.

Tell you local health care provider if you develop any of these symptoms:

- Fever over 38 degrees C.
- Sore throat or cough.
- Respiratory distress or failure.

Be sure to tell the health official that you have been involved in poultry culling.

Figure 1 below summarizes the activities in Step 5 and the rationale for each activity.

Figure 1 Actions Taken During Step 5 of the Job Cycle and the Rationale for those Actions

ACTIONS	WHY
Declare the infected zone (farms or areas infected with avian influenza virus).	<ul style="list-style-type: none"> • Keeps the virus within a certain area. • Specific actions on the farms/area can be carried out.
Declare the control zone to watch, make notes, sample and record (see picture).	<ul style="list-style-type: none"> • Provides early warning of an infected farm/area. • Controls the spread of the avian (bird) virus. • Information with supervisors can be shared. • If there is an outbreak, actions can be taken.
Inform the farmer from the suspect premises about the outbreak and ad-	<ul style="list-style-type: none"> • Keeps the virus within a certain area.

ACTIONS	WHY
<p>advise all farm personnel not to visit other farms or poultry holding houses and not to allow anybody to visit suspect premises (see picture).</p>	<ul style="list-style-type: none"> • Creates a barrier between uninfected birds and the infected area.
<p>STOP ALL movement of birds into and out of infected site.</p>	<ul style="list-style-type: none"> • The infected premises are immediately quarantined - NO movement in or out of birds because infected birds can spread the virus to other birds or people. • The virus can be carried to other farms by hands/skin, clothing or shoes which have been infected – either directly from birds or from manure/bedding. • The virus can be in the air when contaminated dust and soil is kicked up by wind, walking or movement. • Transmission of the virus has been strongly linked to transporting live birds, contaminated dead birds, or litter in vehicles. • Creates a barrier between uninfected birds and the infected area.
<p>STOP the movement of crews, carcasses, manure, and contaminated vehicles. Specify what items can be moved:</p> <ul style="list-style-type: none"> • Under what conditions they can move • What treatments must be applied 	<ul style="list-style-type: none"> • The virus can be easily carried from one contaminated location to other uncontaminated locations. • The virus can survive, at cool temperatures, in contaminated manure for days and even weeks. • In water the virus can survive for up to 4 days at 22°C and more than 30 days at 0°. (See page 197 of this workbook for more information).
<p>STOP sale of birds or bird products, egg, equipment, supplies, feeds, etc out</p>	<ul style="list-style-type: none"> • The avian (bird) influenza virus can be spread directly through

ACTIONS	WHY
of the suspect premises.	contaminated feed, water and equipment.
<p>Cull (stamp out) birds on infected premises using personal protective equipment (PPE) and in an environmentally acceptable manner (see pictures 1 and 2 page-8):</p> <ul style="list-style-type: none"> • When: within 24 hours of detecting infection • What: birds; use PPE equipment and other culling equipment • Where: as close as possible to where birds are housed • Who: health status of cullers, number of cullers; veterinary health official • How: safe, humane and efficient (dislocation of neck with gloved hands, gassing with carbon dioxide) <p>Apply country stamping out policy to specified radius of infected premises.</p>	<ul style="list-style-type: none"> • Delayed culling of birds on infected premises results in production of large quantities of virus that increases the likelihood of spread. • Timely culling reduces the amount of virus present. • PPE keeps the worker safe and healthy. • Workers who use PPE are protecting their families, communities, farms businesses and themselves. • The avian influenza virus can infect a person through their eyes, nose or mouth.
Dispose of carcasses by burial, burning or composting in an environmentally acceptable manner using personal protective equipment (PPE).	<ul style="list-style-type: none"> • Prevents risk for further spread of the avian (bird) influenza virus to other birds either by direct or indirect means, e.g. by scavengers or by contamination of food or water. • PPE keeps the worker safe. • Workers who use PPE are protecting their families, communities, farms and businesses. • The avian influenza virus can infect a person through their eyes, nose or mouth.
Decontaminate by thoroughly cleaning and disinfecting the premises	<ul style="list-style-type: none"> • Avian influenza virus can be killed by most disinfectants (with proper

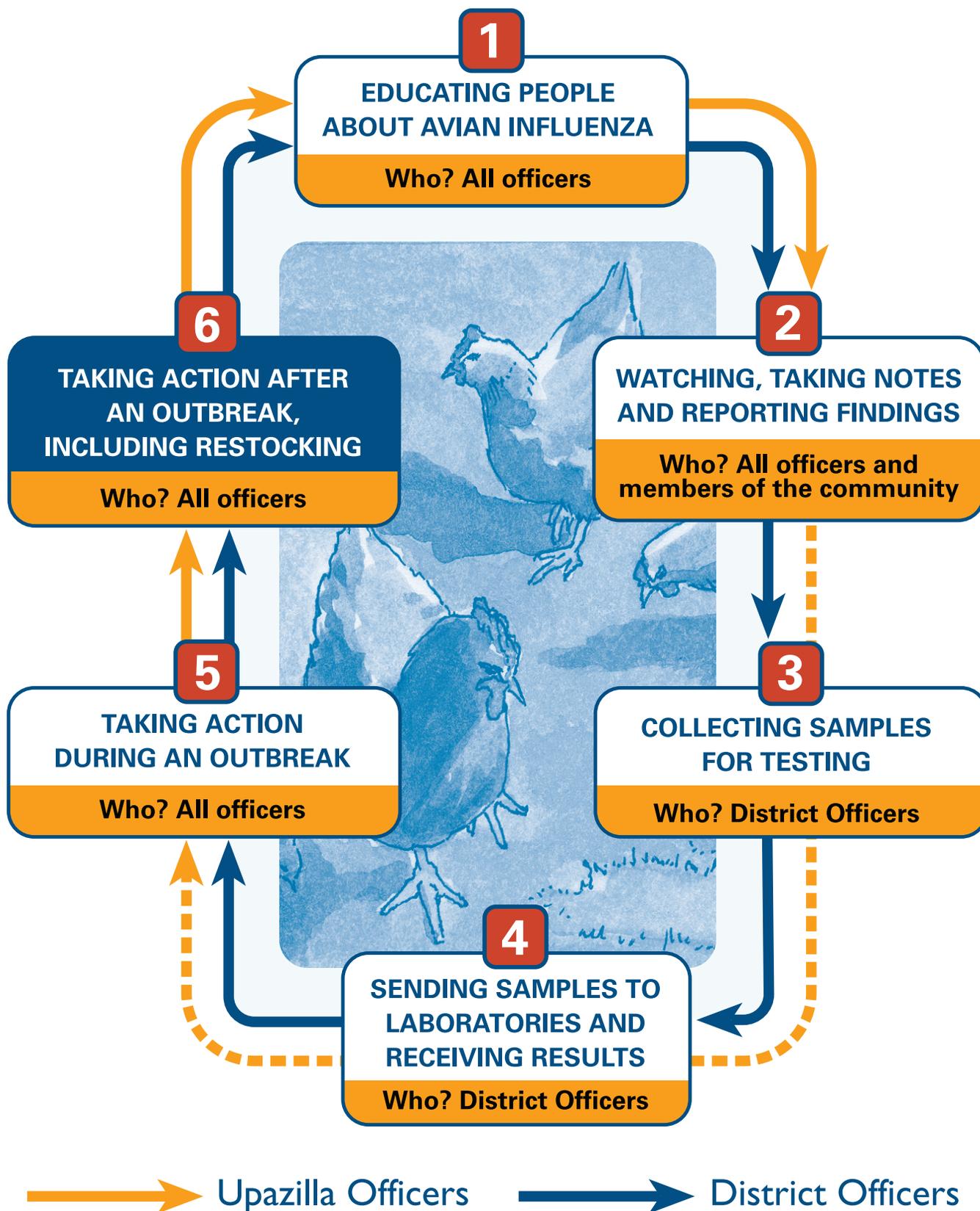
ACTIONS	WHY
<p>and equipment that had contact with birds with protective clothing.</p> <ul style="list-style-type: none"> • Follow recommended cleaning methods and disinfectants • Avian (bird) influenza virus survives best in a moist or soiled environment • Avian (bird) influenza virus also survives in water <p>Make sure all culling, disposal of carcasses (burying or burning) and cleaning/disinfection of the area is completed before removing PPE.</p>	<p>concentration).</p> <ul style="list-style-type: none"> • Infected birds can spread the virus to other birds or people. • Disinfection prevents the virus from being spread or carried. • Prevents contamination of people and the environment. • Prevents further spread of the virus during an outbreak. • Allows safe repopulation of flocks after a proper quarantine period. • Bird feces are the biggest danger for spreading the avian influenza virus.
<p>Ensure that all stray animals within the suspect premises are put on a leash or caged.</p>	<p>Prevents these animals from roaming out of the suspect premises, or from entering into suspect premises.</p>
<p>Enforce minimum hygiene standards at culling points in live bird markets.</p>	<p>Because avian influenza virus is spread to uninfected birds through infected feces, saliva, mucous or other animal fluids, it is important to practice strict sanitary procedures.</p>
<p>Encourage cullers, supervisors, farmers and families to monitor their health for at least 7 days after contact with sick or dead birds, and visit the nearest health facility (advising the health care provider of exposure to sick or dead birds) if any of the following symptoms appear:</p> <ul style="list-style-type: none"> • Fever over 38°C • Sore throat or cough • Respiratory distress or failure 	<p>Encourages early detection and treatment of possible infection of avian (bird) influenza virus.</p>
<p>Carry out publicity campaigns.</p>	<p>Informs public on avian influenza and actions being taken to prevent the spread of infection.</p>

There are certain procedures to follow once the avian influenza outbreak has been brought under control. These activities are found in ***Step 6: Taking Action after an Outbreak***

CHECKLIST REVIEW

- ✓ What actions does the District Officer take when there is a confirmed positive test result for avian influenza?
- ✓ What actions does the Upazilla Officer take when there is a confirmed positive test result for avian influenza?
- ✓ Why is it important to take these actions?

The Job Cycle for District and Upazilla Officers



JOB CYCLE-STEP 6

TAKING ACTION AFTER AN OUTBREAK, INCLUDING RESTOCKING

Step 6: Taking Action after an Outbreak, involves all officers, Step 6 leads to the repetition of the whole Job Cycle with **Step 1: Prevention and Control of Avian Influenza**. Besides starting the Job Cycle over, Step 6 emphasizes recovery and restocking.

Successful recovery after an outbreak focuses on preserving farms, protecting birds, and resuming business as quickly as possible.

Recovery measures also include:

- waiting before restocking flocks;
- determining the source of infection;
- restocking flocks;
- correcting deficiencies;
- reviewing transport issues;
- compensation to those who lost flocks;
- restoring confidence in the farmers and the community; and
- fostering change of industry practices; and strengthening/continuing on-going education and training programs.

Figure 1 describes these activities in more detail and includes the rationale behind each activity.

Figure 1

ACTIONS	WHY
<p>After culling, disposing, cleaning, disinfecting (and if the outbreak has been brought under control) replacement or restocking of flocks can be considered. Actual replacement of flocks can not occur for 3 months.</p>	<ul style="list-style-type: none"> • Ensures the virus is no longer alive. • Reduces the risk of infecting the new/replacement flocks.
<p>Inspect market before restocking by someone knowledgeable about avian influenza (bird flu).</p>	<ul style="list-style-type: none"> • Ensures the site has been fully cleaned and disinfected accordingly.
<p>Add 2 % of birds first and check daily for disease</p> <ul style="list-style-type: none"> • If birds remain healthy, more birds can be added. • If disease is observed, notify authorities to determine cause. • Add new birds from tested and trusted sources. • Monitor birds to make sure infection has not returned. 	<ul style="list-style-type: none"> • Ensures that the farm/infected premise is completely free from AI virus before restocking. • If virus still exists, only a small amount of birds will be affected.
<p>Determine source of infection:</p> <ul style="list-style-type: none"> • Observe in detail the farm and its surroundings. • Interview the farm workers to determine normal practices. • Establish the time/date the disease was first detected. • Trace the movements of people, poultry, poultry bi-products, feeds, eggs and equipment onto and from the farm before and after the first signs of disease. • Cover movements in the period 14–21 days before the outbreak and all movements off the farm after the disease was first detected. 	<ul style="list-style-type: none"> • Provides information to prevent and control further outbreaks. • Promotes changes of practices. • Helps to implement changes of practices.

ACTIONS	WHY
<p>Restocking</p> <p>For restocking, sentinel birds will be kept in the poultry farm as per accommodation of the farm (2% of the house population capacity for commercial poultry operation) or 5 heads (for backyard poultry operation) in the Infected Premises and in selected locations within the Infected Zone.</p> <p>The type of day old poultry to be placed shall depend on the previous type of poultry. However, for game fowl, layer and duck farms, day old cockerels shall be used for restocking.</p> <p>The poultry shall be vaccinated for Newcastle Disease (ND or Ranikhet Disease) and Infectious Bursal Disease (IBD). An optional vaccination for Mycoplasma and Infectious Bronchitis can be done if the virus challenge is high for the particular premises.</p> <p>Restocking continued</p> <p>The sentinel bird shall be grown to a minimum period of 42 days. At least 30 blood samples and 30 cloacal swab samples from the same set of sentinel bird shall be taken on days 21 and 42. These samples shall be tested for antibody to avian influenzas by ELISA and avian influenza antigen by a rapid test for influenza A. If necessary, virus isolation shall be attempted.</p> <p>Any incidence of mortality has to be monitored daily and reported to Upazilla Livestock Office.</p>	

ACTIONS	WHY
<p>In the event the signs are indicative of a potential repeat of infection in the infected premises, the growing of the poultry shall be immediately terminated. The same procedures as in the culling activity shall be implemented.</p> <p>Upon completion of the 42-days period and confirmation by the Bangladesh Livestock Research Institute (BLRI) of the absence of the avian influenza virus, the premises shall be subjected to cleaning and disinfection in preparation for a repopulation.</p> <p>Repopulation of the previously Infected Premises can be carried out upon approval by the Chief Veterinary Officer and the Department of Livestock Services based on results of growing the sentinel poultry.</p>	
<p>Correct any deficiencies:</p> <ul style="list-style-type: none"> • Promote the continued and correct use of PPE (see picture) • Prevent domestic flocks from mixing with wild birds. • Remove items that attract wildbirds & pests (standing water, weeds, spilled feed, etc). • Control pests and predators that may spread disease. • Restrict the movement of animals, manure, poultry, poultry bi-products, feeds, eggs, equipment and people between farms and markets. • Practice basic hygiene such as hand-washing with soap or 	<ul style="list-style-type: none"> • PPE keeps the worker safe, protects families, communities, farms and businesses when used properly. • Promoting continued use of PPE helps prevent complacency. • Preventing the mixing of domestic and wild flocks, restricting movement, and practicing hand-washing helps to prevent transmission and spread of disease.

ACTIONS	WHY
detergent (see picture 1)	
<p>Review transport</p> <ul style="list-style-type: none"> • Promote use of plastic or metal (not wood) for the construction of cages for carriage of birds from farms to markets for easy cleansing and disinfecting. • During transport, implement measures to reduce the risk of fecal contamination of the area around markets where cages and poultry are off-loaded. • Put in place facilities for cleaning and disinfecting transport cages before they are taken back to farms. • Monitor, with an official system, the effectiveness of cleaning and disinfection. 	<ul style="list-style-type: none"> • The presence of feces or dirt makes it much more difficult for the disinfectant to destroy the virus. Therefore, feces and dirt must first be removed by scrubbing or brushing with soap and water. • The virus can be carried to other farms by hands/skin, clothing, shoes or vehicles which have been infected.
<p>Compensate Compensate for losses – provided by government, communities, or co-ops.</p>	<ul style="list-style-type: none"> • Encourages participation and cooperation. • Helps farming industries and communities. • Helps restore confidence with farmers and community.
<p>Restore Confidence (see picture) Communicate effectively with consumers and the public to restore their confidence.</p>	<ul style="list-style-type: none"> • Helps to restore consumer confidence. • Restoring confidence is critical to the recovery of business.
<p>Foster change of industry practices (e.g. poultry production, transportation and marketing) that facilitate viral spread.</p>	<ul style="list-style-type: none"> • Helps to prevent and control further avian (bird) influenza outbreaks.

ACTIONS	WHY
<p>Strengthen public extension/education programs through organizing meetings, seminars, workshop, conference, leaflets, posters, stickers, TV and Radio advertisements etc.</p>	<ul style="list-style-type: none"> • Correct and timely information in the community helps to prevent and control future avian (bird) influenza outbreaks.
<p>Continue training programs.</p>	<ul style="list-style-type: none"> • Ensures correct and timely information transfer to others to help prevent and control future avian (bird) influenza outbreaks.

CONCLUDING REMARKS

This workbook and its guidelines should not be considered an end to your understanding and education concerning the management of avian influenza. Continue to develop your skills in your local community. Take advantage of any trainings being conducted in your village or district.

Like your education on avian influenza, these materials are also a work in progress. The value of the lessons and information included in this workbook is truly attained when District and Upazilla officers become the instructors and leaders concerning avian influenza management in their communities. Share all that you have learned with your family, friends, neighbors and other officers.

This workbook has provided a comprehensive set of guidelines through which District and Upazilla officers learn to effectively address the issues of avian influenza in their local communities. Through the six steps of the job cycle, Upazilla officers have learned how to:

- promote healthy behaviors in the home and farmyard,
- communicate effectively with the community on risk reduction tactics,
- develop appropriate reporting techniques,
- collect and manage samples for testing:
- define the necessary steps to respond effectively to an outbreak, and
- implement strategies in overall harm reduction after an outbreak occurs.

However, completion of this workbook is not meant to serve as the final point in officers' education and training in the prevention and control of avian influenza. Graduates of this training process are encouraged to continue to develop their knowledge, skills, and tools in the local arena to ensure that Officers are as effective as possible. Good luck in your efforts in implementing the knowledge you have gained from this and may they aid in your continued protection of animal and human health.

ANNEX I: DISEASE INVESTIGATION FORM

Government of the People's Republic of Bangladesh
Department of Livestock Services

Disease Investigation Form

Reference No.						Date:		
Name of the Farm:								
Name of the Farm Owner:								
Address:								
Contact telephone number:								
Type of Farm: (Tick)		GP Stock	Parent stock	Commercial layer	Broiler	Backyard		
Number of sheds in the farm:				Number of sheds affected:				
Type of shed: (Tick)		Environmentally controlled			Open sided			
Information about affected flock(s)								
Shed #	Species	Type of Birds	Breed	Source of Chicks (Hatchery)	Flock Size	Age (Wk's)	Litter/Cage/scavenging system	Date of onset of outbreak
Number of birds affected and died (date wise):								
Date	Shed #		Number affected			Number died		
Vaccination history of the affected flock:								
Vaccinated against		Age at vaccination/Date of vaccination				Name of vaccine		

ANNEX 2: WHAT TO DO AND WHAT NOT TO DO

Note to the Upazilla Officer:

Make and distribute copies of this at the village level. list. Share it with farmers, neighbors and villagers well in advance, so that they know what to do and what NOT to do while they are waiting for the Upazilla Officer to arrive on the scene.

WHAT TO DO AND WHAT NOT TO DO WHEN WAITING FOR THE UPAZILLA OFFICER TO ARRIVE

- Do not dispose of dead poultry. The Upazilla Officer or Veterinarian Surgeon will want to see it.
- Children should not be allowed to visit the farm where birds are sick or have died.
- Do not sell the sick or dead birds.
- Do not prepare the sick or dead birds to eat.
- Do not sell or eat eggs from the sick or dead birds.
- Protect the rest of your flock by taking the sick or dead birds out of the flock.
- When removing the sick or dead bird from the rest of the flock, make sure you cover your hands with gloves or a plastic bag. Cover your mouth and nose with a mask (N-95 if possible) or a cloth.
- Do not pick up sick or dead birds with your hands. Use a shovel to pick up the sick or dead birds and take them to an area away from the rest of the flock. Then, place the dead bird(s) in a bag or other container away from other farm animals. Do not leave the dead birds in the farm yard or in an open field. They must be contained in a bag or other closed container. Do not dispose of the bag. The Upazilla Livestock Officer or Veterinarian Surgeon will want to study the contents.

