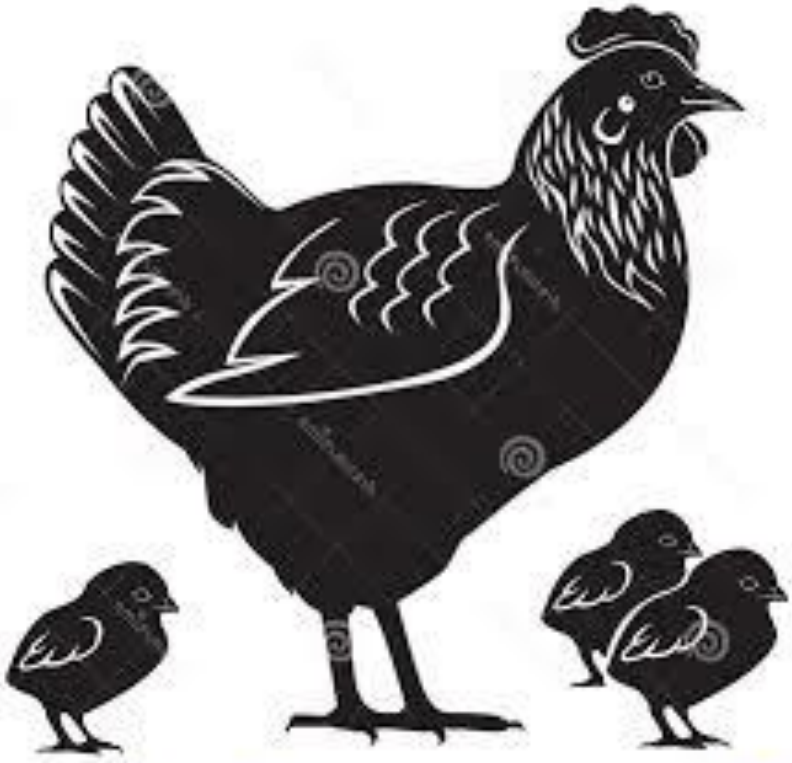

NATIONAL INFLUENZA PANDEMIC PREPAREDNESS PLAN
&
STANDARD OPERATING PROCEDURES

NIPPP & SOPs
2020

DEPARTMENT OF LIVESTOCK

Ministry of Agriculture & Forests

Royal Government of Bhutan

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National Centre for Animal Health

Department of Livestock

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2nd July 2020

FOREWORD

I am happy to note that the National Centre for Animal Health, Secretariat to the National Command Committee for Avian Influenza has taken the lead in revising and updating the National Influenza Pandemic Preparedness Plan & Standard Operating Procedures for Avian Influenza. Since the publication of the first version of the document in 2011, Bhutan has recorded at least 13 outbreaks of the Highly Pathogenic Avian Influenza at district level which was successfully contained from further spread into the interior parts of the country. The virus has also evolved necessitating changes in the prevention and control strategies. With the emergence of cases of Avian Influenza H7N9 in humans in China and subsequent isolation of the virus from poultry, it was necessary to incorporate strategies for preparedness for H7N9 into the NIPPP document so that all stakeholders are aware of the additional requirements for surveillance and control for this novel virus.

I would like to extend my appreciation to all the organizations and individuals who contributed towards revising and updating this very important document. The NIPPP 2020 is revised in line with the provisions of the Disaster Management Act of Bhutan 2013 specifically for the incident command structure and fund mobilization aspects. The revisions also took into account the issues and challenges encountered during responding to the HPAI outbreaks thus, the revised NIPPP 2020 is comprehensive. New SOPs have been added to provide information to the field staff while implementing surveillance and containment activities

I hope this revised plan document will be useful as a ready reference to all those involved in the preparedness, surveillance and containment of Highly Pathogenic Avian Influenza and other notifiable Avian Influenza viruses in the country

Dasho Rinzin Dorji
Chairman & Secretary
National Incident Command Committee / MoAF