- Do not throw dead birds or animals into a river, pond or any other body of water.
- Clean the shovel and other tools used to move sick or dead birds with soap and water or disinfectant. Wash anything that came into contact with the sick or dead birds.
- Wash your hands with soap and water after removing the sick or dead birds from your flock.
- Wash off your shoes, especially the bottoms of shoes.
- If at all possible, restrict the contact of native and wild birds to the suspected farms. This may only be possible if farm poultry can be kept away in a shed or chicken coop.

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ANNEX 3: SAMPLE SUBMISSION FORM

**Government of the People's Republic of Bangladesh
Department of Livestock Services**

Sample Submission Form

Collection date:			Submission date:		
Submitting veterinarian:					
Designation:					
Address:					
Contact telephone number:					
Farm/Owner:					
Address:					
Species of bird	Type	Breed	Age		
No. of birds in affected flocks	No. affected	No. died	No. of birds sampled		
			Sick	Dead	
History of Outbreak:					
Clinical signs:					
Necropsy findings (if done)*:					
Preliminary diagnosis:					
Details of samples submitted:					
Specimen type	Specimen ID#	Tests requested	Specimen type	Specimen ID#	Tests requested
Date:			Signature:		

***Necropsy should be done only at designated places. Special protection must be taken if AI is suspected**

****Information on preservatives, antibiotics, pooling of samples, etc.**

If commercial feed supplied, specify brand:								
If custom formulated feed supplied, give information:								
If any supplement is given in feed or water, give details:								
If any treatment already given in the present outbreak, give details:								
History of any previous outbreak in the farm:								
Shed #	Flock size	Age at outbreak	Disease diagnosed	Duration of outbreak	No. of birds affected	No. of birds died	Treatment given	Vet consulted

CLINICAL OBSERVATIONS

Clinical signs:					
Necropsy findings (if any)*:					
Samples collected:					
Specimen type	Number of specimens	Laboratory referred to	Date of Shipment	Date of Shipment **	Test requested for:
Investigating veterinarian's name:					
Date:			Signature:		

***Necropsy should be done only at designated places. Special protection must be taken if AI is suspected**

****Must accompany Sample Submission Form**

ANNEX 4: PERSONAL PROTECTIVE EQUIPMENT (PPE)

SEVEN-STEP PROCESS FOR PUTTING ON PPE

Before you begin putting on your PPE, it is important to designate a clean location to put on the equipment, preferably away from anything that could be contaminated with avian influenza or other viruses. Also try to wash your hands with soap and water, if it is available, before you begin, and remove watches and other non-smooth jewelry like bracelets.

I. COVERALLS

Put on coveralls first. Step into the “feet” of the coveralls first, and pull them up. Zip up the front of the coveralls. You should keep your regular clothing and shoes on under the coveralls.



2. SHOE COVERS

Put on shoe covers second. They should fit over your coverall feet, giving you another layer of protection to protect your shoes from contamination.



3. RESPIRATORS

Put the respirator under your chin with the nosepiece up. Pull the bottom strap over your head, and place it around your neck below the ears. Then pull the top strap over your head and rest it high at the top back of your head. Place your fingertips from both hands at the top of the metal nosepiece. Using two hands mold the nose area to the shape of your nose by pushing inward while moving your fingertips down both sides of the nosepiece.



4. GOGGLES

Goggles are adjustable and should fit snugly over and around your eyes. If you wear personal eye glasses, the goggle can be placed over your eye glasses.



Then pull coverall hood over the head, the elastic should hold it in place



5. APRON

Aprons are provided to fit over the coveralls. They are in a small packet that you will open up, place the apron over



6. INNER GLOVES

Put on the inner pair of gloves first (usually white or clear).



7. OUTER GLOVES

Will usually be a different color than the inner gloves and may be thicker. Pull them over the inner gloves. Pull the edge of the gloves over the cuff of your coveralls or gown, if possible.



NOW READY TO ENTER THE CONTAMINATED AREA.



TEN- STEP PROCESS FOR REMOVING AND DISPOSING OF PPE

Before you begin the process of removing your PPE, you should find an area that is not contaminated with blood, soil, feces or other waste.

I. OPEN THE POUCH WITH THE GERMICIDAL WIPE



And use it first on your outer gloves



Then on your outer boots



Place it in the red biohazard bag when done.



2. REMOVE AND DISPOSE OF YOUR APRON



3. REMOVE AND DISPOSE OF YOUR OUTER SHOE COVERS IN THE RED BIOHAZARD BAG.



4. REMOVE AND DISPOSE OF YOUR OUTER GLOVES IN THE RED BIOHAZARD BAG.



5. UNZIP AND ROLL DOWN YOUR COVERALL UNTIL IT IS INSIDE-OUT, AND THEN STEP OUT OF IT. PLACE THE USED COVERALLS INTO THE RED BIOHAZARD BAG.



6. REMOVE YOUR GOGGLES BY PULLING THEM UP OVER YOUR HEAD. YOU SHOULD HANDLE THEM BY THE HEAD BAND OR EAR PIECES. PLACE THEM IN THE RED BIOHAZARD BAG.



7. REMOVE YOUR RESPIRATOR BY GRABBING THE TOP AND THEN THE BOTTOM ELASTIC BANDS AND PULLING THEM UP OVER YOUR HEAD. PLACE THE RESPIRATOR AND GOGGLES IN THE RED BIOHAZARD BAG.



8. REMOVE YOUR INSIDE GLOVES. BEGIN WITH ONE HAND, ROLLING DOWN THE GLOVE (WITH YOUR OTHER HAND), STARTING AT THE WRIST UNTIL THE GLOVE IS INSIDE OUT. HOLD THE REMOVED GLOVE IN YOUR HAND IN A LITTLE BALL, AND THEN ROLL DOWN THE OTHER GLOVE – STARTING AT THE WRIST – WITH YOUR FIRST HAND. PLACE THEM IN THE RED BIOHAZARD BAG.



9. CLOSE THE RED BIOHAZARD BAG BY TYING A KNOT AT THE TOP OR OTHERWISE TYING IT SHUT. THE RED BIOHAZARD BAG SHOULD BE PLACED AT A DESIGNATED LOCATION SO THAT IT CAN BE COLLECTED AND BURNED OR BURIED.

10. WASH YOUR HANDS, FOREARMS AND FACE WITH SOAP AND WATER.



ANNEX 5: CULLING, DECONTAMINATION AND DISPOSAL

Note: Annex 5 consists of 4 Handouts.

HANDOUT #1

CULLING PROCEDURE

Birds for destruction will vary in their size and structure. For small numbers of birds the preferred method is dislocation of the neck using forceps, pliers or by hand. For larger numbers of birds in commercial poultry units the preferred method is gassing with CO₂ in containers.

CO₂ METHOD:

- The objective is the humane destruction of large numbers of birds in a short time. Birds should be unconscious in one minute and dead within 3 to 5 minutes.
- CO₂ gas has human health and safety risks and both a safety officer and First Aid should be available.
- The effort should be organized into teams with specific jobs such as leader, catchers, gas re-supply, gas operators, record keeper (number of birds) and animal welfare.
- A site plan is needed before beginning and should include access to birds keeping movement to a minimum, no birds should escape, enough bins, gas, etc. and easy bin removal. It is expected that most birds will be killed in containers.
- It may be easier to place them in plastic garbage bags for transfer to bins for CO₂.
- If they can be driven into a corner it is also easier for catchers.
- Caged birds are more difficult and progress will be slower. Each catcher can remove 3 or 4 birds and carry them directly to the bins.

- Containers or apparatus should allow the required gas concentration to be maintained and accurately measured.
- The equipment used should be designed, constructed, and maintained in such a way as to avoid injury to the animals and allow them to be observed.
- Animals should be introduced into the container or apparatus after it has been filled with the required CO₂ concentration, and held in this atmosphere until death is confirmed. 17.5 kg gas saturates 1000 cubic meter area.
- The bins need to be pre- charged with CO₂ for about 5 minutes before any birds are received. Each bin should have a secured gas cylinder or each end.
- Hot water should be readily available in case the regulators freeze.
- Place the gas hoses in the bins, about 300 mm above the level of the birds, adjusting as the bins fill with birds.
- Team members should ensure that there is sufficient time allowed for each batch of animals to die before subsequent ones are introduced into the container or apparatus.
- Only one layer of birds should be placed in a bin at once-inspect for death after 20 minutes.
- Additional layers of birds can be added, one at a time, until the bin is 70 to 90% filled.
- Seal the lids for containment and transfer to the disposal site. Care must be taken that no birds are buried alive.
- Please keep in mind that it is not advisable to try to kill water birds such as ducks with CO₂. Cervical dislocation is preferred.
- Also care must be taken that fertilized eggs do not hatch.

HANDOUT #2

METHODS FOR DISPOSING OF CARCASSES

SELECTION OF A DISPOSAL METHOD

The first choice, by far, would be on – site burial. In a larger outbreak one common burial site should be considered. Consideration include amount to bury, site availability, soil type, water table, nearby wells or ponds and digging equipment available.

The second choice is burning but this may be influenced by fire restrictions, prevailing winds, a small site and the availability of cremation fuel. Burning may be quicker, cheaper and a way to avoid a high water table.

BURIAL SITE SELECTION

Important considerations for burial site selection include:

- Access to the site – for both equipment to dig the burial pit and for the delivery of livestock, carcasses or other materials to be buried
- Environmental- distance to water sources, bores and wells; height of water-table; proximity to buildings, especially houses; proximity to neighbors or public lands including roads; slope of the land drainage to and from the pit; permeability of soil; sufficient space for temporary storage of overburden; and direction of prevailing wind
- Construction considerations- avoid rocky areas (slows digging and increases costs) but select soils with good stability capable of withstanding the weight of equipment used to construction of diversion banks if required. Similar banks should be constructed to prevent any liquids escaping from the burial site. Fencing may be necessary to exclude animals until the site is safe for use.

EARTH MOVING EQUIPMENT

- Locally available earth moving equipment will be used.

BURIAL PIT CONSTRUCTION

- The dimensions of the burial pit will be dependent on the equipment used, site considerations and the volume of material to be buried. The preferred dimensions are for pits to be as deep as practically possible (Reach of machinery, soil type and water-table level being the usual constraints), with vertical sides.
- A pit of 2 meters wide, 2 meters deep and 2 meters long would accommodate 1800 birds. If the pit is made one meter deeper the capacity would increase up to 3000 birds.
- Make sure that no bird is still alive when dropped into the burial pit. If this happens, birds must be immediately caught and humanely killed.
- Carcasses should be covered by about 400 mm of soil then an unbroken layer of slaked lime $\{Ca(OH)_2\}$. If this lime is applied directly to carcasses the decomposition process will be significantly delayed.
- When closing the pit, surplus soil should be heaped over the pit as overfill. The weight of soil acts to stop carcasses rising out of the pit due to gas entrapment, prevents scavengers digging up carcasses, helps filter out odors and assists in absorbing the fluids of decomposition. After pit subsidence it will be necessary to replace any topsoil not utilized during pit closure.
- Disinfectants need to be sprayed on equipment used and on the pathway used to take carcasses to the pit. The way to dispose of PPE, tools and animal carcasses and animal parts may be different in each situation or location. District and Upazilla Officials will decide on how best to dispose of dead birds and other items that have come in contact with blood or other animal parts.

HANDOUT #3

DECONTAMINATION

The first consideration would be to decontaminate contaminated areas. The following steps should be taken in order and under site supervisor direction.

PROPERTY ASSESSMENT

This should be detailed starting with making a map and marking in the location of electrical and water lines, drains, effluent run off, identification of a decontamination site as well as a clean unloading site, an estimate of the degree of contamination of each area, the amount of poultry manure and feed and especially heavy or light areas of expected contamination.

PRELIMINARY DISINFECTION

This is designed to quickly start and rapidly reduce the amount of virus present up to the completion of slaughter.

- Any area known or suspected to be contaminated is sprayed. This includes barn feeders, waters (after draining), and access roadways and around the outside of houses and poultry barns as soon as the birds are removed for disposal.
- Spraying should be repeated up to 5 times a day. For disinfection Virkon R S is treated as wonderful disinfectant but other locally available disinfectants are also effective against AIV. These include the use of soaps and detergents as well as phenols, Dettol and quaternary ammonia compounds used after proper cleaning.
- All contaminated materials and surfaces should be allowed to soak with disinfectants for at least 24 hours.

Be sure to disinfect any tools that will be used again and discard PPE by burning or burying it.

- Potassium permanganate is in wide use in the country especially for footbath disinfection. Literature review has revealed that this practice is of limited value against AIV and it is recommended that a commonly available product like Dettol (R) be used instead.

CLEAN-UP

The aim is to remove, without using water, all manure, debris, feed, etc, to expose surfaces for a second round disinfection. This is very important as organic material reduces any disinfectant effectiveness. These types of materials, such as feed and manure, should be buried with the birds. The next step is a wash down with a low pressure sprayer using a detergent or bleaching powder.

FULL SCALE DISINFECTION

- Disinfectant to be sprayed in the following order – roof, walls and finally the floor.
- Inspection is carried out to ensure that everything has been completed- repeat clean -up and disinfection if there is doubt.
- Another round of full disinfection 7 to 14 days later.
- Final disinfection before restocking.

DECONTAMINATION OF EQUIPMENT AND VEHICLES

- The other consideration is the decontamination of contaminated equipment. The primary concern would be for anything used during stamping out. This would include items like CO2 tanks, bins, excavators, back hoes, trucks and other vehicles. Use the decontamination site previously selected. Apply the same principles including cleaning first followed by a low pressure detergent spray, inspection then disinfection spray. Repeat the inspection and disinfectant spray.
- If any trucks, vehicles, motor cycles or rickshaw vans, egg tray are on the contaminated side they must be decontaminated before leaving the premise. Particular attention needs to be paid

to mats under driver's feet. Vehicle interiors, including trunks, can be wiped down with disinfectants on cloths as required. All under parts and wheels of cars should be sprayed with water and disinfection.

- The wicker baskets used to transport poultry are of particular concern as they appear almost impossible to disinfect. Burial or burning appears to be the only solution. Similar attention must be paid to egg flats - burial would be the best solution.

HANDOUT #4

DECONTAMINATION PROCEDURES OF VARIOUS OBJECTS

Disinfectant/chemical selections and procedures for avian influenza outbreak.

ITEM TO BE DECONTAMINATED	DISINFECTANT/CHEMICALS/PROCEDURE
Live Birds	Euthanasia
Carcasses	Bury or burn
Animal Housing/Equipment	Soap & Detergents, Sodium hypochlorite, Calcium hypochlorite, Virkon, Alkalis
Humans	Soap & Detergents
Electrical Equipments	Formaldehyde gas
Water Tanks	Drain to pasture where possible
Dams	Drain to pasture if practical
Feed	Bury
Effluent, manure	Bury or burn, Acids, Alkalis.
Human housing	Soap & Detergents, Sodium hypochlorite, Calcium hypochlorite, Virkon.
Machinery, vehicles	Soap & detergents, Alkalis.
Clothing	Soap & Detergents, Sodium hypochlorite, Calcium hypochlorite, Virkon, Alkalis
Aircrafts	Soap & Detergents, Virkon.

ANNEX 6: ABBREVIATIONS

ABBREVIATIONS	
AI	Avian Influenza
BLRI	Bangladesh Livestock Research Institute
CDIL	Central Disease Investigation Laboratory
CVO	Chief Veterinary Officer
DLS	Department of Livestock Services
DOC	Day Old Chick
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food & Agriculture Organization
FDIL	Field Disease Investigation Laboratory
GPS	Grand Parent Stock
HPAI	Highly Pathogenic Avian Influenza
HA	Haemagglutinin Antigen
NA	Neuraminidase Antigen
OIE	World Organization for Animal Health
PME	Post Mortem Examination
PS	Parent Stock
PPE	Personal Protective Equipment
RDADT	Rapid Direct Antigen Detecting Test
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
ULO	Upazila Livestock Officer
USAID	United States Agency for International Development
VS	Veterinary Surgeon

ANNEX 7: THE EPIDEMIOLOGY OF AVIAN INFLUENZA

Avian Influenza (AI) is caused by specified viruses under the family Orthomyxoviridae, in the genus Influenzavirus A.

AETIOLOGY

There are three influenza genera-A, B, C based on differences between their nucleoprotein and matrix protein antigens. AI viruses belong to Type A. Influenza viruses are further categorized into subtypes according to the antigens of the haemagglutinin (H) and neuraminidase (N) projections on their surfaces. There are 16 haemagglutinin subtypes and 9 neuraminidase subtypes of influenza A viruses (Hence H5N1). And AI viruses have representative in all of these subtypes.

DEFINITION OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI)

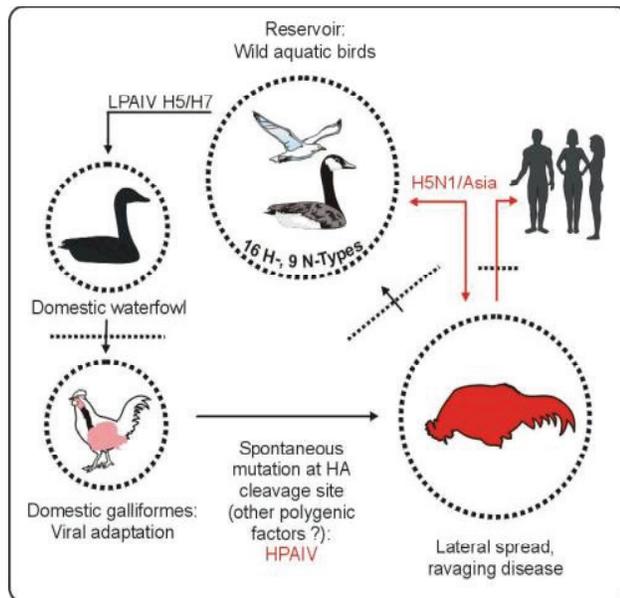
HPAI viruses are those who can cause severe illness and high mortality in poultry. More recently, some HPAI viruses (e.g. H5N1) have been found to cause no illness in some poultry, such as ducks. Avian Influenza A viruses of the subtypes H5 & H7, including H5N1, H7N7 and H7N3 viruses, have been associated with HPAI.

HOW DO OUT BREAKS START?

Most outbreaks start with direct or indirect contact of domestic poultry with water birds which are either non-pathogenic or mildly pathogenic. A virulent strain may emerge either by genetic mutation or by reassortment of less virulent strains.

Once AI is established in domestic poultry, it is highly contagious disease and wild birds are no longer an essential ingredient for spread. Infected birds excrete virus in high concentration in their faeces and also in nasal and ocular discharges. Once introduced into a flock, the virus is spread from flock to flock but the usual methods involving the movement of infected birds, contaminated equipment, egg flats, feed trucks, and service crews, to mention a few.

Transmission may occur if birds are in close proximity and with appropriate air movement. Birds are readily infected via instillation of virus into the conjunctival sac, nares (nose), or the trachea.



INCUBATION PERIOD

The incubation period is 3 to 5 days in general but may be longer. Maximal incubation period is 21 days.

SURVIVAL OF AVIRUS

After an outbreak of Avian Influenza (bird flu), the virus can survive for:

- At least 35 days at 4°C in poultry droppings (feces)
- Several days in carcasses at air or room temperature
- Up to 4 days at 22°C and more than 30 days at 0°C in water
- In general, warmer temperatures kill the virus more quickly, while colder temperatures help it live longer. This is important to know in locations where AI can survive potentially longer in the environment.

HOW INFLUENZA VIRUSES CHANGE – DRIFT AND SHIFT

Influenza viruses are dynamic and continuously evolving. They can change in two different ways: antigenic drift and antigenic shift. They are changing by antigenic drift all the time, but antigenic shift happens only occasionally. Influenza type A viruses undergo both kinds of changes; Influenza type B viruses change only by antigenic drift.

ANTIGENIC DRIFT

Antigenic drift refers to small, gradual changes that occur through point mutations in the two genes that contain the genetic material to produce the main surface proteins, haemagglutinin and neuraminidase. These point mutations occur unpredictably and result in minor changes to these surface proteins. Antigenic drift produces new virus strains that may not be recognized by antibodies to earlier influenza strain. This process works as follows: a person infected with a particular influenza virus strain develops antibody against that strain. As newer virus strains appear, the antibodies against the older strains might not recognize the ‘newer’ virus, and infection with a new strain can occur. This is one of the main reasons why people can become infected with influenza viruses more than one time and why global surveillance is critical in order to monitor the evolution of human influenza virus strains for selection of which strains should be included in the annual production of influenza vaccine. In most years, one or two of the three virus strains in the influenza vaccine are updated to keep up with the changes in the circulating influenza viruses. For this reason, people who want to be immunized against influenza need to be vaccinated every year. It is also why every one should get flu vaccine every year, specially those working with AI so that people do not become infected with human and avian influenza. The virus mix for a super virus potentially start human pandemic.

ANTIGENIC SHIFT

Antigenic shift refers to an abrupt, major change to produce a novel influenza A virus subtype in humans that was not currently circulating among people. Antigenic shift can occur either through direct animal (poultry) to human transmission or through mixing of human influenza A and animal influenza A virus genes to create

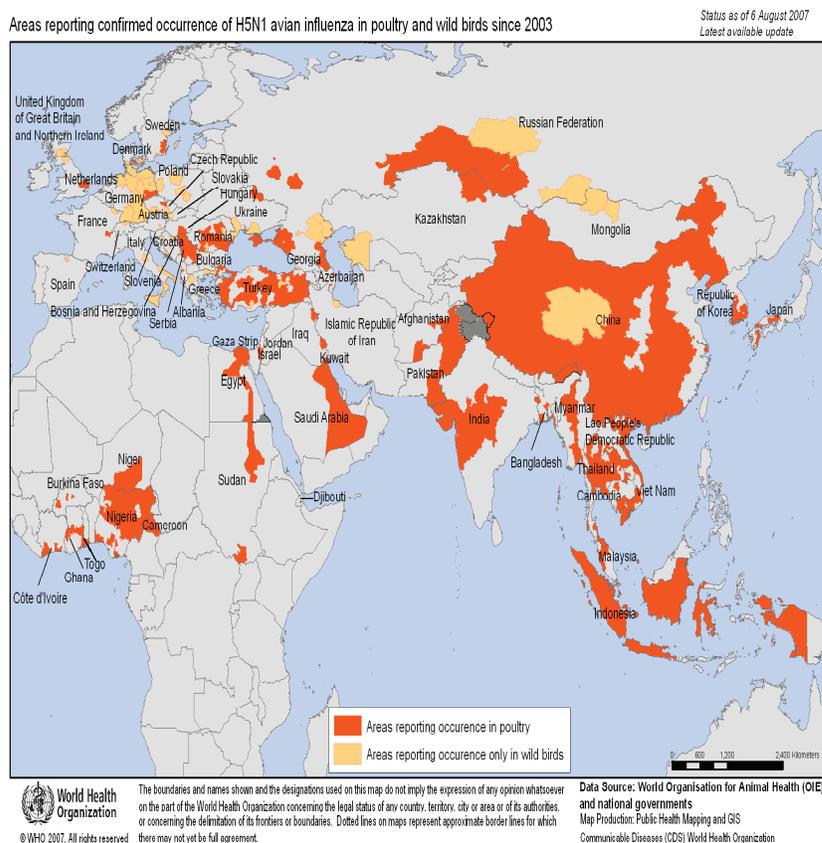
a new human influenza A subtype virus through a process called genetic reassortment. Again this is why especially poultry workers should be vaccinated so that people do not get co-infected that leads to pandemic. Antigenic shift results in a new human influenza A subtype. A global influenza pandemic (worldwide spread) may occur if three conditions are met.

NATURAL HOSTS

Domestic fowl, ducks, geese, turkeys, guinea fowl, quail and pheasants are susceptible. Disease outbreaks occur most frequently in Domestic fowl and turkeys. Many species of wild birds particularly water and sea birds are also susceptible, but infections in these birds are generally sub clinical.

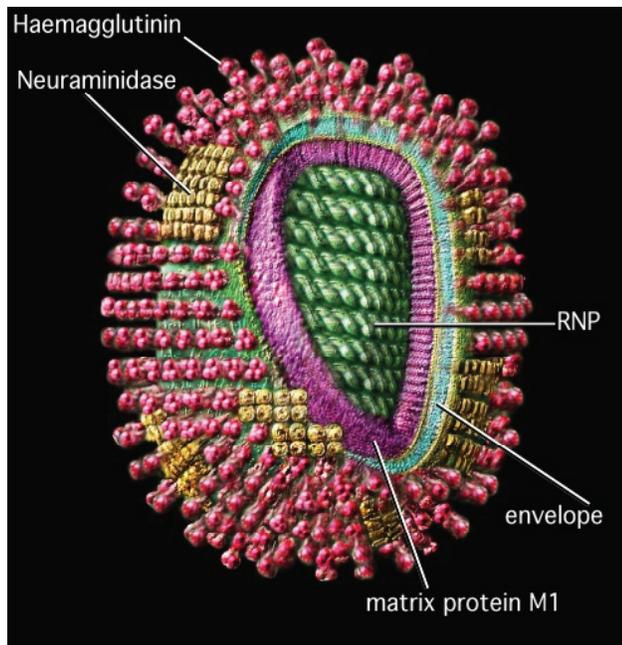
WORLD DISTRIBUTION

AI viruses are probably ubiquitous in wild water birds. Pathogenic strains could emerge and cause disease in domestic poultry in any country at any time without warning. In fact, outbreaks have occurred at irregular intervals on all continents. See Map below.

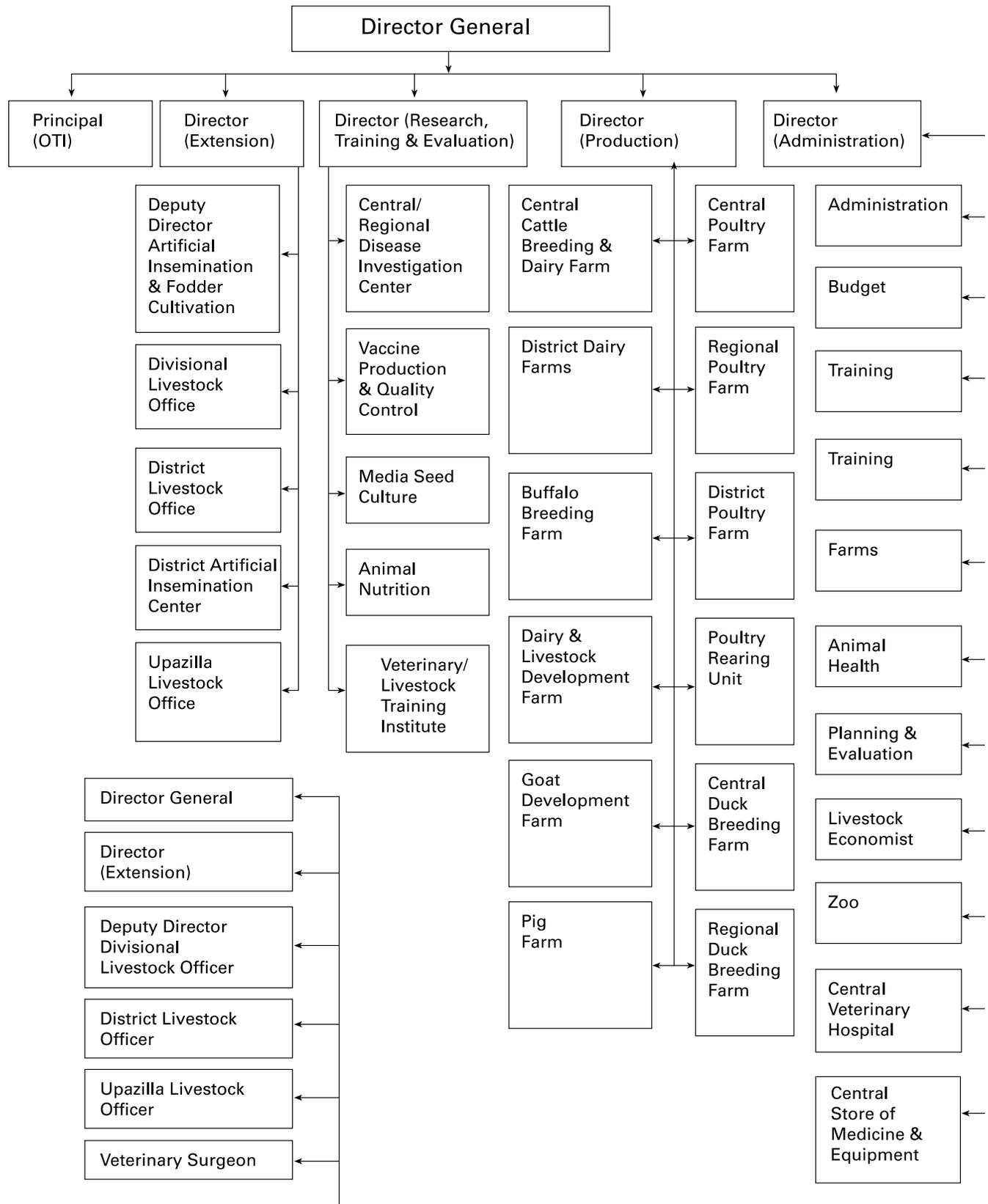


EXPLANATION OF PICTURE

The green core (RNP-ribonucleoprotein) contains the genetic information of the virus wrapped up in protein. This combination of genetic material and protein is called the nucleocapsid. In influenza, the nucleocapsid is helical. The genetic information is stored as single stranded -ve sense RNA. The full complement of genetic information is called the genome and in influenza the genome is divided into eight segments. These segments are assumed to link together (possibly in an ordered fashion) to form a helix when the virus assembles at the cell surface. Overlying the nucleocapsid is a layer of matrix protein, M1, shown in purple. Overlying the matrix is the viral envelope (blue-green and edged in yellow) derived from the host cell membrane (the nucleocapsid and the matrix proteins become wrapped in cell membrane as they bud from the infected cell). The characteristic “spikes” of the influenza virus are haemagglutinin. They radiate all over the surface and are interspersed (in some types) by clusters of neuraminidase. These (HA and NA) molecules are thought to pass through the envelope and interact with the underlying matrix protein, M1.



ANNEX 8: ORGANIZATIONAL CHART DIVISION OF LIVESTOCK SERVICES (DLS)



ANNEX 9: FREQUENTLY ASKED QUESTIONS

Note to reader:

As officers or villagers ask questions about avian influenza you can use these handouts to help answer their questions. They are based on the most frequently asked questions and answers from other animal and health workers around the world.

FREQUENTLY ASKED QUESTIONS

AVIAN INFLUENZA IN BIRDS

1. What is Avian Influenza?

The disease commonly referred to as “bird flu” is an animal infection caused by the H5N1 virus. The virus occurs naturally among birds. Wild birds carry the virus in their intestines, but usually they do not get sick. But some domesticated birds - like chickens, - get very sick and can die from the virus.

2. Which birds carry the virus?

Avian influenza can kill domesticated birds, including chickens, ducks, geese, and turkeys. Traditionally, wild waterfowl and shorebirds have been credited as the sources for the many strains of avian influenza, but rarely fell ill. The current H5N1 strain has caused mortality in 40 species of wild birds, including geese, storks, egrets, herons, and falcons, and some mammals.

3. How does it spread?

The virus can remain viable in droppings for long periods, spreading among birds and animals through ingestion or inhalation of the droppings. Virus can also be excreted from the eyes, nose and mouth of infected birds.

Transmission from flock to flock is usually by humans – avian influenza viruses can be spread by manure, equipment, vehicles,

egg flats, crates, and people whose clothing or shoes have come in contact with the virus.

4. How can humans contract avian influenza?

There are several ways humans can get the virus: contact with infected poultry; eating contaminated poultry meat or eggs; drink from water sources that have been contaminated by bird feces or feathers; breathing in feces, blood, or mucous from infected birds.

5. Why should I worry about Avian Influenza when it is a bird disease?

Current outbreaks of the virus have been happening among the bird populations since 2003. Animal and human health experts are concerned that the virus is crossing the species barrier and is infecting humans. There have been reported human cases and deaths in Indonesia, Vietnam, China, Egypt, and Cambodia. Scientists and the medical community are closely monitoring the virus to see if it mutates which will make it easier to spread from human to human.

6. How is Avian Influenza virus spread?

Avian influenza is not an airborne disease. Most of the current evidence suggests that the virus spreads mainly through the movement of poultry and birds, poultry products like eggs and feathers, people and the vehicles used for transport. This is why human contact with poultry and poultry products needs to be controlled and good bio-security practiced so the virus will not spread.

7. How could avian influenza reach a country?

There are several ways that the influenza can be introduced into your country or community:

1. By Importing poultry or eggs that are infected;
2. By Illegal trade and importation of live birds that are infected;
3. By a person who has been at a facility or farm that has infection and carries the virus on his or her clothes, shoes, or equipment such as car/truck tires.

4. Through the handling and slaughter of infected live-poultry.
5. Wild birds migrating are another source but studies show they are less of a threat than earlier believed.

8. Can the virus be contained?

Yes, the most common practice to contain the spread of the virus is: culling of all infected or exposed birds; proper disposal of carcasses; and the quarantining and rigorous disinfection of farms and poultry markets.

Vaccination has also been used but is impractical outside commercial settings and the vaccine requires regular updating.

The virus is killed by heat (56 degrees C for three (3) hours or 60 degrees C for 30 minutes) and common disinfectants, such as formalin and iodine compounds. Thorough cooking of any poultry meat will destroy the virus, however, if poultry appears sick or is dead do not prepare it for cooking or consumption. Dispose of the poultry properly.

9. What can I do to protect my poultry?

The number one practice is to observe good bio-security practices – keep your yard clean; your poultry separated from other wild and domestic birds; clean tires and equipment when it enters your farm, and control the introduction of new birds and eggs to your farm by keeping new poultry separate for at least 14 days.

10. Is it safe to buy and eat poultry?

Yes, poultry products should be properly cooked and properly handled during preparation – that includes covering your face with a mask or cloth so you won't breathe in blood, mucous, feathers and feces and wear gloves or use plastic bags. Meat should be well-cooked (not pink) and eggs should not be eaten raw or with runny yolks. If poultry is cooked at normal temperatures – about 70 degrees Centigrade for about 30 minutes – the heat and cooking will kill the virus. Be safe and do not eat birds that have recently died.

11. If my poultry dies can I eat it? We do when our birds die from Exotic Newcastle Disease.

No, this is not like other virus that kill of your flocks. You should not prepare dead birds for eating. Preparing and eating infected poultry could make you infected too.

12. What should I do if I think my flock is infected with avian influenza?

Call the relevant authorities immediately.

Because the signs of avian influenza are so variable, it is important to get the help of an expert for diagnosis. Keep children and pregnant women away from the birds. If you are instructed to handle or dispose of a dead or infected bird: Wear protective equipment and clothes (including gloves) and Place the dead birds into a bag. Dead birds should not be disposed of in a river or a pond, or left in the yard.

Protective clothing or equipment should be kept away from other people and thoroughly disinfected after use.

FREQUENTLY ASKED QUESTIONS

AVIAN INFLUENZA IN PEOPLE

1. Are people at risk for avian influenza?

To date, most human cases have been limited to people who have had contact with infected poultry or contaminated surfaces. Many of these human cases have occurred in rural or suburban areas where households keep small poultry flocks.

2. What can people do to reduce the risk of getting avian influenza?

There are several key behaviors people who come in close contact with poultry can adopt to reduce the risk of contracting the virus:

- Protect healthy flocks from the introduction of new poultry by quarantining new poultry for 14 days;
- Separate ducks from chickens;
- keep poultry in a closed building,
- clean up yards and coops daily to remove droppings;
- wash their hands with soap before and after handling birds; and
- clean off their shoes before entering their homes

If possible, children and pregnant women should be kept away from poultry and poultry parts, and should not handle eggs.

If poultry appears sick, people should not touch it or handle it. Call the local authorities. (Keep in mind that ducks often do not show symptoms of the virus.)

If people must handle a dead bird, they should:

- Wear protective equipment and clothes (including gloves) and
- Place the dead birds into a bag.

- Do not dispose dead birds in a river or a pond, or left in the yard.
- If you think you have been exposed, try to minimize contact with others.

3. Why is there so much concern about this virus?

Although the current outbreaks have been happening since mid-2003, beginning in Asia and spreading around the world, this is the first time that so many countries been affected at the same time by this virus. The animal and human health experts' concern is that the virus is crossing the species barrier and is infecting humans. Scientists are closely monitoring the virus to see if it will mutate, making it easier to spread from human to human.

4. What is the difference between regular, seasonal flu and avian influenza?

These are different viruses. The difference that makes the most distinction to the layman is that that avian influenza is transmitted from birds to birds and birds to humans, but at this point not human to human. That is one of the reasons it is being watched so carefully to see if the virus changes - or mutates - and can be transmitted from human to human. Unlike normal seasonal influenza, where infection causes mild respiratory symptoms in most people, H5N1 has been found to cause more severe symptoms and leads to faster deterioration in condition. In the present outbreak, many of those infected with the virus have died, and many cases have occurred in previously healthy children and young adults.

5. Can we treat avian influenza?

There is some evidence that recent H5N1 viruses are susceptible to a class of antiviral drugs called neuraminidase inhibitors – oseltamivir (also known as Tamiflu) and zanamivir (also known as Relenza). H5N1 appears to be resistant to the alternative M2 inhibitors – amantadine and rimantadine. Most experts agree that neuraminidase inhibitors will be vital in controlling a future pandemic. However, flu viruses can become resistant to drugs.

6. Is there an avian influenza vaccine for people?

Not yet. There are several potential vaccines for protecting humans from infection with bird flu, at various stages of testing. Whether they would be suitable for use against a new pandemic flu strain depends on how much that strain may have mutated from the original H5N1 virus strain. In addition, due to production issues, it is not likely that an effective vaccine would be widely available until several months after the start of a pandemic.

7. Will a regular flu shot protect against avian influenza?

No. The annual flu vaccination will not provide protection against avian influenza. Current vaccines protect only against circulating human strains.

8. What are the symptoms of avian influenza in people?

The symptoms are similar to those of other forms of influenza: Including fever, sore throat, cough, headache and muscle aches and pains. These symptoms may vary in severity. If you think you may have been exposed, minimize your contact with others.

9. What should I do if I think I have avian influenza?

Keep in mind that people get respiratory infections quite regularly, and that the chances that your symptoms are from avian influenza are low. But if you have recently been near chicken or other poultry or have returned from an area where avian influenza in humans has been reported and you are experiencing any of the symptoms outlined above, you should seek medical advice. Tell your health care provider of your recent travel and activities, including any visits to farms or markets.

10. Is it safe to buy and eat chicken?

Yes, as long as import controls are strictly enforced. In countries where avian influenza has been reported, poultry and poultry products should be properly cooked and handled during food preparation. Normal temperatures used for cooking (70 degrees C for at least 30 minutes) will kill the virus. Make sure that all parts of the poultry are fully cooked (no “pink” parts) and eggs are also properly cooked (no “runny” yolks).

Remember you should not buy, eat or prepare poultry that is sick or has died.

FREQUENTLY ASKED QUESTIONS

PANDEMIC RISK

1. What are the chances that avian influenza could cause a human pandemic?

Not likely. There are several critical steps that must occur before a human pandemic can happen. These include: a new influenza virus subtype emerges; it infects humans, causing serious illness; and it spreads easily and is sustainable among humans. The H5N1 virus has met the first two criteria, but it has not yet efficiently and sustainably infected humans. The risk that the H5N1 virus will acquire this ability remains as long as there are opportunities for human infection; however, control measures that are being undertaken worldwide continue to minimize these risks.