ABBREVIATIONS

AI	Avian Influenza
BAFRA	Bhutan Agriculture & Food Regulatory Authority
BVT	Border Vigilance Team
CP	Contact Premises
DDM	Department of Disaster Management
DoL	Department of Livestock
DoPH	Department of Public Health
DuDMC	Dungkhag Disaster Management Committee
DDMC	Dzongkhag Disaster Management Committee
DDMO	Dzongkhag Disaster Management Officer
GDMC	Gewog Disaster Management Committee
HPAI	Highly Pathogenic Avian Influenza
IOC	Incident Operation Centre
IP	Infected premises
MoAF	Ministry of Agriculture & Forests
MoF	Ministry of Finance
MoH	Ministry of Health
MoHCA	Ministry of Home & Cultural Affairs
NCAH	National Centre for Animal Health
NDMA	National Disaster Management Authority
NEOC	National Emergency Operations Centre
NICC	National Incident Command Committee
NIPPP	National Influenza Pandemic Preparedness Plan
OIE	Office International des Epizooties (World Organization for Animal Health)
PLQO	Plant and Livestock Quarantine Office
PPE	Personal Protection Equipment
RLDC	Regional Livestock Development Centre
RRT	Rapid Response Team
SOP	Standard Operating Procedures
TAC	Technical Advisory Committee
VO	Veterinary Officer
VVT	Veterinary Vigilance Team

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1 RATIONALE FOR DEVELOPMENT OF NIPPP

Following the outbreaks of highly pathogenic avian influenza (HPAI-H5N1) in Southeast Asian countries in January 2004, Ministry of Agriculture & Forests (MoAF) and Ministry of Health (MoH) initiated contingency measures to prevent incursion of H5N1 virus into the country and to strengthen the surveillance system in the animal health and human health sectors in order to detect and respond to any outbreaks. Focal officers were identified from the Department of Livestock (DoL), Bhutan Agriculture & Food Regulatory Authority (BAFRA) of MoAF, and the Department of Public Health (DoPH) of MoH to facilitate collaboration between the two ministries to implement contingency measures. Risk assessments were carried out by DoL from January to February 2004. The assessment at that time indicated a very low risk for the incursion of the virus into the country. The risk had greatly increased from 2006 onwards due to frequent outbreaks of HPAI in Indian states of West Bengal and Assam.

Realizing the pandemic potential of H5N1 virus and considering the imminent threat of incursion of HPAI into the country, MoAF and MoH initiated the development of the National Influenza Pandemic Preparedness Plan (NIPPP) under the World Bank-supported National Influenza Preparedness and Response Project. Through this project the draft plan was tested through a series of desktop and field simulation exercises and core capacities were developed to respond effectively to an outbreak of HPAI and pandemic influenza in Bhutan. Although the first edition of the NIPPP document was printed out in 2011, the country was well prepared with the necessary preparedness in responding effectively to the first outbreak of HPAI H5N1 in Bhutan in February 2010. With the changing epidemiological pattern of disease (H5N1/ H7N9) and challenges faced in the field, the NIPPP document was revised in 2014 and in 2019. The experiences gained and the lessons learned during containment of various previous HPAI outbreaks are incorporated into this document.

The objectives of this NIPPP document are to:

- Strengthen surveillance for early warning, detection and response to HPAI H5N1/H7N9 and other Notifiable Avian Influenza Viruses;
- Rapidly contain or prevent/ delay spread of the virus at the source;
- Reduce the opportunities for human infection;
- Minimize morbidity, mortality and social disruption, and
- Monitor and evaluate the response capacity.

This NIPPP was developed to ensure that all the required resources, expertise and services are mobilized and deployed rapidly to reduce the morbidity, mortality and social disruption to the minimum. In addition, establishment and strengthening of core capacities to pre-empt and control the next pandemic would also be useful in dealing with other infectious disease epidemics and public health emergencies of international concern as required under the International Health Regulations (IHR, 2005).