2. Can a pandemic be averted?

Yes. That is why so much attention by governments and the health professionals is being placed on how to prevent and control the virus.

The first priority is to reduce opportunities for human exposure to infected or potentially infected poultry. **Computer modeling has suggested that a human pandemic could be stopped or slowed with concerted action such as**

- washing your hands with soap and water before and after handling poultry, separating ducks and chickens,
- keeping poultry fenced or penned in,
- keeping new poultry separated from existing flocks for 14 days.

ANNEX 10: STANDARD OPERATING PROCEDURES (SOP) FOR COLLECTING AND SUBMITTING SAMPLES FOR TESTING

The only way to confirm that there is an avian influenza outbreak in your area is to collect good samples of the virus and to make sure that they get to the proper location for testing. The samples that are sent to international laboratories will also be tested and used to do genetic typing, which will help researchers track the virus and its spread, as well as provide information to people who are developing vaccines for avian influenza.

One of the most important things to remember is that you should only use the tests on sick birds or humans. The tests will not be effective if you use them on animals or humans that do not have any symptoms that match the case definition of the avian influenza virus.

OBTAINING SAMPLES FROM BIRDS

For animals, the preferred way to obtain a sample is **from the trachea or the respiratory tract of a living bird**. Because avian influenza can affect the large intestinal tract of birds (in addition to the respiratory tract), cloacal samples from sick or recently dead birds is also acceptable.

Fecal specimens (or droppings) can be collected from cages or from the environment, but this is only if you cannot obtain tracheal or cloacal samples. With fecal samples, you cannot be certain that it came from birds living in a cage or roaming in environment.

If the birds are dead, you can obtain samples by lung lavage, tissue from the trachea and lung, or from fecal droppings.

OBTAINING SAMPLES FROM HUMANS

For humans, there are three ways to obtain specimens to diagnose avian influenza in the respiratory tract:

- nasal swab
- throat swab
- tracheal swab

To take samples, many supplies are needed including both personal protective equipment (PPE) and sampling and testing equipment.

PERSONAL PROTECTIVE EQUIPMENT	QUANTITY
Infectious waste bag	4 per kit
Alcohol pads, 70%	16 per kit
PDI Sani Cloth virucidal wipe	1 case of 150 wipes per kit
DuPont Tyvek white coverall with hood and boots	4 per kit
DuPont Proshield III shoe covers	4 pairs per kit
Vinyl inner gloves, 4 mil	4 pairs per kit
Chemical splash goggles with indirect vents	4 pairs per kit
N-95 respirator	4 pairs per kit
Respirator fit test kit (Bitrex solution)	1 test per kit
Plastic apron	4 per kit
SAMPLING AND TESTING EQUIPMENT	
Synbiotics Avian Influenza Type A Antigen Test Kit	1 box of 30 tests
BD Universal Viral Transport Combo Kit	1 box of 50 kits
Biological Sampling Module	1 per kit
Directigen Human BD EZ Flu A+B Test Kit	1 box of 30 tests
Tissue Forcep 1x2 Teeth Econ 5-1/2"	4 per kit
Scissors, general surgical, straight, CRS	5 per kit
Poultry shears, Heritage cutlery 7220	4 per kit
Saline Solution	1000 mL container per kit
Polyurethane Swabs	5 swabs (2.7 inch) per kit
Pipettes	5 pipettes (5 mL) per kit
PACKAGING AND SHIPPING EQUIPMENT	
Mailing labels	1 roll of 100 labels
Safe-T-Pak STP-310 insulated infectious shipper	1 box of 4 shippers
Safe-T-Pak STP 314 freezer gels	1 case per kit

Ammonium nitrate cold packs	12 per kit
Sharpie markers	1 dozen per kit
Rubber bands	1 bag of 25
Whirl-Pak 5x12" sterile sample collection bags	Pack of 500
Igloo cooler pack	1 per kit
Packaging tape 3"	1 roll per kit

PERFORMING RAPID DIAGNOSTIC TESTS

Rapid testing can be performed on both animals and humans. Testing should always be performed while wearing PPE.

The test is highly specific and sensitive test for the detection of Type A avian influenza virus. It is called a rapid test because you get results in 15 minutes. Other unique features of the test are:

- It is easy to learn to use
- It can be used in both the laboratory and on the farm
- It is highly sensitive, even to very small amounts of virus
- It detects all subtypes of Type A influenza virus, which is the type of influenza that has caused all of these avian influenza outbreaks
- It can be used to monitor all types of birds, including flocks of chickens, turkeys, ducks or migratory birds, for active exposure to avian influenza virus

The name of the test is called Flu Detect and was created by Synbiotics Corporation. It has been used around the world, including by the USAID as well as by the UN Food and Agriculture Organization (FAO) in infected and high-risk countries.

The Flu Detect Kit was designed to test tracheal samples, but can be used with both tracheal and cloacal samples.

FLU DETECT – TESTING OF SINGLE TRACHEAL SWAB

Tracheal samples should be taken from behind the tongue area of the bird,



Collecting a tracheal sample.

The trachea of live birds is swabbed by inserting a swab into the trachea and gently swabbing the wall. The swab is then placed in transport medium.

It is also acceptable to take tracheal swabs from dead animals after you remove the lungs and trachea from the carcass. The trachea is held in a gloved hand and the swab inserted to its length with vigorous swabbing of the wall. The swab is then placed in transport medium.

All samples should be at normal “room temperature” before running the test.

- Place 8 drops (approximately 250 μ l) of Extraction Buffer in a clean test tube.
- Place the swab containing the specimen in the tube and rotate the swab 5 – 10 times in the buffer.
- When removing the swab from the tube, press the swab against the side of the tube repeatedly until no more liquid comes from the swab.
- Discard the swab in a biohazard container (such as the red biohazard bag that comes with your PPE).
- If the samples will not be tested immediately, cap the tube with the provided cap and store the sample in the cooler provided in your kit.

- Insert the test strip into the tube.
- Allow the tube to sit at room temperature for 15 minutes.
- Read test strip results.

FLU DETECT – TESTING OF SINGLE CLOACAL SWAB

Cloacal samples should be taken from within the cloacal area of the bird, avoiding excess solid fecal material or visible blood.



Collecting a cloacal sample.

A cloacal swab from a live bird is taken by inserting a swab deeply into the vent and vigorously swabbing the wall. The swab should be deeply stained with fecal material. The swab is then placed in transport medium.

If fecal specimens from the cages of sick poultry in bird markets or from wild birds in the field are collected from freshly deposited wet feces, the swab should be heavily coated with feces. The swab is then placed in transport medium as above.

THE PROCESS FOR TESTING A SINGLE CLOACAL SWAB ON SITE

All samples should be at typical “room temperature” before running the test

- Pour 0.5 ml of BHI or Viral Transport Media into a clean test tube

- Dip 1 cloacal swab into BHI or Viral Transport Media.
- Place the swab containing the specimen in the tube and rotate the swab 5 – 10 times in the buffer.
- When removing the swab from the tube, press the swab against the side of the tube repeatedly until no more liquid comes from the swab
- Discard the swab in a biohazard container such as the red biohazard bags provided with your PPE.
- Drop 200ul of extracted sample into a second test tube
- Add 3 drops of extraction buffer into the tube and mix it thoroughly
- If the samples will not be tested immediately, cap the tube with the provided cap and store the sample in the cooler provided in your kit.
- Insert the test strip into the tube.
- Allow the sample to sit at room temperature for 15 minutes.
- Read test strip results.

HOW TO SAMPLE AND PERFORM RAPID DIAGNOSTIC TESTS ON HUMANS

The test kit is called the Directigen EZ Flu A + B. The Directigen EZ Flu A+B test can detect influenza A and B virus from nasal and throat swabs of people who have symptoms of avian influenza. As most of you know, influenza symptoms generally include the sudden onset of fever, chills, headache, fatigue and cough.

This test can tell the difference between influenza A virus and influenza B virus, by using one sample and placing it in the testing device, it is possible to find out whether the person being tested has influenza B, influenza A or both. The results are available in 15 minutes, which is why it is called a rapid test.

It is important to determine if influenza A or B is causing symptomatic disease. If the person tests positive for influenza B they do not have avian influenza (at least not HPAI circulating now). It means that they have another type of influenza that is causing their symptoms.

The test manufacturer recommends that all negative test results should be confirmed.

In each box of the Directigen EZ Flu A+B Test Kit there are 30 tests. Each of them contains the following items:

- one 4.5 mL bottle Reagent E
- one 2.8 mL bottle A+/B- Control
- one 2.8 mL bottle A-/B+ Control
- 30 Directigen EZ Flu A+B Devices
- 30 DispensTube Tubes
- 30 DispensTube Tips.

Each Directigen EZ Flu A+B device contains both positive and negative internal/procedural controls to let you know whether the test is working properly. For example, if you see a reddish purple control line in the Flu A and/or Flu B windows at the Control “C” position, it means that the test is working properly, and that the correct test procedure was followed.

The area surrounding the Flu A and/or Flu B test and control lines is the internal negative control for the device. If the background area is white to light pink, this means that the test is working correctly.

If the kit controls do not perform as expected, do not report patient results. Try to use another test that is working properly.

There are a few other important points to keep in mind with this test. First, the reagents in the kit contain sodium azide, which is harmful if you breathe it in, swallow it, or touch it with your skin. If the reagents come in contact with your skin, you should wash immediately with plenty of water. If you are disposing the reagents down pipes that might be made with either lead or copper, make sure to flush it down the pipes with large amounts of water.

Other things to watch for are:

- You should not use kit components beyond the expiration date.
- You should not mix reagents from different kit lot numbers.
- You should not reuse the device.
- You should not use the kit if the Control A+/B- and Control B+/A- do not show appropriate results.

CONDUCTING HUMAN SAMPLES FROM THE NOSE OR THE THROAT

For nasal samples, insert a dry swab into the nostril, parallel to the palate, and then leave it in place for a few seconds. Slowly remove the swab, with a rotating motion, down the inside of the nose. It is all right to obtain samples from both nostrils with the same swab. The tip of the swab is then put into a vial containing 2–3 ml of transport medium and the applicator stick is broken off.

For a throat swab, the back of the throat is swabbed vigorously, and the swab is placed in transport medium.

HOW TO PREPARE SAMPLE COLLECTION VIALS

- First you begin with sterile plastic screw-cap vials.
- Drop 1.0–2.0 ml of transport medium into each vial. It is preferable to store these vials at –20 °C until you are ready to use them. They can also be stored at 4°C for 48–96 hours or at room temperature for short periods of time, such as 1 or 2 days.
- Vials should be assigned a number that corresponds to that on the Field Data Collection Sheet. When possible, the following information should be recorded on the Field Data Collection Sheet: type of animal sampled, species, type of sample, date of collection, and geographical location of the sample collection.
- The samples should then be collected (as we discussed during the sampling techniques section), and added to transport medium. All specimens should be kept on ice or at 4° C.

Note: Tissue samples should be frozen immediately without transport medium.

STORAGE OF SAMPLES

Samples should be kept in the viral transport medium at 4° C and transported to the laboratory as soon as possible. If the samples are taken to the laboratory within 2 days, they may be kept at 4 °C. Maintain this temperature by using the cooler and cold packs.

If it will take longer than two days to send the samples to a laboratory, freeze them at or below –70 °C until they can be

delivered to the laboratory. Avoid repeated freezing and thawing, as this might destroy the samples.

STORING A BLOOD SAMPLE

Blood samples can be stored at 4 °C for approximately one week, but for longer than one week, they should be frozen at –20 °C. Allow the blood to clot and then use a centrifuge at 2500 rpm for 15 minutes to separate red blood cells and serum. The serum should be removed with a pipette, and the red cells may be discarded.

PREPARING SAMPLES FOR SHIPPING

To prepare samples for shipping or delivery to a laboratory, pack them into Safe-T-Pak insulated shipper boxes, along with freezer gels or cold packs.

Influenza samples should not be stored or shipped in dry ice (solid carbon dioxide) unless they are sealed in glass or sealed, taped and double plastic-bagged. This is because carbon dioxide can kill influenza viruses if it comes into contact with the samples. In addition, shipping with dry ice is hazardous.

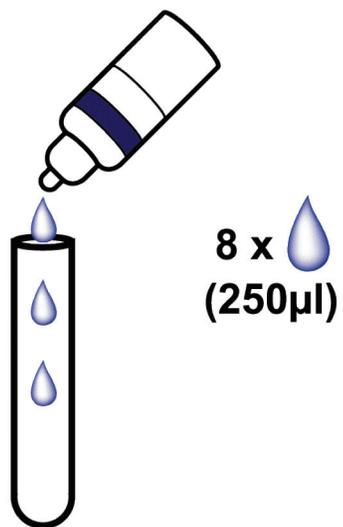
It is important to pack the samples into the shipper box so that they cannot break, be punctured or leak their contents, assuming “normal” transportation movement. Place the samples into an inner package, and then place it into a final, outer package surrounded with material that can act as a cushion and protect the samples from damage. Any containers used should be leak-proof.

The World Health Organization has guidelines on the transport of infectious disease samples, and each country usually has regulations on transporting infectious materials within its borders, so you should become familiar with the procedures in your country.

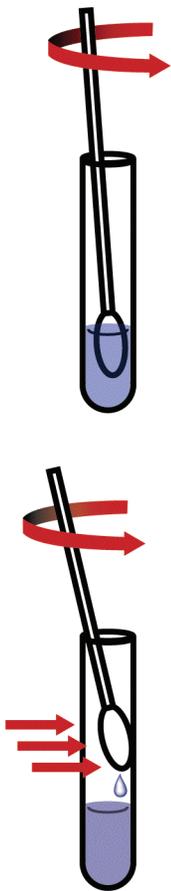
FLU DETECT TEST KIT FOR INFLUENZA A DIAGNOSIS (FOR ANIMALS)



USING THE FLU DETECT TEST STRIP (FOR ANIMALS)

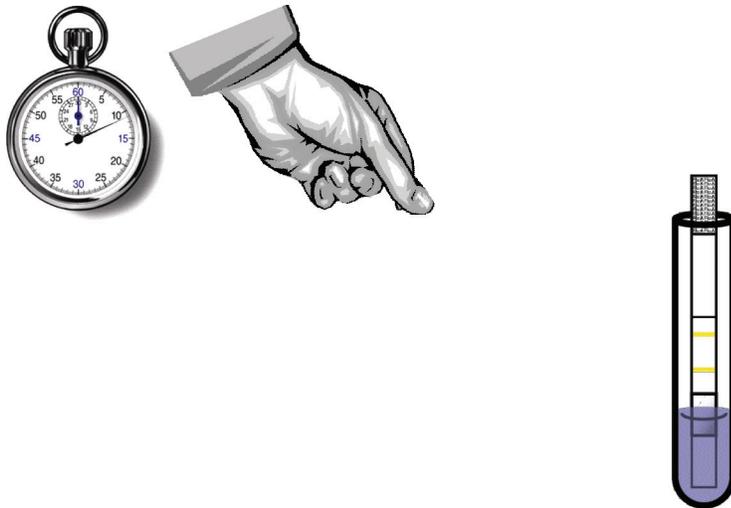


Step 1
8 drops (~250µl) extraction buffer into test tube



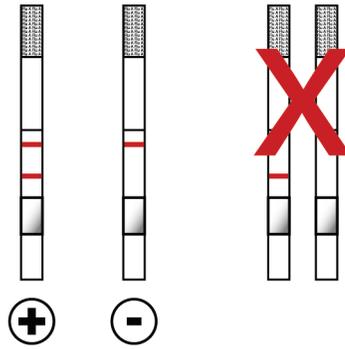
Step 2

Insert swab, rotate. Press swab against side of tube to extract liquid. Dispose of swab.



Step 3

Insert test strip, labeled side up, so that pink pad is just submerged into extracted sample. Incubate for 15 minutes. Remove strip and read results.



Step 4

Reading results and validation:

Control line at top, closest to handle.

Absence of control line indicates invalid test.

QUESTIONS AND ANSWERS ON USING SYNBIOTICS FLU DETECT (FOR ANIMALS)

1. Under what temperature conditions should the Synbiotics Flu DETECT Antigen Capture Test Strips be shipped and stored?

The Flu DETECT Test Strips are designed to be shipped, stored, and run at normal room temperatures: 15 to 25°C. For longer storage, the kit can be stored at refrigerated temperatures: 4°C.

2. What is the shelf life of the Synbiotics Flu DETECT Antigen Capture Test Strips?

The shelf life of the Flu DETECT Test Strips is 18 months from the date of manufacture.

3. Is it possible to pool multiple samples and test with a single Test Strip?

Yes. However, it is recommended that no more than six (6) swabs be tested at one time.

The following procedure is recommended:

- Add 2.5ml of BHI broth or Viral Transport Media to tube, after adding each swab, swirl, remove.

- Transfer 200µl of the BHI pooled sample material to another tube.
- Add 3 drops of extraction buffer from kit.
- Run the Test Strip as per the package insert.
- The remaining pooled sample material can be used for RT-PCR or VI.

4. What if I want to test pooled tracheal samples with Virus Isolation or RT-PCR at the same time?

If testing samples with Virus Isolation, the following procedure is recommended:

- Pool no more than 6 sample swabs at once. Add 2.5 ml of BHI broth or Viral Transport Medium to the 6 swabs.
- Remove 200 µl of the broth and mix with 150µl of extraction buffer.
- Run the Test Strip as per the package insert.
- The remaining sample BHI or Viral Transport Media can be used for Virus Isolation or RT-PCR.

5. How can I store samples for transporting them to a laboratory?

After collection, samples may be extracted, or swabs may be removed from the handle (by breaking the handle just above the swab) and stored in a capped test tube for testing at a later time. If the sample swabs are also needed for Virus Isolation, then add BHI broth or Viral Transport Medium to the tube, add the swab, break the swab handle and cap the tube. To test, follow the procedures outlined above.

6. Is it possible to use the Synbiotics Flu DETECT Antigen Capture Test Strips for testing other avian hosts, such as turkeys, ducks, geese, migratory birds, etc.?

Yes. The Flu DETECT Test Strips will detect Type A Influenza from these avian hosts just as well as with chickens.

7. Is it possible to use the Synbiotics Flu DETECT Antigen Capture Test Strips for the general identification of candidate AIV isolates in chicken embryo materials?

Yes. Remove 100µl of allantoic fluid, transfer to a sample tube, and then add 100µl of extraction buffer. Run the Test Strip as per the package insert.

DIRECTIGEN EZ FLU A+B KIT (FOR HUMANS)

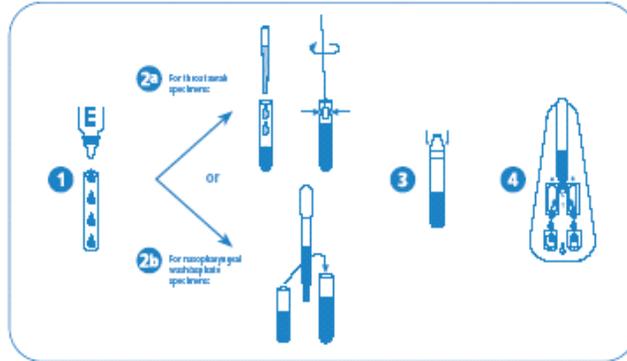
The following components are included in the Directigen EZ Flu A+B kit

BD Flu A+B Devices	30 Devices	Foil pouched device containing two reactive strips. Each strip has a test line of monoclonal antibody specific to either Flu A or Flu B influenza viral antigen and a control line of anti-species antibody.
Reagent E	4.7 mL	Detergent, with 0.2% sodium azide (preservative).
Control A+/B-	2.8 mL	Flu A Positive and Flu B Negative Control, inactivated influenza A antigen (recombinant nucleoprotein) with 0.1% sodium azide (preservative).
Control B+/A-	2.8 mL	Flu B Positive and Flu A Negative Control, inactivated influenza B antigen (recombinant nucleoprotein) with 0.1% sodium azide (preservative).
DispensTube Tubes	30	Tubes for specimen processing and sample delivery into devices.
DispensTube Tips	30	Tips to filter sample when delivered into devices.

Directigen . EZ Flu A+B
 For the Differentiated, Direct Detection of
 Influenza A and B Viral Antigens

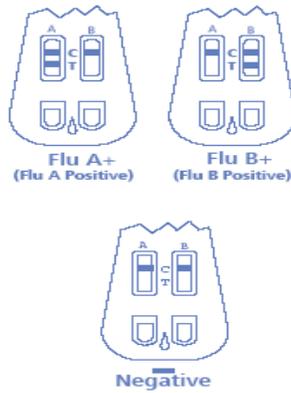
Easy to follow instructions:

1. Add 4 drops of Reagent E.
- 2a. Add 300 µL normal saline to DispensTube™. Swirl 6-8 times, remove swab while squeezing;
- or
- 2b. Pipette 300 µL specimen into DispensTube.
3. Cap tube with DispensTube tip, mix thoroughly.
4. Add 3 drops to each sample well, read results at 15 min.



Directigen . EZ Flu A+B

INTERPRETATION OF RESULTS



Directigen. EZ Flu A+B

INTERPRETATION OF RESULTS



Flu A Positive

ANNEX 11: STANDARD OPERATING PROCEDURES (SOP) FOR PACKING, LABELING AND SHIPPING SAMPLES

PACKAGING AND SHIPPING OF SAMPLES TO INTERNATIONAL REFERENCE LABORATORIES

Before packing and shipping avian influenza samples it is important to be able to:

- Recognize the abbreviations and definitions used in packaging and shipping samples of infectious substances.
- Understand exactly who is involved in transporting dangerous goods.
- Understand your responsibilities as the sender or shipper.
- Understand how to classify substances as category A or category B.
- Demonstrate proper packaging of samples of infectious substances following International Air Transport Association (IATA) Dangerous Goods Regulations (DGR).
- Demonstrate proper labeling of samples of infectious substances following IATA Dangerous Goods Regulations (DGR).
- Demonstrate proper documentation of samples of infectious substances following IATA Dangerous Goods Regulations (DGR).
- Practice preparing shipping labels and documentation of infectious substances.
- Practice classifying, packaging, labelling and documenting of infectious substances and Exempt Patient/Animal Specimens.
- Participate in the IATA shipping training certification test if it is offered in your area.

ABBREVIATIONS AND DEFINITIONS USED IN PACKAGING AND SHIPPING SAMPLES OF INFECTIOUS SUBSTANCES

Many abbreviations and definitions are used in the packaging and shipping of infectious substances.

ABBREVIATIONS

ICAO – International Civil Aviation Organization (UN organization)

IATA – International Air Transport Association

DGR – Dangerous Goods Regulations

49CFR – Code of Federal Regulations, US Department of Transportation

DEFINITIONS

Infectious Substances

Substances which are known to contain, or are reasonably expected to contain, pathogens.

Pathogens

Micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals

Cultures

The result of a process by which pathogens are intentionally propagated. This definition does not include human or animal patient specimens.

Patient specimens

Those collected directly from humans or animals, including, but not limited to, excreta, secretions, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention”

Medical or clinical wastes

Wastes derived from the medical treatment of animals or humans or from bio-research.

Biological products

Those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines.

Regulation

A rule, ordinance or law.

Applicable Regulations for Packaging and Shipping of Samples

1. Dangerous goods are carried routinely by air worldwide
2. In order to protect the safety of everyone, that includes, cargo handlers, airline personnel, passengers and the general public, it is important to have uniform regulations in place that can be followed by everyone.
3. Regulations associated with the transport of dangerous goods are detailed in the UN Recommendations on the Transport of Dangerous Goods.
 - Developed by the United Nations Committee of Experts on the Transport of Dangerous Goods
 - Applicable to all modes of transport
4. The shipment of dangerous goods is governed by the UN's International Civil Aviation Organization (ICAO)
 - Broad principles that require dangerous goods be carried in accordance with the Technical Instructions for the Safe Transport of Dangerous Goods by Air (the "Technical Instructions")
5. The ICAO Technical Instructions reflect amendments made to the Infectious Substances requirements published in the UN Model Regulations

- The ICAO TIs define the legal requirements for the safe transport of dangerous goods by air
- As part of the ICAO TIs is the requirement that initial and recurrent in-depth training must be taken by shippers and their agents, packers, freight forwarders, cargo agents, operators (or airlines), agencies handling operators and performing cargo acceptance function.
- The ICAO TIs require that recurrent training must take place within 24 months of previous training to ensure that the knowledge is current

6. International Air Transport Association (IATA) is a global airline trade Organization that is responsible for:

- Aircraft operations
- Passenger services
- Cargo
- Safety and security

As part of their services they produce a manual of Dangerous Goods Regulations

- Updated yearly – the 48 Edition: January 2007
- The IATA Dangerous Goods Regulations (DGR) guide is the trusted source to help classify, mark, pack, label and document dangerous shipments, based on international and national air regulations as well as airline-specific requirements.
- Regulations are not available on-line have to purchase it – US\$ 179

7. The IATA Dangerous Goods Regulations

- A “field manual” version of the ICAO Technical Instructions must be followed if you ship specimens by air. If you comply with these, you also comply with ICAO TIs

ICAO TIs

- Written and edited by airline dangerous goods experts



- Presents the requirements for shipping dangerous goods by air in a user friendly, easy to interpret format
- It includes additional information that can assist shippers in making sure their consignments are in compliance and will be accepted quickly and easily by the airlines.
- Since IATA airlines are stricter in their requirements than the ICAO Technical Instructions, adherence to these regulations will ensure that you comply with any mode of transportation

List of Resources

- http://www.unece.org/trans/danger/publi/unrec/rev14/English/00E_Intro.pdf
- IATA e-mail address - information@iata.org and IATA Hotline - 514-390-6770

WHAT IS THE SHIPPER RESPONSIBLE FOR?

The shipper or sender is responsible for:

1. Proper Classification
2. Proper Packaging
3. Proper Labeling
4. Proper Documentation

WHAT ARE DANGEROUS GOODS?

There are 9 classes of dangerous goods, including:

- Explosives
- Gases
- Flammable Liquids
- Flammable Solids
- Oxidizing substances and Organic Peroxides
- **Toxic and infectious substances (including animal specimens like HPAI and human specimens)**
- Radioactive material
- Corrosives
- Miscellaneous Dangerous Goods – like dry ice

I. HOW TO CLASSIFY A SUBSTANCE

A SUBSTANCE FALLS INTO CATEGORY A IF...	A SUBSTANCE FALLS INTO CATEGORY B IF...	NOT SUBJECT TO IATA DANGEROUS GOODS REGULATIONS
When exposure to it occurs, it is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.	When exposure to it occurs, is NOT capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals	Substances, which do not contain infectious substances, or substances, which are unlikely to cause disease in humans or animals
Infectious Substance, affecting humans, UN 2814 (based on the known medical history and symptoms of the source, human or animal, endemic local conditions, or professional judgment)	Biological substance assigned the following proper shipping name and UN number UN 3373	Substances containing micro-organisms, which are non-pathogenic to humans or animals
Infectious Substance, affecting animals only, UN 2900 (based on the known medical history and symptoms of the source, human or animal, endemic local conditions, or professional judgment)		Substances in a form that any present pathogens have been neutralized or inactivated such that they no longer pose a health risk
If the substance affects both humans and animals then it is UN 2814		Environmental samples (including food and water samples), which are not considered to pose a significant risk of infection.
If there is any doubt of whether the pathogen is dangerous or not.		Dried blood spots, and blood or blood components which have been collected for the purposes of transfusion or for the preparation of blood products to be used for transfusion or transplantation and any tissues or organs intended for use in transplantation.

		<p>Patient specimens for which there is minimal likelihood that pathogens are present if the specimen is transported in Packaging for Exempt Patient Specimens</p>
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PROPER CLASSIFICATION

- It is the shipper’s responsibility to properly classify the shipment.
 - In most cases, this responsibility falls on the laboratory personnel sending the sample – they are the only one who have the “professional judgment concerning individual circumstances of the source”
- Shipments can only be classified as Class A or B:

Class A

- An infectious substance, if exposed to, is capable of causing permanent disability, life-threatening or fatal disease in healthy humans or animals
- They are assigned the following proper shipping names and UN number:
 - Infectious Substance, affecting humans, UN 2814; or
 - Infectious Substance, affecting animals only, UN 2900;
 - If the substance affects both humans and animals then it is UN 2814
- Assignment to UN 2814 or UN 2900 is based on the known medical history and symptoms of the source, human or animal, endemic local conditions, or professional judgment concerning individual circumstances of the source - human or animal
- If there is any doubt as to whether or not a pathogen falls within this category it must be transported as a Category A Infectious Substance
- To assist in the assignment of an infectious substance into Category A, IATA has put together an Indicative List in Table 3.6.D provided in 3.6.2 Division 6.2—Infectious Substances (provided as reference material with this training)

- This list is not exhaustive! Infectious substances, including new or emerging pathogens, that do not appear in the table but which meet the same criteria must be assigned to Category A

Class B

- An Infectious Substances that does not meet the criteria for inclusion in category A
- They are assigned the following proper shipping name and UN number UN 3373:
 - **Biological Substance, Category B;**
- The shipping name “**Diagnostic Specimen** or **Clinical Specimen**” will not be permitted by the IATA after 1 January 2007 and packages labeled as such will not be accepted by the carrier!

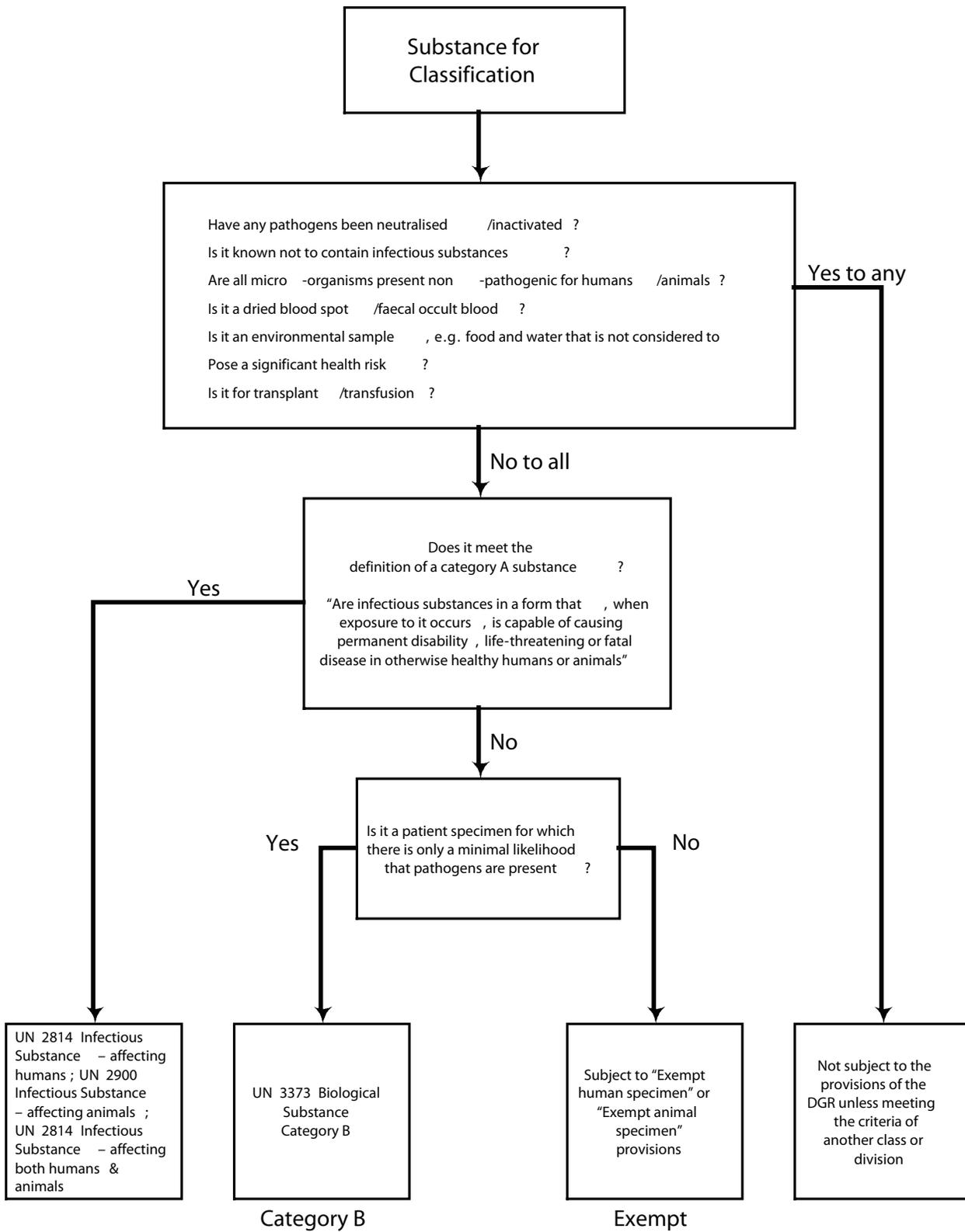
CATEGORY A INFECTIOUS SUBSTANCES – 3.6.2 DIVISION 6.2

- **Examples of Class A infectious substances (Table 3.6.D)**
 - Remember a Class A infectious substance is an infectious substance, if exposed to, is capable of causing permanent disability, life-threatening or fatal disease in healthy humans or animals. According to IATA a substance is Class A if it is “known or reasonably suspected to contain” and of the following substances that affect humans and/or animals:
- **UN 2814** Infectious substance, affecting *humans*
 - Clostridium botulinum (cultures only)
 - Hepatitis B virus (cultures only)
 - Human immunodeficiency virus (HIV) (cultures only)
 - Mycobacterium tuberculosis (cultures only)
 - Yersinia pestis (cultures only)
 - Any infectious substance, if exposed to, is capable of causing permanent disability, life-threatening or fatal disease in healthy humans or animals
- **UN 2814** Infectious substance, affecting *humans and animals*
 - Highly pathogenic avian influenza virus (cultures only)
 - Brucella abortus and melitensis (cultures only)
 - Bacillus anthracis (cultures only)
 - Rabies virus (cultures only)

- UN 2900 Infectious substance, affecting *animals*
 - Foot and mouth disease virus (cultures only)
 - Avian paramyxovirus Type 1 – Velogenic Newcastle disease virus (cultures only)
 - Rinderpest virus (cultures only)

EXAMPLES OF CLASS B INFECTIOUS SUBSTANCES (FROM THE IATA GUIDANCE DOCUMENT)

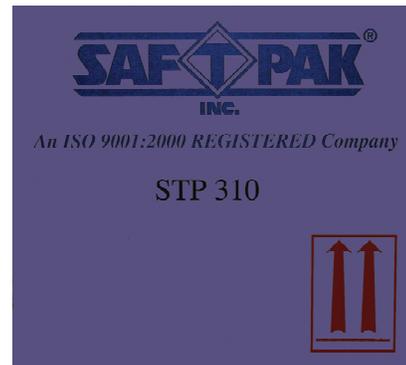
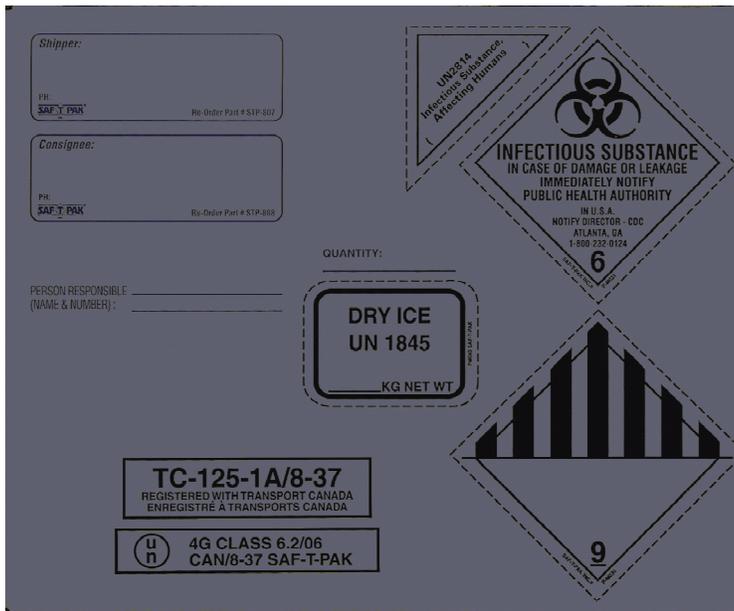
- Remember a Class B infectious substance is “an Infectious Substance that does not meet the criteria for inclusion in category A”
 - It can be difficult to make this distinction sometimes
 - If there is any **doubt** as to whether or not a pathogen being shipped falls within this category it *must be transported as a Category A Infectious Substance*



2. HOW TO PACK A SHIPMENT BASED ON CATEGORY

PACKING CATEGORY A	PACKING CATEGORY B	PACKING EXEMPT PATIENT/ ANIMAL SPECIMENS
Primary watertight receptacle of glass, metal, or plastic	Primary receptacle: leak proof or sift proof	Primary receptacle: leak proof or sift proof
Watertight secondary packaging	Secondary packaging: leak proof or sift proof (e.g. sealed plastic bag or other intermediate packaging)	Secondary packaging: leak proof or sift proof (e.g. sealed plastic bag)
Rigid outer packaging	Rigid outer packaging	Outer packaging
Absorbent packing material (for liquids)	Absorbent packing material (for liquids) Cushioning material	Absorbent packing material (for liquids)
UN Package Certification Mark	Package Mark	Exempt human specimen or exempt animal specimen
Infectious Substance label: Class 6		
Proper Shipping Name and UN number	Name and telephone number of a person responsible (or written document such as an air waybill).	
Shipper of Consignee Identification number		
Outer packaging not less than 100 mm	One surface of outer packaging 100 mm x 100mm	At least 100 mm in the smallest external dimension

CLASS A INFECTIOUS SUBSTANCES



LABEL	DESCRIPTION	POSITION
SAF-T-PAK INC. An ISO 9001: 2000 REGISTERED Company	Manufacturer of the box and packing material	
STP 310	Box Part Number	
	Two (2) up arrows on opposite sides of the box	Place on opposite sides of the box