This NIPPP document has been developed jointly by the disease experts of MoAF and MoH. Consultations have been made extensively with other relevant sectors including the Department of Disaster Management (DDM) under the Ministry of Home and Cultural Affairs

(MoHCA) especially for the fund mobilization aspects in line with the Disaster Act 2013. As the government needs to be very clear in its preparedness and response plans when pandemic strikes, an explicit chain of command and incident command structures for an influenza pandemic and HPAI outbreak in the animal was developed including their roles and responsibilities. The standard operating procedures (SOPs) have also been developed for rapid response teams for responding to an influenza pandemic and HPAI outbreak in animals to avoid confusion and disagreement in times of crisis. Every agency and individual involved in crisis management are fully trained and equipped to initiate their actions immediately.

2 KEY UPDATES/REVISIONS IN THE REVISED NIPPP 2020

The key revisions/updates made in this document as compared with the NIPPP 2014 are:

- The incident command system has been updated in line with the Disaster Management Act of Bhutan 2013. The National Disaster Management Authority (NDMA) replaces the National Steering Committee as the highest authority on NIPPP. Linkages and coordination mechanisms have been built in the command system with the Disaster Management Committees at the National, District, Dungkhag and Gewog level in order to maintain coherence for the implementation of response activities in line with the Disaster Management Act of Bhutan 2013.

- The roles and responsibilities of the National Incident Command Committee (NICC) have been defined for both normal (peace) times and during an outbreak.

- The composition of the National Incident Command Committee (NICC) and Incident Operation Centre (IOC) have been reviewed and updated. The number of technical members in NICC was reduced with the formation of the Technical Advisory Committee (TAC).

- The National One Health Technical Committee will function as Technical Advisory Committee to guide NICC, IOC and field offices on prevention and control of HPAI during outbreaks. The Terms of Reference for TAC is developed and included in this document.

- Roles of the National Centre for Animal Health and Regional Livestock Development Centre are specified.

- A timeline has been indicated from the time a case of avian influenza is suspected until deactivation of the IOC/NICC in order to have a common understanding of the roles and responsibilities at all levels of the command system.

- To ensure that the compensation is fair, transparent and timely to all eligible farmers/owners, compensation committee members are categorized into Dzongkhag and Thromde level.

- The protocol for information sharing and reporting has been included.

- The fund mobilization and release mechanisms have been explained clearly in line with the Disaster Management Act of Bhutan 2013.

- The Standard Operating Procedures (SOPs) have been updated based on the latest scientific evidence/knowledge. New SOPs have been added on vehicle disinfection, movement of poultry/poultry products during outbreak time, surveillance on Avian Influenza (AI) H7N9.

- In view of the emergence of AI H7N9 in China and the imminent threat to Bhutan, the surveillance and response mechanisms for this novel influenza virus has also been incorporated in this document.

- Information on the historical outbreaks of HPAI H5N1 in Bhutan has been included along with the virus clades circulating in the country.

- Dzongkhag Disaster Focal person has been included as a member of the compensation committee and IOC.

- A flow chart for budgeting and disbursement process for compensation has been developed for reference by the IOC/NICC

- The forms under Standard Operating Procedures are pretested, revised based on the field experience and are attached as printable forms