Sender: _____

 Ph: _____

Person responsible _____
 Telephone number _____

Receiver: _____

 PH: _____

Quantity ____ ml/gm

Biological substance , Category B

UN 3373

9

UN 1845 DRY ICE
NET WT

OVERPACK

75

Exempt patient or animals specimens

Sender: _____

 Ph: _____

Receiver: _____

 PH: _____

Exempt Human Specimen

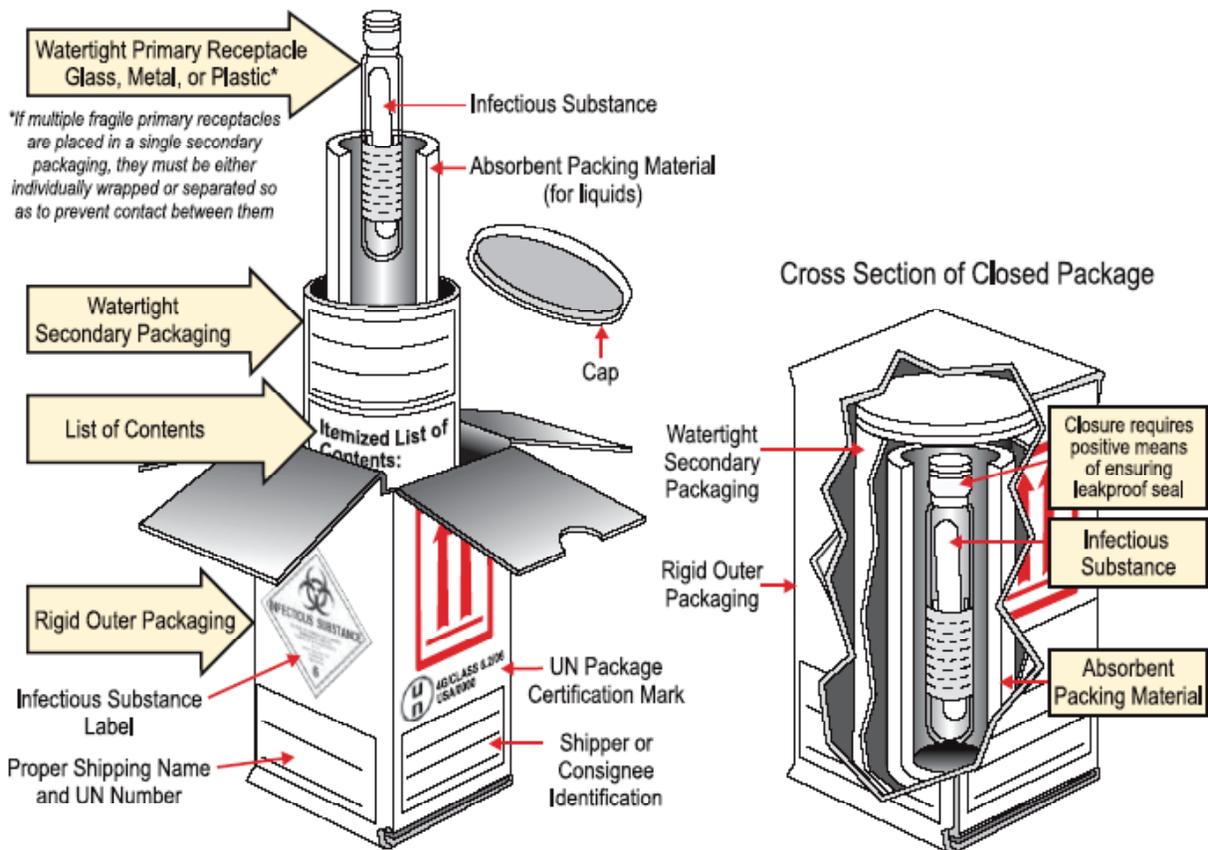
9

UN 1845 DRY ICE
NET WT

OVERPACK

75

PACKING – CATEGORY A INFECTIOUS SUBSTANCES – PI 602



- Note 1:** The smallest external dimension of the outer packaging must not be less than 100 mm (3.9 inches)
- Note 2:** The primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa
- Note 3:** Follow package manufacturer's closure instructions

PROPER PACKING FOR CATEGORY A AND CATEGORY B

Category A Packing Requirements

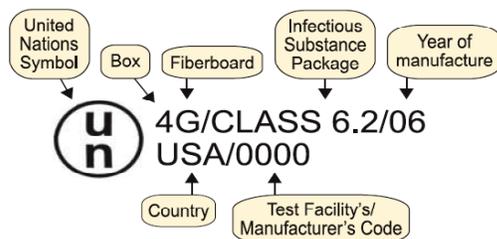
- **Must use** Packing Instructions (PI) 602 in the DGR
 - This instruction is provided as part of your reference material
- PI 602 states shippers must ensure that “Shipments are prepared in such a manner that they arrive at their destination in good condition and that they present no hazard to persons or animals during shipment”
- Used for both UN 2814 and UN 2900 infectious substances

Category A Infectious Substances – PI 602

- **Must have** Triple Packaging
 - 1st Package - A watertight primary receptacle(s)
 - * The receptacle must have sufficient room for expansion of liquids
 - * Must have a positive means of ensuring a leak-proof seal
 - Reinforce screw caps with adhesive tape
 - * If shipped at ambient temperatures or higher the receptacle can be made of glass, metal or plastic, it must have a leak-proof seal
 - * If shipped refrigerated or frozen on dry ice, the receptacle must be capable of maintaining its structural integrity
 - Some plastics can break when frozen in dry-ice
 - Glass can break as the liquid expands
 - Tape can become brittle and fail
 - * For packages containing large body parts/whole organs, items shipped in liquid nitrogen (e.g., dry shippers), lyophilized refer to PI 602
 - * Multiple primary receptacles must be wrapped individually
 - 2nd Package - A watertight secondary packaging (container)
 - 3rd Package – Rigid outer packing of adequate strength for its capacity, weight and intended use
 - * At least one surface must have a minimum dimension of 100 mm x 100 mm

- The maximum quantity of infectious substance in the package being shipped *can not* exceed:
 - 50 ml or 50 gm when transported in a passenger plane
 - 4 liters or 4 kg when transported by cargo plane
 - * Need to indicate that the shipment can only go by cargo on the outside of the box
 - Under A81 – The quantity limits do not apply to body parts, organs or whole bodies.
- The Primary and Secondary Packaging Containers *must be* capable of withstanding, without leakage, an internal pressure which produces a pressure differential of not less than 95 kPA in the range of -40° C to +55°
- The outer package *must* be of sufficient strength to meet Subsection 6.5 of the DGR – deals with criteria to be met for drops, penetrations, etc.
 - This package must bear Specification Markings as required by 6.0.6 for shipments of infectious substances – Referred to as the “UN Package Certification Mark” or “UN Specification Mark”

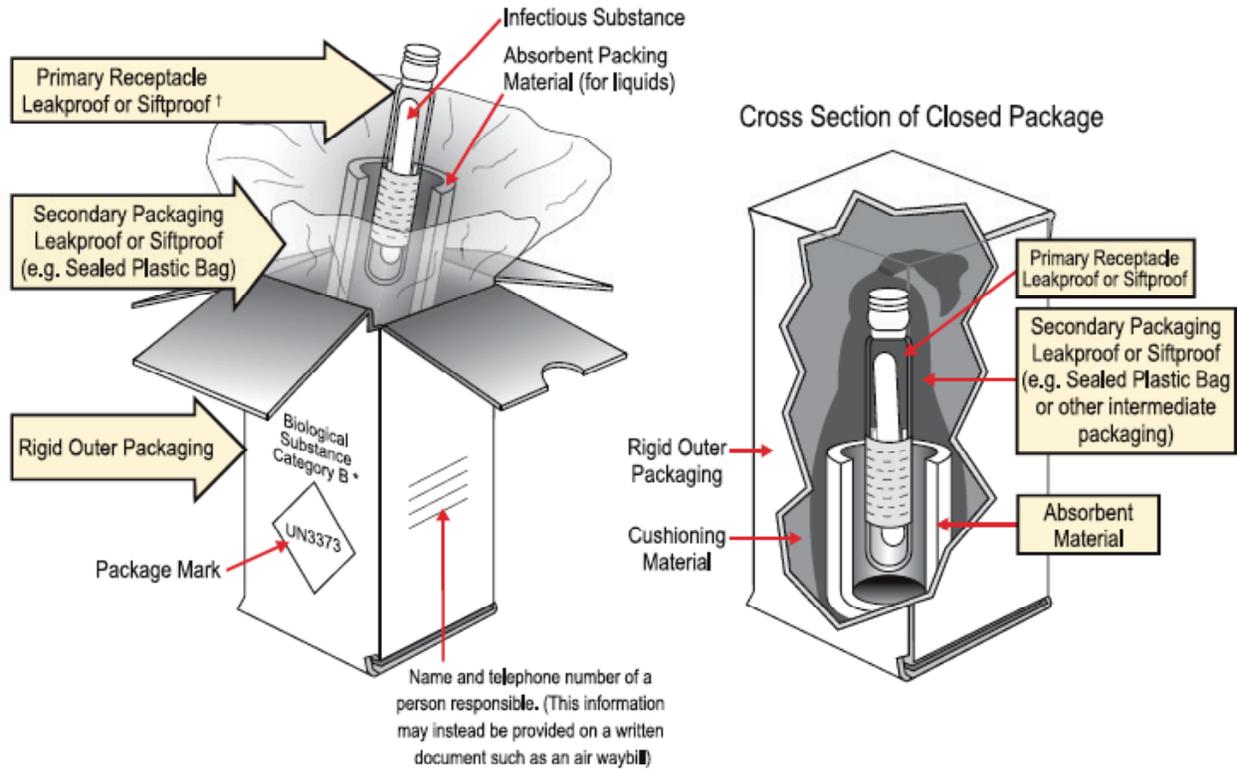
SAMPLE OF UN PACKAGE CERTIFICATION MARK



- The packaging must also include:
 - An absorbent material between the primary receptacle and the secondary packaging
 - * Must be of sufficient quantity to absorb all of the liquid in the primary packaging
 - * Exceptions:
 - Large body parts and whole organs – these require special packaging
 - Not required for solid substances

- An itemized list of the infectious substance being shipped in the package is placed between the secondary and outer container
- Additional requirements:
 - The name and telephone number of the person responsible for the shipment must be on the outside of the box – this individual has to be available 24 hours a day until the shipment is received by the consignee
 - Shipper has to make advance arrangements with the consignee and operator to ensure that the shipment can be transported and delivered without unnecessary delays – Required to indicate this on the shipper declaration
 - Under A140 – When the infectious substances shipped are unknown but are suspected of meeting the criteria for inclusion in Category A, the word “Suspected Category A infectious substance” must appear in parenthesis following the proper shipping names in the shipper’s declaration for dangerous goods.
- If refrigerants are used in the shipment:
 - Refrigerants like wet ice, prefrozen packs, dry ice (Carbon dioxide, solid) or other refrigerants can be placed either around the secondary packaging or in an overpack (we will talk more about overpacks later on)
 - There must be interior support provided to secure the secondary packaging(s) so it stays in its original position after the ice or dry ice has dissipated.
 - If ice is used, the outer packaging or overpack must be leak-proof.
 - If dry ice is used, the outer packaging or overpack must permit the release of carbon dioxide gas.
 - The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the refrigerant used

PACKING CATEGORY B INFECTIOUS SUBSTANCES—PI 650



* The proper shipping names "Biological Substance, Category B"; "Clinical Specimen"; and "Diagnostic Specimen" are authorized until December 31, 2006. From January 1, 2007 only the proper shipping name "Biological Substance, Category B" will be authorized.

† If multiple fragile primary receptacles are placed in a single secondary packaging they must be either individually wrapped or separated to prevent contact

Note: Follow package manufacturer's closure instructions

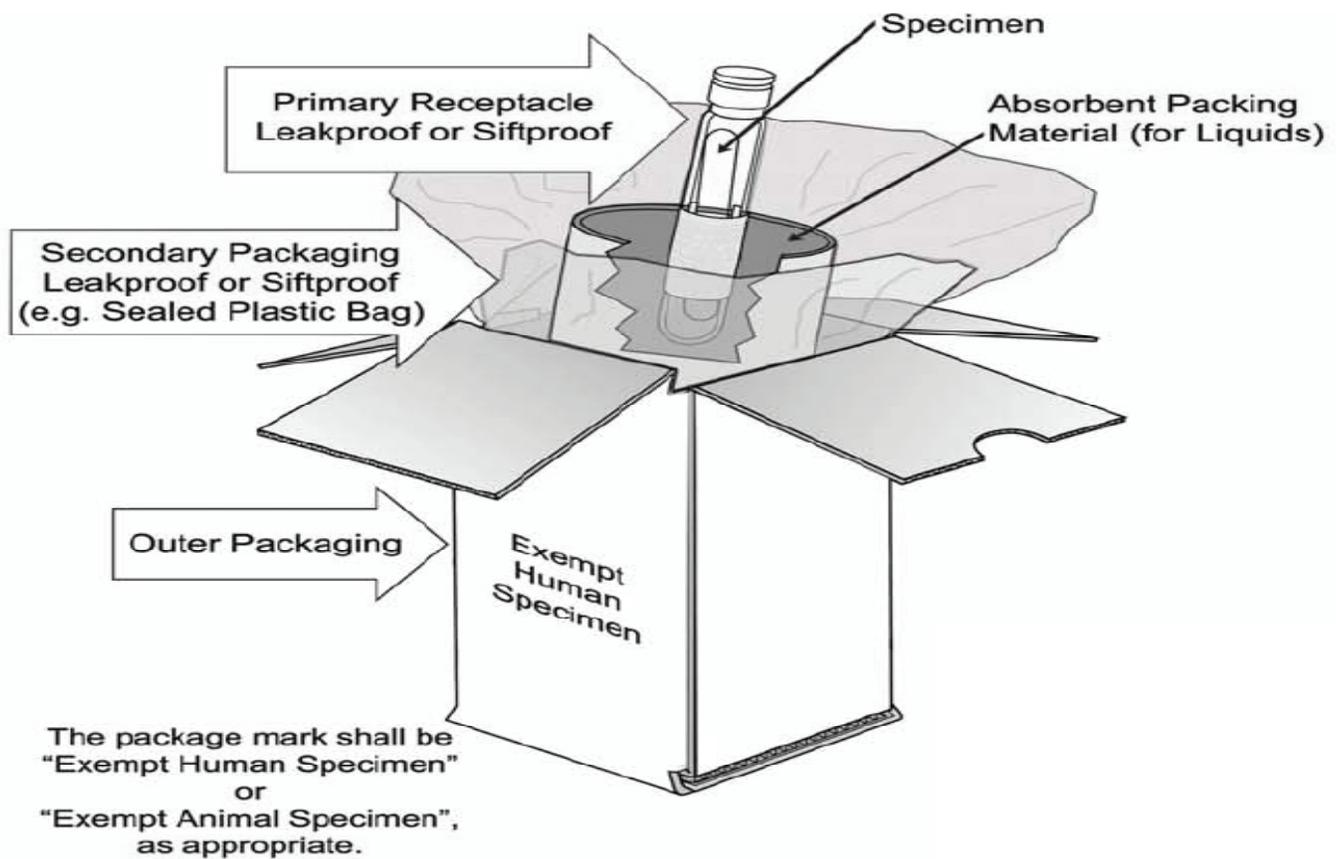
Note 1: At least one surface of the outer packaging must have a minimum dimension of 100 mm x 100 mm (3.9 inches)

Note 2: For liquid shipments by aircraft, the primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa

PROPER PACKING

Category B packing Requirements

- **Must use** Packing Instructions (PI) 650 in the DGR
 - This instruction is provided as part of your reference material
- PI 602 states “the packaging must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including transshipment between transport units and between transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure”.
- Used UN 3373 infectious substances
- **Must have** Triple Packaging
 - Liquid substances
 - * Primary (1st) Receptacle
 - Must be leak-proof
 - May be glass, metal or plastic
 - Must have a positive means of ensuring a leaf-proof seal - Reinforce screw caps with adhesive tape
 - Must not contain more than 1 liter
 - Multiple primary receptacles must be wrapped individually
 - * Secondary (2nd) Packaging
 - Must be leak-proof if liquid or sift proof if a solid
 - * Outer (3rd) Packaging – Rigid and be appropriately sized
 - This package must be at least 100 mm in the smallest overall external dimension



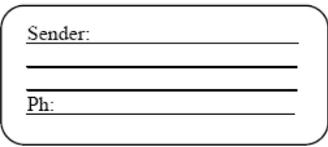
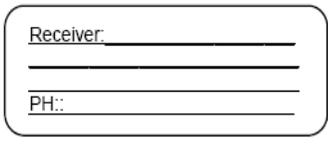
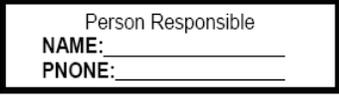
Overpacks

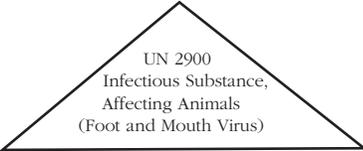
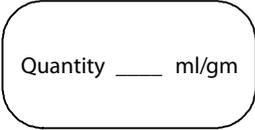
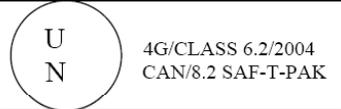
- An overpack may be used to combine several infectious substance packages (comprised of the primary, secondary and outer package) into one large package.
 - This is a convenient way to send several smaller packages to the same destination
- Overpacks can also be used to ship infectious disease packages in dry ice or ice bags with a cooler (plastic or Styrofoam)
 - If dry ice is used then the overpack has to be packed as described in IATA Packing Instruction 904
- The overpack box must have all of the required labels and markings that are found on the infectious substances boxes) contained inside as defined in PI 950 or 602
- Two (2) up arrows must be on opposite sides of the box
- The total volume of sample shipped in the overpack can not exceed the volumes detailed in PI 950 or 602

Shipping infectious substance packages with dry ice.

- Dry ice is a hazard.
 - Explosion hazard - due to the sublimation of the CO₂ gas
 - make sure that the container is ventilated so the gas can escape
 - Suffocation hazard – CO₂ gas released from the package can displace oxygen in enclosed areas
 - Contact hazard – the dry ice blocks can cause severe frost bite of skin
- Shipments containing dry ice must be packed according to IATA Packing Instruction 904 (provided as a reference material)
 - Class 9 “Miscellaneous” label for dry ice
 - Dry Ice label

3. PROPER LABELING: CLASS A INFECTIOUS SUBSTANCES

LABEL	DESCRIPTION	POSITION
 <p>Sender: _____ _____ Ph: _____</p>	<p>The full name, address and phone number of the sender</p>	<p>Place in upper left hand quadrant of box.</p>
 <p>Receiver: _____ _____ PH: _____</p>	<p>The full name, address and phone number of the receiver</p>	<p>Place below sender label in upper left hand quadrant of box.</p>
 <p>Person Responsible NAME: _____ PHONE: _____</p>	<p>Emergency contact – the name and phone number of the emergency contact must be available 24 hours a day until the shipment is received</p>	<p>Place below receiver label in upper left hand quadrant of box.</p>
	<p>Class 6 “Infectious Substance” Label</p>	<p>Place in upper right quadrant of box. Label must be 4” square.</p>
	<ul style="list-style-type: none"> • UN 2814, Infectious Substance, affecting humans • If the substance affects both humans and animals then it is considered UN 2814 • Technical name • A recognized name currently used in scientific and technical handbooks, texts, journals, etc. Typically the technical name of the agent is the proper genus and species or virus name – 	<p>Place adjacent (left) to Class 6 Infectious Substance label.</p>

	<p>UN 2900, Infectious Substance, affecting animals</p>	<p>Place adjacent (left) to Class 6 Infectious Substance label.</p>
	<ul style="list-style-type: none"> • The total quantity of infectious substance shipped in the box • Can only ship up to 50 ml or 50 gm in one box if shipped on a passenger plane • Can ship up to 500 ml on a cargo plane 	<p>Place adjacent (left) to Class 6 label.</p>
	<p>The box must have the UN compliant packaging certification mark</p>	<p>Not a label but marking on the box printed by the box manufacturer</p>

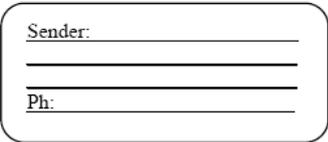
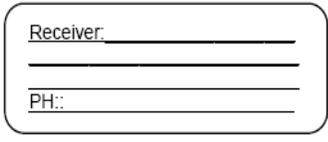
The following additional labels are used on packages that contain dry ice

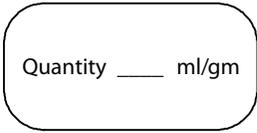
LABEL	DESCRIPTION	POSITION
	<p>Class 9 “Miscellaneous” label for dry ice</p>	<p>Place in upper right quadrant of box. Label must be 4” square.</p>
	<p>Dry ice label</p>	<p>Quantity (in kg) of Dry Ice must be written on label. Place adjacent to the Class 9 label.</p>

<p style="text-align: center; border: 1px solid black; padding: 5px;">OVERPACK</p>	<p>For convenience and lower costs, one or more complete packages may be shipped in a single box (an “overpack”). Each package in the overpack must be a complete package properly prepared and labeled according to applicable IATA regulations</p>	<p>The “overpack label” must be clearly visible on the package.</p>
	<p>Packages that must be shipped only by cargo aircraft according to the IATA DGR</p>	<p>The “DANGER” DO NOT LOAD IN PASSENGER AIRCRAFT LABEL must be clearly visible on the package.</p>

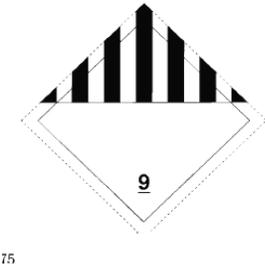
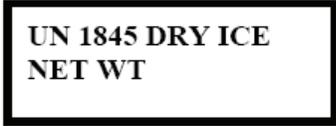
PROPER LABELING: CLASS B INFECTIOUS SUBSTANCES

The outside of every box must be labeled/marked with the following:

LABEL	DESCRIPTION	POSITION
 <p>Sender: _____ _____ Ph: _____ _____</p>	<p>The full name, address and phone number of the sender</p>	<p>Place in upper left hand quadrant of box.</p>
 <p>Receiver: _____ _____ PH: _____ _____</p>	<p>The full name, address and phone number of the consignee</p>	
<p>A UN 3373 Label</p>  <p>UN3373</p>	<p>This label must be displayed on the external surface of the outer packaging on a background of a contrasting color and must be clearly visible and legible. The mark must be in the form of a square set an angle of 45° (diamond-shaped) with each side having a length of at least 50 mm, the width of the line must be at least 2 mm, and the letters and numbers must be at least 6 mm high.</p>	<p>Place in upper right quadrant of box. Label must be 4” square.</p>
<p>Biological substance, Category B</p>		<p>This must be on the outer package adjacent to the diamond-shaped UN 3373 label in letters at least 6 mm high</p>

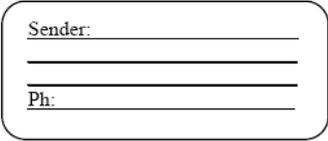
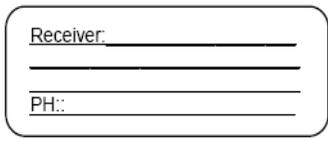
OPTIONAL MARKINGS		
	<p>The name and phone number of the emergency contact (this person must be available 24 hours a day until the shipment is received by the consignee). If this is not placed on the outside of the box it has to be included in the airway bill.</p>	<p>Place below receiver label in upper left hand quadrant of box.</p>
	<p>The total quantity of infectious substance shipped in the box</p>	

The following additional labels are used on packages that contain dry ice.

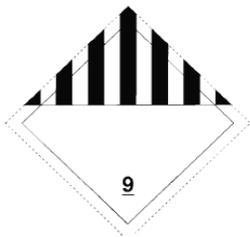
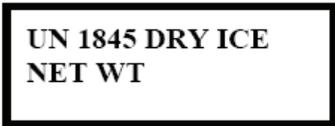
LABEL	DESCRIPTION	POSITION
	<p>Class 9 “Miscellaneous” label for dry ice</p>	<p>Place in upper right quadrant of box. Label must be 4” square.</p>
	<p>Dry ice label</p>	<p>Quantity (in kg) of Dry Ice must be written on label. Place adjacent to the Class 9 label.</p>
<p>If Overpack is needed</p> 	<p>For convenience and lower costs, one or more complete packages may be shipped in a single box (an “overpack”). Each package in the overpack must be a complete package properly prepared and labeled according to applicable IATA regulations</p>	<p>The “overpack label” must be clearly visible on the package.</p>

PROPER LABELING: EXEMPT PATIENT OR ANIMALS SPECIMENS

The outside of every box must be labeled/marked with the following:

LABEL	DESCRIPTION	POSITION
 <p>Sender: _____ _____ Ph: _____</p>	<p>The full name, address and phone number of the sender</p>	<p>Place in upper left hand quadrant of box.</p>
 <p>Receiver: _____ _____ PH: _____</p>	<p>The full name, address and phone number of the consignee</p>	
<p>Exempt Human Specimen</p>	<p>Mark as “Exempt Patient Specimen” or “Exempt Animal Specimen”</p>	<p>This must be on the outer package</p>

The following additional labels are used on packages that contain dry ice.

LABEL	DESCRIPTION	POSITION
 <p>75</p>	<p>Class 9 “Miscellaneous” label for dry ice</p>	<p>Place in upper right quadrant of box. Label must be 4” square.</p>
	<p>Dry ice label</p>	<p>Quantity (in kg) of Dry Ice must be written on label. Place adjacent to the Class 9 label.</p>
<p>If Overpack is needed</p> 	<p>For convenience and lower costs, one or more complete packages may be shipped in a single box (an “overpack”). Each package in the overpack must be a complete package properly prepared and labeled according to applicable IATA regulations</p>	<p>The “overpack label” must be clearly visible on the package.</p>

4. PROPER DOCUMENTATION

AIRLHM-1 UNCL		7		8		
Airport of Destination:				Shipment type: (delete non-applicable) NON-RADIOACTIVE RADIOACTIVE		
NATURE AND QUANTITY OF DANGEROUS GOODS						
Dangerous Goods Identification				Quantity and type of packaging	Packing Inst.	Authorization
UN or ID No.	Proper Shipping Name	Class or Division (subsidiary Risk)	Packing Group			
9a	9b	9c	9d	9e	9f	9g

- 7** Enter the full name of the airport or city of destination. *This information may be entered or changed by the shipper, shipper's agent, the airline, or the airline's handling agent.* (IATA 8.1.6.7)
- 8** Delete "Radioactive" to indicate the shipment does not contain radioactive material. *This information must be entered by the shipper.* Radioactive material must not be included on the same Declaration form as other dangerous goods, except as authorized in IATA 8.1.6.8.
- 9** Enter the required information strictly in accordance with IATA 8.1.6.9. *This information must be entered by the shipper.* NOTE: Columns indicated are those in the List of Dangerous Goods (IATA 4.2).
- UN Number or ID Number (Column A), preceded by "UN" or "ID" as appropriate.
 - Proper Shipping Name (Column B), with technical name(s) if appropriate.
 - Class Number or Division Number as appropriate, for Class 1 - Compatibility Group (Column C). Subsidiary Risk(s) as indicated (Column D) in parentheses following the class or division.
 - Number of packages (of the same packaging type and content) and their type of packaging (either spelled out in full, or using the UN Packing Specification Code) and the net quantity (as specified in IATA 8.1.6.9 Step #5).
When two or more different dangerous goods items are packed in the same outer package, the words "All Packed in One" must immediately follow the relevant entries.
When an overpack is used, the wording "Overpack Used" must be inserted on the declaration form immediately after all relevant entries relating to the packages within the overpack.
 - Number of the Packing Instruction or of the Limited Quantity Packing Instruction (Column G, I, or K as appropriate).
 - Authorizations as applicable (in accordance with IATA 8.1.6.9 Step #8).
 - The word(s) "Limited Quantity" or "Ltd. Qty." if using limited quantity provisions.
 - The Special Provision Number if the special provision is A1, A2, A51, A81, or A109 [IATA 8.1.6.9.4(b)].
 - Authorizations and/or exemptions which must accompany your shipment must either

Additional Handling Information	
Emergency Telephone Number	10
I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labeled/placarded, and are in all respects in proper condition for transport according to applicable International and National Governmental Regulations. I declare that all of the applicable air transport requirements have been met.	
Name/Title of Signatory	11
Place and Date	12
Signature <i>(see warning above)</i>	13
FOR RADIOACTIVE MATERIAL SHIPMENT ACCEPTABLE FOR PASSENGER AIRCRAFT, THE SHIPMENT CONTAINS RADIOACTIVE MATERIAL INTENDED FOR USE IN OR INCIDENT TO RESEARCH, MEDICAL DIAGNOSIS, OR TREATMENT.	

- 10** Enter any special handling information relevant to your shipment.
This information must be entered by the shipper.
- All dangerous goods shipments to, from, within, or transiting through the U.S. must include a 24-hour emergency response telephone number (IATA 2.9.2, USG-12). *Magnetized material (UN 2807), dangerous goods for which no Shipper's Declaration is required, and Other Regulated Materials (ORM-D) as defined in 49 CFR 173.144 are exempt from this requirement.*
 - When shipping Division 4.1 self-reactive substances, other substances having similar properties, and Division 5.2 organic peroxides with Special Provision A20, the shipper must indicate that the packages containing such substances must be protected from direct sunlight and stored away from all heat sources in a well ventilated area (IATA 8.1.6.11.1).
When a sample of a self-reactive substance or an organic peroxide is transported, a statement to this effect must be included in the "Additional Handling Information" box.
 - The statement "I declare that all of the applicable air transport requirements have been met" must either be after the certification statement or in the Additional Handling section.
- 11** Enter the name and title of the person actually signing the Declaration. *This information which must be entered by the shipper, may be printed or stamped (IATA 8.1.6.13).*
- 12** Enter the place and date of signing the form. *This information must be entered by the shipper (IATA 8.1.6.14).*
- 13** Sign the Shipper's Declaration.
This information must be entered by the shipper (IATA 8.1.6.15).
- The signature should be written by hand; however, facsimile signatures are acceptable where applicable laws and regulations recognize the legal validity of facsimile signatures (IATA 8.1.4.1).
 - Typewritten signatures are not acceptable.
 - The Shipper's Declaration must be completed and signed by the shipper.
- NOTE:** When shipping with FedEx Express, Shipper's Declarations must be typewritten or computer generated.
FedEx Express does not accept handwritten forms. See **IATA Variation FX-12** for more details and exceptions to this FedEx Express requirement (IATA 2.9.4).

PASSENGER PROVISIONS

Category A or B Infectious Substances are not permitted for transport in carry-on or checked baggage and must not be carried on a person.

Packages containing Exempt human or animal specimens may be carried in checked or carry-on baggage provided that they meet the applicable packaging requirements.

EMERGENCY PROCEDURES – IATA GUIDELINES

As a shipper you may be called, typically by the carrier or freight forwarder, to assist if there is an “accident” with your shipment or someone else’s

- In the USA and Europe there are emergency response personnel trained to deal with HAZHAT spills, these people are usually available to the carrier in the event of an accident

MITIGATION PROCEDURES

- **Advise personnel not to clean-up or dispose of infectious substances, except under the supervision of a specialist – typically that will be you!**
- Isolate spill or leak area immediately.
- Keep unauthorized personnel away.
- Obtain identity of substance involved if possible and report the spill to the appropriate authorities.
- Do not touch or walk through spilled material.
- Do not touch damaged containers or spilled material unless wearing appropriate protective clothing.
- Be particularly careful to avoid contact with broken glass or sharp objects that may cause cuts or abrasions that could significantly increase the risk of exposure.
- Damaged packages containing solid CO₂ (dry ice) used as a refrigerant may produce water or frost from condensation of air. Do not touch this liquid as it could be contaminated by the contents of the parcel.

- Liquid nitrogen may be present and can cause severe burns.
- Absorb spilled materials with earth, sand or other non-combustible material while avoiding direct contact.
- Cover damaged package or spilled material with damp towel or rag and keep wet with liquid bleach or other disinfectant. Liquid bleach will generally effectively inactivate the released substance.

FIRST AID:

- Move exposed person(s) to a safe isolated area. CAUTION: Exposed person(s) may be a source of contamination. Persons administering first aid should take precautions to avoid personal exposure or secondary contamination of others.
- Call emergency medical services.
- If clothing and/or shoes are significantly contaminated, remove and isolate them. However, do not allow this to delay other first aid interventions.
- In case of contact of the substance to skin, eyes, nose or mouth, immediately flush the exposed area with copious amounts of running water. Continue this until emergency medical services arrives. Follow their advice for further decontamination.
- Most effects of exposure (inhalation, ingestion or skin contact) to substance are likely to be delayed.
- Ensure that medical personnel are aware of the substances involved so they can take precautions to protect themselves.

CONTACT THE APPROPRIATE PUBLIC HEALTH OR VETERINARY HEALTH AUTHORITIES AS SOON AS POSSIBLE

PACKING INSTRUCTIONS – CLASS 6 – TOXIC AND INFECTIOUS SUBSTANCES

Source: www.iata.org

Packing Instructions—Class 6—Toxic and Infectious Substances
PACKING INSTRUCTION 650

STATE VARIATIONS: BHG-02 CAG-05 DQG-03 FRG-06 GBG-05
VCG-04

OPERATOR VARIATIONS: AF-02 AM-06 AM-10 AS-08 BR-14 CI-01
CO-07 CS-07 FX-09 IJ-06 IJ-10 JJ-06 JK-03 KE-06 LA-07 LH-12 MN-03
MS-03 MX-06 MX-11 OO-01 OU-12 OU-16 PX-08 SQ-10 TK-08 TY-03
UA-14 UU-05 XK-05

This instruction applies to UN 3373 on passenger and cargo aircraft
and CAO.

GENERAL REQUIREMENTS

The packagings must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including trans-shipment between transport units and between transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packagings must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

The packaging must consist of three components:

- (a) a primary receptacle(s);
- (b) a secondary packaging; and
- (c) a rigid outer packaging.

Primary receptacles must be packed in secondary packagings in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings must be secured in outer packagings with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the

cushioning material or of the outer packaging.

Packages must be prepared as follows:

(A) FOR LIQUID SUBSTANCES:

- The primary receptacle(s) must be leakproof and must not contain more than 1 L;
- The secondary packaging must be leakproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C (-40°F to 130°F).

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***Note:** The capability of a packaging to withstand an internal pressure without leakage that produces the specified pressure differential should be determined by testing samples of primary receptacles or secondary packagings. Pressure differential is the difference between the pressure exerted on the inside of the receptacle or packaging and the pressure on the outside. The appropriate test method should be selected based on receptacle or packaging type. Acceptable test methods include any method that produces the required pressure differential between the inside and outside of a primary receptacle or a secondary packaging. The test may be conducted using internal hydraulic or pneumatic pressure (gauge) or external vacuum test methods. Internal hydraulic or pneumatic pressure can be applied in most cases as the required pressure differential can be achieved under most circumstances. An external vacuum test is not acceptable if the specified pressure differential is not achieved and maintained. The external vacuum test is a*

generally acceptable method for rigid receptacles and packagings but is not normally acceptable for

- flexible receptacles and flexible packagings
- receptacles and packagings filled and closed under a absolute atmospheric pressure lower than 95 kPa
- The outer packaging must not contain more than 4 L. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.

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(B) FOR SOLID SUBSTANCES:

- The primary receptacle(s) must be siftproof and must not exceed the outer packaging weight limit;
- The secondary packaging must be siftproof;
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them;
- Except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4 kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold;
- If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a packaging suitable for liquids, including absorbent materials, must be used.

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

At least one surface of the outer packaging must have a minimum dimension of 100 mm x 100 mm (4 in x 4 in).

The completed package must be capable of successfully passing the drop test described in 6.5.1.1 except that the height of the drop must not be less than 1.2 m. Following the appropriate drop sequence, there must be no leakage from the primary receptacle(s) which must remain protected by absorbent material, when required, in the secondary packaging. For transport, the mark illustrated below must be displayed on the external surface of the outer packaging on a background of a contrasting color and must be clearly visible and legible. The mark must be in the form of a square set at an angle of

45° (diamond-shaped) with each side having a length of at least 50 mm (2 in), the width of the line must be at least 2 mm and the letters and numbers must be at least 6 mm high. The proper shipping name “Biological Substance, Category B” in letters at least 6 mm high must be marked on the outer package adjacent to the diamond-shaped mark.

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Unless all package markings are clearly visible, the following conditions apply when packages are placed in an overpack:

- the overpack must be marked with the word “Overpack”; and
- the package markings must be reproduced on the outside of the overpack.

A Shipper’s Declaration for Dangerous Goods is not required.

SPECIFIC REQUIREMENTS

Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen:

- When dry ice or liquid nitrogen is used to keep specimens cold, all applicable requirements of these Regulations must be met. When used, ice or dry ice must be placed outside the secondary packagings or in the outer packaging or an overpack. Interior supports must be provided to secure the secondary packagings in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or overpack must be leakproof. If dry ice is used, the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packagings.
- The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures, which could result if refrigeration were to be lost.

Infectious substances assigned to UN 3373 which are packed and marked in accordance with this packing instruction are not subject to any other requirement of these Regulations except for the following:

- (a)** the name and address of the shipper and of the consignee must be provided on each package;

- (b) the name, and telephone number of a person responsible must be provided on the air waybill or on the package;
- (c) the classification must be in accordance to 3.6.2;
- (d) the incident reporting requirements in 9.6.1 must be met; and
- (e) the inspection for damage or leakage requirements in 9.4.1 and 9.4.2.

Note: *When the shipper or consignee is also the 'person responsible' as referred to in b) above, the name and address need be marked only once in order to satisfy the name and address marking provisions in both a) and b), above.*

Passengers and crew members are prohibited from transporting infectious substances as or in carry-on baggage, checked baggage or on their person.

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If an Air Waybill is used, the “Nature and Quantity of Goods” box should show “UN 3373” and the text “BIOLOGICAL SUBSTANCE, CATEGORY B”.

Clear instructions on filling and closing such packages must be provided by packaging manufacturers and subsequent distributors to the shipper or to the person who prepares the package (e.g. patient) to enable the package to be correctly prepared for transport. Other dangerous goods must not be packed in the same packaging as Division 6.2 Infectious Substances unless they are necessary for maintaining the viability, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. A quantity of 30 mL or less of dangerous goods included in Classes 3, 8 or 9 may be packed in each primary receptacle containing infectious substances provided these substances meet the requirements of 2.7.1 and 2.7.5. When these small quantities of dangerous goods are packed with infectious substances in accordance with this packing instruction, no other requirements in these Regulations need be met.

ANNEX 12: STANDARD OPERATIONAL PROCEDURES (SOP) FOR RESTOCKING OF POULTRY FARMS IN BANGLADESH¹

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¹ Source: Avian Influenza Operation Manual, Department of Livestock Services, Ministry of Fisheries and Livestock in Bangladesh, with technical assistance by the Food and Agriculture Organization of the United Nations.

RESTOCKING OF POULTRY FARMS AFTER CULLING

Poultry farms are depopulated after confirmation of HPAI outbreaks to inhibit further spread of this highly contagious infectious agent. Since HPAI virus can survive for some time in the environment under certain circumstances therefore, depopulation must be followed by secure disposal of all possibly infected material. (See Annex 1 for more detail)

Before restocking of poultry farms after depopulation it has to be assured that no active avian influenza virus is remaining and circulating on the farm compound or in the neighbourhood.

PRECONDITIONS FOR RESTOCKING

- Complete culling and secure disposal of all poultry kept on the farm after the confirmed outbreak, in accordance with the AI Operation Manual;
- Complete and secure disposal or destruction of all eggs and other poultry products, droppings, litter, animal feed and any material used for poultry production (e.g. straw, rice husks) after the confirmed outbreak, in accordance with the AI Operation Manual;
- Disinfection after outbreak containment accomplished in accordance with the AI Operation Manual;
- Intensified surveillance in the Control Zone conducted in accordance with AI Operation Manual;
- No suspected case of HPAI in poultry or wild birds under current investigation within 10 km radius (Control Zone);
- Pest control carried out in the Infected Zone post outbreak and documented;
- Rodent control carried out in the Infected Zone post outbreak and documented.

- **Village poultry:**

At least 60 days of rest period after final disinfection following culling and disposal;

No confirmed outbreak of HPAI in poultry or wild birds within 10 km radius (Control Zone) during the last 60 days;

- **Commercial farms:**

No confirmed outbreak of HPAI in poultry or wild birds within 10 km radius (Control Zone) since final disinfection following culling and disposal;

Premises proved to be free from HPAI through sentinel birds introduced at least 21 days after final disinfection following culling and disposal

I. SENTINEL BIRDS

SENTINEL BIRDS CAN BE USED TO HELP DETERMINE THE SUCCESS OF DISINFECTION.

To detect remaining active HPAI virus after disinfection, birds that are highly susceptible to HPAI can be used as sentinel birds. Chickens being highly susceptible for HPAI are the most suitable sentinel birds.

Sentinel birds should be used prior to restocking in commercial farms. Their use in village chicken production is of limited value since movement of birds can not be controlled in this extensive production system.

Introduction of sentinel birds in commercial farms can be used to shorten the period of rest prior to restocking.

I.1 WHERE to introduce sentinel birds?

- Commercial farms with confirmed outbreaks; and

- All commercial farms within the Infected Zone (1 km radius).¹

The sentinel birds should be placed in every poultry house on these farms and may be allowed free ranging on a fenced farm compound during daytime to detect HPAI virus in the environment of the disposal site.