3 OUTBREAKS OF HPAI, H5N1 IN BHUTAN

HPAI is listed as a notifiable disease in the country under the Livestock Rules and Regulations of Bhutan 2017 and it is mandatory to report any suspected cases of HPAI in avian population to relevant authorities. Bhutan reported its first HPAI H5N1 outbreak on 23 February 2010 to the World Organization for Animal Health (OIE). The disease was first detected on 18th February 2010 at Rinchending village, Chukka district in free-ranging poultry that was subsequently confirmed by the High-Security Animal Disease Laboratory (HSADL) Bhopal, India and the National Institute of Animal Health (NIAH), Bangkok, Thailand. Subsequently, at least 12 separate outbreaks of HPAI H5N1 were confirmed in twenty-one locations of seven districts (Table 1). All of these outbreaks in Bhutan were rapidly contained following the implementation of the NIPPP and SOPs for a response to HPAI outbreak. Except for the first outbreak, the rest of the cases were first confirmed at the National Centre for Animal Health (NCAH) using real-time reverse transcription PCR (rt-RT-PCR) and later reconfirmed at HSADL. The officials from MoH and related agencies were also equally involved in response to these outbreaks. The strain of virus isolated during the first outbreak was virus

clade 2.2.3 (in 2010) and subsequently clades 2.3.2.1a (2011, 2012, 2013, 2015, 2016, 2018 and 2019) were recorded.

Currently, passive and active clinical surveillance of avian influenza in poultry, wild birds and waterfowls are carried out routinely by the Border Vigilance Team (BVT) of Bhutan Agriculture and Food Regulatory Authority (BAFRA) and Veterinary Vigilance Team (VVT) of Department of Livestock. Active clinical and laboratory surveillance are also carried out in high-risk areas during the high-risk period (beginning October until February end) and also following reports of outbreaks in the neighbouring countries.

The regional and district laboratories are equipped with rapid antigen detection tests for type A and H5 subtypes and all the rapid test positive samples in the field are shipped to the National Centre for Animal Health (NCAH) for confirmation through real-time RT-PCR. These samples are further referred to the OIE regional reference laboratory at HSADL, Bhopal, India for confirmation and virus characterization.

Table 1 Details of the confirmed outbreaks of HPAI H5N1 in Bhutan (2010 - 2019)

Sl no	Year/ Months	District	Sub-districts	Villages	Species	Farming system	Virus clade
1.	February, March 2010	Chukha	Phuentsholing Samphelling	Rinchending Toorsa Ramitey Pasakha Burkhey	Poultry	Backyard	2.2.3
2.	December 2011 and January - March 2012	Chukha	Bjabcho Chapcha Darla Bongo Geling Phuentsholing Samphelling	Tsimasham Mebesa Mebari Chukha Tsimalakha Bunagu Darla Gedu Kamji Ramitey Wangdigatshel Burkhey	Poultry Pigeon Sparrow	Semi-commercial and backyard	2.3.2.1
3.	January 2012	Thimphu	Chang	Chang Gedaphu	Poultry	Backyard	2.3.2.1
4.	March 2012	Mongar	Gongdue	Yangbari	Poultry	Backyard	2.3.2.1
5.	October 2012	Chhukha	Phuentsholing	Rinchending	Poultry	Backyard	2.3.2.1
6.	December 2012	Dagana	Lhamoizingkha	Farmgaon	Poultry	Backyard	2.3.2.1
7.	January 2013	Sarpang	Gelephu Chuzagang	Namkhaling Pelrithang Chuzagang	Poultry	Backyard and semi-commercial	2.3.2.1

8.	February 2013	Samtse	Yoeseltse	Dungkhar	Poultry	Backyard	2.3.2.1
9.	May 2015	Thimphu	Motithang	Motithang	Poultry	Backyard	2.3.2.1
10.	October 2016	Chhukha	Bjabcho	Alubari	Poultry	Backyard	2.3.2.1
11.	March 2018	S/Jongkhar	S/Jongkhar	Zangtopelri	Poultry	Backyard	2.3.2.1
12.	March 2018	Samtse	Phuntshopelri	Kalapani and Pugli	Poultry	Semi-commercial and backyard	2.3.2.1
13.	April 2019	Chhukha	Phuentsholing	Gairigang/ Dhamdara	Poultry	Commercial	2.3.2.1

The map below (Figure 1) shows the approximate locations of the confirmed HPAI cases in Bhutan.