I.2 WHICH BIRDS TO USE AS SENTINEL BIRDS?

- Chickens (no other species to be used as sentinel birds);
- Source of origin should be from outside any control zone;
- Free of disease (no clinical symptoms of any disease);
- Flock of origin vaccinated against Newcastle Disease (ND, Ranikhet Disease) regularly²;
- Complete records on productivity and mortality of the flock of origin are available; and
- Flock of origin checked clinically by ULO in charge within 2 days before the delivery of sentinel birds.

It is recommended to use birds from a few selected farms (preferably governmental farms) as sentinel birds. These farms should be monitored for HPAI regularly:

- Flock of origin checked for HPAI serologically every 3 months (30 samples/flock);

¹ The need of introducing sentinel birds in every commercial farm in the infected zone regardless of detection of HPAI on that farm might be reconsidered.

² Following the instructions of the vaccine manufacturer

I.3 WHAT AGE SHALL THE SENTINEL BIRDS BE?

WHAT AGE SHALL THE SENTINEL BIRDS BE?		
0 to 14 days	For sentinel birds	In broiler rooms or in brooder houses
0 day to 20 weeks	For sentinel birds	in parent or grandparent farms, in grower houses or in layer houses with floor keeping systems
14 to 20 weeks	For sentinel birds	In caged layer houses

I.4 HOW MANY SENTINEL BIRDS PER POULTRY HOUSE?

1% of the poultry house population, but a minimum of 20 birds per poultry house.

2 COURSE OF ACTION: (to be conducted by ULO)

STEP 1: CHECK PRECONDITIONS ACCORDING TO CHECK LIST (ANNEX 3A, 3B).

All preconditions must be fulfilled before moving on to the next step.

For commercial farms proceed with **STEP 2** (introduction of sentinel birds)

For village flocks proceed with **STEP 5** (restocking)

STEP 2 INTRODUCTION OF SENTINEL BIRDS

1. Check whether selection criteria are met, record source of origin (Annex 4).
2. Oropharyngeal/tracheal and cloacal swabs and blood samples from at least 5 birds per unit/shed of where sentinel birds will be introduced have to be collected at the date of introduction.
3. Samples have to be submitted with the sample submission form (Annex 5) to CDIL for diagnosis of HPAI.
4. Advise poultry worker/farm manager to keep daily records on mortality.
5. Advise farm manager to report mortality and clinical symptoms immediately to ULO.

In case of positive serum samples:

Stop the process: Cull the birds, disinfect the premise once and introduce new sentinel birds.

In case of positive swab samples:

Outbreak containment following the AI Operation Manual

STEP 3 WEEKLY EXAMINATION OF SENTINEL BIRDS AFTER INTRODUCTION ON THE FARM (ANNEX 4)

1. Check records on mortality.
2. Clinical examination of the sentinel flock.

In case of mortality:

Every dead sentinel bird has to be submitted with the sample submission form (Annex 5) to FDIL for necropsy and diagnosis of HPAI (PCR mandatory)

In case of clinical symptoms (Annex 2):

1. Collection of oropharyngeal/tracheal and cloacal swabs from all diseased sentinel birds.
2. Samples have to be submitted with the sample submission form (Annex 5) to BLRI for diagnosis of HPAI

In case of positive swab samples after sampling:

Outbreak containment following the AI Operation Manual.

STEP 4 CHECK SENTINEL BIRDS 21 DAYS AFTER INTRODUCTION ON THE FARM

1. Check records on mortality
2. Check clinically
3. Collect oropharyngeal/tracheal swabs, cloacal swabs and blood samples from 5 birds
4. Samples have to be submitted with the sample submission form (Annex 5) to CDIL for diagnosis of HPAI

In case of negative results:

Proceed with **STEP 5**.

In case of clinical symptoms (Annex 2):

1. Collect swabs of ALL sentinel birds of that flock and
2. Send all diseased birds for necropsy with consecutive virological examination
3. Samples have to be submitted with the sample submission form (Annex 5) to BLRI for diagnosis of HPAI

In case of positive serum samples:

1. Collect swabs of ALL sentinel birds of that flock and
2. Samples have to be submitted with the sample submission form (Annex 5) to BLRI for diagnosis of HPAI

**In case of positive swab samples/virological results
after necropsy:**

Outbreak containment following the AI Operation Manual.

STEP 5 RESTOCKING FOLLOWING DLS DECLARATION

For **village flocks** proceed with **STEP 6**

**STEP 6 CLINICAL SURVEILLANCE OF
AT LEAST 10 RANDOMLY SELECTED
HOUSEHOLDS PER RESTOCKED
INFECTED ZONE 7 DAYS AND 21
DAYS AFTER RESTOCKING, FOLLOWED
BY SAMPLING OF SUSPECTED CASES
(ANNEX 2)**

ANNEX A: SURVIVAL OF HPAI VIRUS

(source AUSVETPLAN)

In droppings:	6 days at 37°C (source: OIE Disease Card) 3 days* at 20°C 35 days* at 4°C
In the poultry house:	2 weeks after depopulation up to 5 weeks (within the poultry house environment)
In water:	4 days at 22° Cover 30 days at 0°C
In carcasses, slaughtered birds:	few days at air or room temperature up to 23 days when refrigerated

* depending on the amount of virus much longer,
when the litter contains carcasses of infected birds

ANNEX B: CASE DEFINITIONS OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI)

1. Village/backyard poultry

- Multiple deaths of poultry in a single household or in several neighboring households.

2. Commercial farms with less than 2000 birds

- Daily mortality of 1% or more in a poultry flock for 2 consecutive days or
- More than 2% mortality in a poultry flock within 24 hours.

3. Grandparent stock, parent stock and commercial farms with more than 2000 birds

- Daily mortality of 1% or more in a poultry flock for 2 consecutive days or
- More than 2% mortality in a poultry flock within 24 hours or
- Sudden drop in egg production by 10% or more or
- Sudden reduction in food and water intake by 20% or more.

With or without following symptoms:

- Respiratory signs (coughing, sneezing, difficult breathing).
- Swollen head.
- Cyanosis of comb and wattle (bluish, dark color).
- Hemorrhages in the shanks (small bleedings under the skin).

ANNEX C: RESTOCKING OF VILLAGE POULTRY CHECKLIST

PRECONDITION	YES	NO
Complete culling and secure disposal of all poultry kept on the farm after the confirmed outbreak. ¹		
Disposal site secure, checked by veterinary authorities.		
Disinfection after outbreak containment accomplished ¹ at least 21 days before introduction of sentinel birds, documented.		
No droppings or litter remaining on the farm compound since the outbreak.		
No eggs, feathers or other poultry products remaining on the farm compound since the outbreak.		
No poultry feed remaining on the farm compound since the outbreak.		
Intensified surveillance in the Control Zone conducted. ¹		
No confirmed outbreak of HPAI in poultry within 10 km radius (Control Zone) during the last 21 days.		
No suspected case of HPAI in poultry or wild birds under current investigation within 10 km radius (Control Zone).		
Pest control carried out on the farm post outbreak and documented.		
Rodent control carried out on the farm post outbreak and documented.		

NOTE: If any of these preconditions is not fulfilled (ticked “NO”), introduction of sentinel birds has to be postponed until all conditions are met.

Date

Signature (Livestock officer)

¹ in accordance with the AI Operation Manual

ANNEX D: RESTOCKING OF VILLAGE POULTRY CHECKLIST

PRECONDITION	YES	NO
Complete culling and secure disposal of all poultry kept on the Infected Zone after the confirmed outbreak. ¹		
Disposal site secure, checked by veterinary authorities.		
Disinfection after outbreak containment accomplished ¹ at least 60 days before restocking, documented (by ULO).		
No droppings or litter from possibly infected poultry remaining in the Infected Zone since the outbreak.		
No eggs, feathers or other poultry products from possibly infected poultry remaining in the Infected Zone since the outbreak.		
No poultry feed used for possibly infected poultry remaining in the Infected Zone since the outbreak.		
Intensified surveillance in the Control Zone conducted. ¹		
No confirmed outbreak of HPAI in poultry or wild birds within 10 km radius (Control Zone) during the last 60 days.		
No suspected case of HPAI in poultry or wild birds under current investigation within 10 km radius (Control Zone).		
Pest control carried out in the Infected Zone post outbreak and documented (by ULO).		
Rodent control carried out in the Infected Zone post outbreak and documented (by ULO).		

NOTE: If any of these preconditions is not fulfilled (ticked “NO”), introduction of sentinel birds has to be postponed until all conditions are met.

Date

Signature (Livestock officer)

¹ in accordance with the AI Operation Manual

ANNEX E: SENTINEL BIRDS – PROTOCOL

Farm: _____
(name, address)

Poultry production: broiler farm layer farm
 parent stock farm grand parent stock farm

STEP 1: PRECONDITIONS

Preconditions for introduction of sentinel birds met (Annex 3a) Yes
 No

STEP 2: INTRODUCTION OF SENTINEL BIRDS

Date: _____

Number of Birds: _____ Number of units/sheds: _____

Type of birds: _____ Age of birds: _____

Farm of origin: _____
(name, address)

Oropharyngeal/tracheal and cloacal swabs and blood samples collected from sentinel birds at date of introduction Yes
 No

_____ samples submitted to _____
(number) (name of laboratory)

Laboratory results: SWABS

all swabs tested negative for HPAI
 samples tested positive for HPAI

No. of swabs tested positive: _____

BLOOD SAMPLES

- all blood samples tested negative for HPAI
- samples tested positive for HPAI

No. of samples tested positive _____

STEP 3: WEEKLY EXAMINATION

1. EXAMINATION (one week after introduction)

Date: _____

Mortality records: no mortality in the sentinel flock

_____ carcasses submitted for laboratory examination

Clinical examination: no sign of clinical disease in any sentinel bird

clinical symptoms observed _____ in birds as described below:

- coughing _____
- sneezing _____
- difficult breathing _____
- swollen head _____
- blueish comb, wattle _____
- shanks with hemorrhages _____
- others (describe) _____

Signature ULO

In case of clinical disease:

Oropharyngeal/tracheal and cloacal swabs collected from all diseased birds Yes
 No

_____ samples submitted to _____
(number) (name of laboratory)

Laboratory results: SWABS

- all blood samples tested negative for HPAI
- samples tested positive for HPAI

No. of swabs tested positive: _____

CARCASSES

- all submitted birds tested negative for HPAI
- birds tested positive for HPAI

No. of swabs tested positive: _____

2. EXAMINATION (two week after introduction)

Date: _____

Mortality records: no mortality in the sentinel flock

_____ carcasses submitted for laboratory examination since the last visit

Clinical examination: no sign of clinical disease in any sentinel bird

clinical symptoms observed _____ in birds as described below:

- coughing _____
- sneezing _____
- difficult breathing _____
- swollen head _____
- blueish comb, wattle _____
- shanks with hemorrhages _____
- others (describe) _____

Signature ULO

In case of clinical disease:

Oropharyngeal/tracheal and cloacal swabs collected from all diseased birds Yes
 No

_____ samples submitted to _____
(number) (name of laboratory)

Laboratory results: SWABS

- all blood samples tested negative for HPAI
- samples tested positive for HPAI

No. of swabs tested positive: _____

CARCASSES

- all submitted birds tested negative for HPAI
- birds tested positive for HPAI

No. of swabs tested positive: _____

STEP 4: EXAMINATION & SAMPLING 21 DAYS AFTER INTRODUCTION

1. EXAMINATION (three week after introduction)

Date: _____

Mortality records:

- no mortality in the sentinel flock
- _____ carcasses submitted for laboratory examination since the last visit
- _____ carcasses disposed without laboratory examination since the last visit

Clinical examination:

- no sign of clinical disease in any sentinel bird
- clinical symptoms observed _____ in birds as described below:

- coughing _____
- sneezing _____
- difficult breathing _____
- swollen head _____
- blueish comb, wattle _____
- shanks with hemorrhages _____
- others (describe) _____

Signature ULO

In case of clinical disease:

Oropharyngeal/tracheal and cloacal swabs collected from all diseased birds Yes
 No

ALL diseased birds sent for necropsy with subsequent virological examination Yes
 No

_____ samples submitted to _____
(number) (name of laboratory)

Laboratory results: SWABS

all blood samples tested negative for HPAI
 samples tested positive for HPAI

No. of swabs tested positive: _____

BLOOD SAMPLES

all blood samples tested negative for HPAI
 samples tested positive for HPAI

No. of swabs tested positive: _____

CARCASSES

all submitted birds tested negative for HPAI
 birds tested positive for HPAI

No. of swabs tested positive: _____

STEP 5: FARM DECLARED FREE FOR RESTOCKING BY DLS

Date: _____

Date

Signature (Livestock officer)

ANNEX F: SAMPLE SUBMISSION FORM

**Government of the People's Republic of Bangladesh
Department of Livestock Services**

Sample Submission Form

Collection date:			Submission date:		
Submitting veterinarian:					
Designation:					
Address:					
Contact telephone number:					
Farm/Owner:					
Address:					
Species of bird	Type	Breed	Age		
No. of birds in affected flocks	No. affected	No. died	No. of birds sampled		
			Sick	Dead	
History of Outbreak:					
Clinical signs:					
Necropsy findings (if done)*:					
Preliminary diagnosis:					
Details of samples submitted:					
Specimen type	Specimen ID#	Tests requested	Specimen type	Specimen ID#	Tests requested
Date:			Signature:		

***Necropsy should be done only at designated places. Special protection must be taken if AI is suspected**
****Information on preservatives, antibiotics, pooling of samples, etc.**

If commercial feed supplied, specify brand:								
If custom formulated feed supplied, give information:								
If any supplement is given in feed or water, give details:								
If any treatment already given in the present outbreak, give details:								
History of any previous outbreak in the farm:								
Shed #	Flock size	Age at outbreak	Disease diagnosed	Duration of outbreak	No. of birds affected	No. of birds died	Treatment given	Vet consulted

CLINICAL OBSERVATIONS

Clinical signs:					
Necropsy findings (if any)*:					
Samples collected:					
Specimen type	Number of specimens	Laboratory referred to	Date of Shipment	Date of Shipment **	Test requested for:
Investigating veterinarian's name:					
Date:			Signature:		

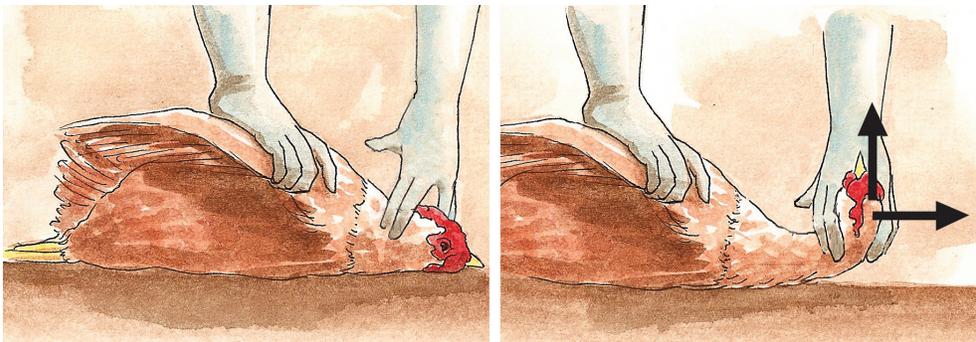
***Necropsy should be done only at designated places. Special protection must be taken if AI is suspected**
****Must accompany Sample Submission Form**

ANNEX 13: CERVICAL DISLOCATION

Cervical dislocation is considered a humane method of poultry euthanasia and is the most common method for killing birds.

Use the following steps to perform a cervical dislocation:

1. Place the bird breast-down on a flat surface (or hold the bird against your hip).
2. Use one hand to hold both wings behind the bird's back.
3. Using your other hand, hold the head between your middle and ring fingers, with the middle finger on the back of the chicken's head.
4. Sharply turn the head 90 degrees while at the same time pulling it firmly and quickly away from the body (in a motion like stretching the neck). See diagram below. You will feel the vertebra separate.
5. Hold the bird in this position until agonal flapping stops.



Others might be more comfortable using this grip:

1. Direct the bird's head toward you. Grasp the bird's head with a handshake grip.
2. Place your thumb behind the head at the base of the skull, allowing the remaining fingers to extend under the throat.

3. Hold the bird's feet with the other hand.
4. Stretch the bird until you feel the head separating from the neck vertebrae. You will probably need to bend the head back slightly while stretching the bird.
5. Be careful to stop pulling when the spine separates or the head may be pulled off.
6. The bird dies immediately when the spine separates.

Source: Manual on Procedures for Disease Eradication by Stamping Out, FAO Corporate Document Repository, www.fao.org/docrep/004 from the Notifiable Avian Influenza Hazard Specific Plan, Appendix J: Blood Collection and Euthanasia Technique in Poultry, Canadian Food Inspection Agency, www.inspection.gc.ca

ANNEX 14: CULLING USING CO₂

Birds for destruction will vary in their size and structure. For small numbers of birds the preferred method is dislocation of the neck using burdizzos, forceps, pliers or by hand.

For larger numbers of birds in commercial poultry units the preferred method is gassing with CO₂ in containers. This method involves lining large garbage waste bins with plastic sheeting so that it also forms a canopy over the top of the bin. CO₂ is pumped into the bottom of the bins, through a 2.5 cm garden hose fitted to the top of the CO₂ cylinders. The CO₂ should be released in 30-45 second bursts. Do not release the gas too quickly, or the bottles will freeze when they become about 1/2 empty. The concentration of carbon dioxide must be in the range of 60-70 % in the waste bin, with the lid tightly closed for 1-2 minutes to properly stun and kill the birds.

Usually, 1/2 of 45 kg cylinder of CO₂ is needed for the three cubic meter bins, and three or more cylinders are needed for the 20 cubic meter bins. Carbon dioxide should be added so that all birds are dead before others are placed on top of them. The bins should be 75% filled with birds, sealed, and transported to the disposal sight. Usually infected birds are slaughtered first, followed by birds in contact with infected birds, and then the remaining birds in the flock.

CO₂ METHOD:

- The objective is the humane destruction of large numbers of birds in a short time. Birds should be unconscious in one minute and dead within 3 to 5 minutes.
- CO₂ gas has human health and safety risks and both a safety officer and First Aid should be available.
- The effort should be organized into teams with specific jobs such as leader, catchers, gas re-supply, gas operators, record keeper (number of birds) and animal welfare.
- A site plan is needed before beginning and should include access to birds keeping movement to a minimum, no birds

should escape, enough bins, gas. etc. and easy bin removal. It is expected that most birds will be killed in containers.

- It may be easier to place them in plastic garbage bags for transfer to bins for CO₂.
- If they can be driven into a corner it is also easier for catchers.
- Caged birds are more difficult and progress will be slower. Each catcher can remove 3 or 4 birds and carry them directly to the bins.
- Containers or apparatus should allow the required gas concentration to be maintained and accurately measured.
- The equipment used should be designed, constructed, and maintained in such a way as to avoid injury to the animals and allow them to be observed.
- Animals should be introduced into the container or apparatus after it has been filled with the required CO₂ concentration, and held in this atmosphere until death is confirmed. 17.5 kg gas saturates 1000 cubic meter area.
- The bins need to be pre-charged with CO₂ for about 5 minutes before any birds are received. Each bin should have a secured gas cylinder or each end.
- Hot water should be readily available in case the regulators freeze.
- Place the gas hoses in the bins, about 300 mm above the level of the birds, adjusting as the bins fill with birds.
- Team members should ensure that there is sufficient time allowed for each batch of animals to die before subsequent ones are introduced into the container or apparatus.
- Only one layer of birds should be placed in a bin at once-inspect for death after 20 minutes.

- Additional layers of birds can be added, one at a time, until the bin is 70 to 90% filled.
- Seal the lids for containment and transfer to the disposal site. Care must be taken that no birds are buried alive.
- Please keep in mind that it is not advisable to try to kill water birds such as ducks with CO₂. Cervical dislocation is preferred.
- Also care must be taken that fertilized eggs do not hatch.