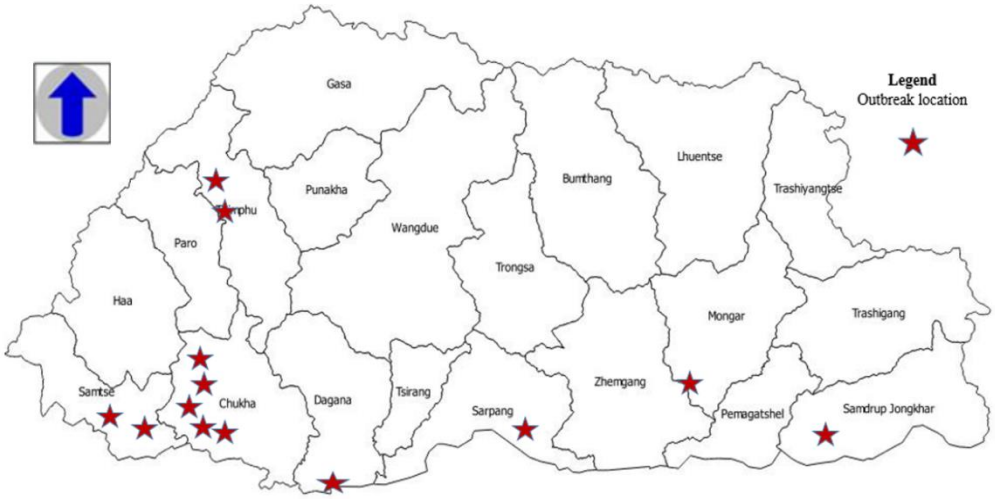


Figure 1 Locations of the confirmed outbreaks of HPAI H5N1 between 2010 and 2019 in Bhutan

4 INCIDENT COMMAND STRUCTURES AND INSTITUTIONAL ARRANGEMENTS

The Incident Command Structure for the veterinary response to pandemic influenza and HPAI outbreak respectively has been developed in line with the Disaster Management Act of Bhutan 2013 and considering the requirement of highly technical and sector-based response for HPAI outbreak as shown in Figure 2.

A pandemic due to avian influenza or other influenza would be considered a national disaster and therefore activation of the National Disaster Management Authority (NDMA) chaired by the Hon'ble Prime Minister and members comprising of high-level decision-makers from key sectoral agencies is essential for smooth implementation of NIPPP, particularly at times of pandemic. In line with the Disaster Act 2013, the NDMA shall replace the National Steering Committee (NSC), given in the NIPPP 2011 document, as the highest policy-making body for NIPPP (Figure 2).

4.1 NATIONAL DISASTER MANAGEMENT AUTHORITY (NDMA) FOR THE NIPPP

The NDMA is the highest authority for the implementation of the NIPPP and will facilitate the implementation of the NIPPP during Phases 4 to 6 of pandemic influenza or as advised by the National Incident Command Committee (NICC). The NDMA shall be chaired by the Prime Minister and shall include members as stipulated in sections 7 and 8 of Chapter 2 of the Disaster Act 2013. The NDMA will make policy decisions during the three main WHO pandemic Phases. The members of the NDMA will be as prescribed in the National Disaster Act 2013.

Incident Command Structure

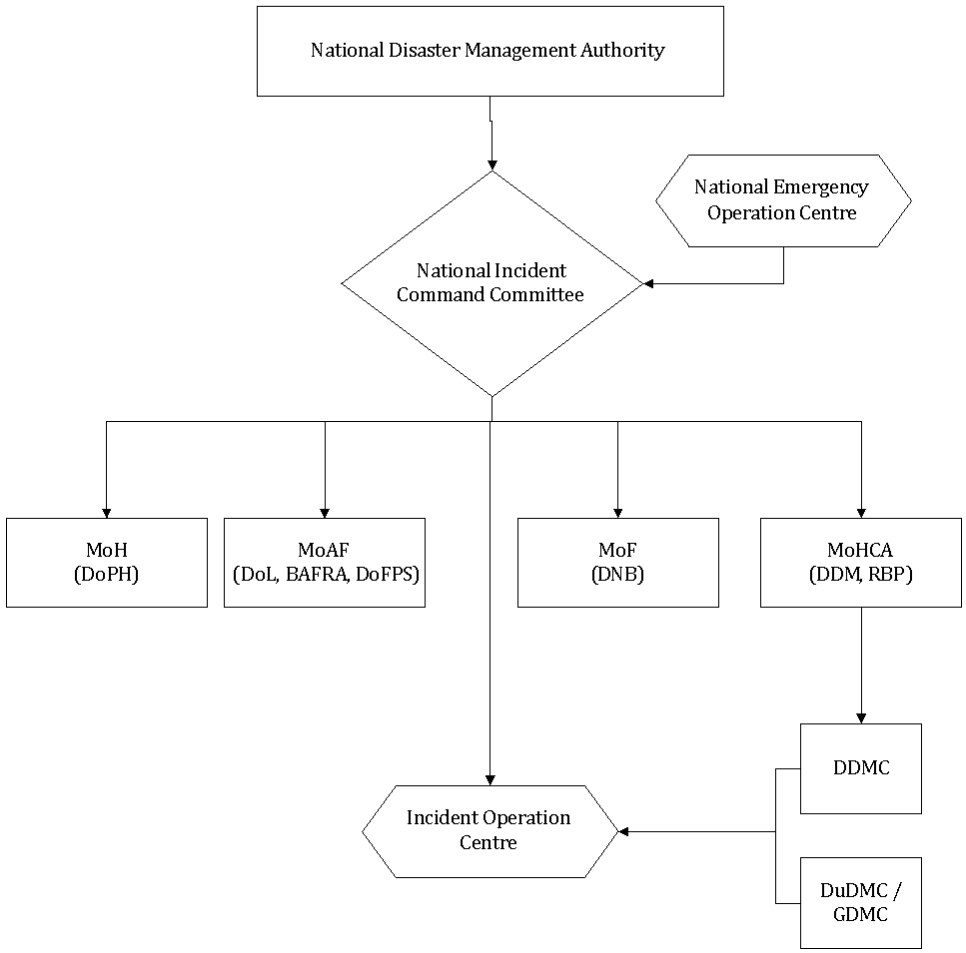


Figure 2 Incident Command Structure for NIPPP

4.1.1 Roles and Responsibilities of NDMA:

- Make policy decisions for the implementation of the NIPPP as recommended by the NICC
- Mobilize required resources for the implementation of the NIPPP as recommended by NICC
- Endorse the updates of NIPPP
- Approve inter-sectoral lead agencies and ensure coordination amongst all relevant sectors which would be involved in effective implementation of the NIPPP during Phase 4 and above of the WHO pandemic phases
- Declare different Phases of pandemic based on the recommendations of the National Technical Committee if human-to-human infection occurs (Phase 4 above)
- Direct NICC to form committees and task forces as necessary to deal with HPAI
- Ensure inter-sectoral collaboration and partnership with international, regional and bilateral agencies including the World Health Organization (WHO), the United Nation's Children Fund (UNICEF), the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), the World Bank (WB), the Asian

Development Bank (ADB), and the South Asian Association for Regional Cooperation (SAARC).

4.1.2 Meeting and procedures:

The NDMA shall meet once a year and as and when required. The Chairperson of the NDMA may call a special meeting, if:

- The Influenza Pandemic reaches to Phase 4 and beyond
- A written request is made by the NICC; or
- The Chairperson of NICC considers necessary

A simple majority shall constitute the quorum for convening the meeting. The NDMA shall seek technical recommendations from experts within MoAF and MoH for decision-making processes. The Incident Command Structures have been adopted for proper coordination of the key stakeholders during the operation. The incident command structure will allow a smooth flow of information from the national level to the incident area and vice versa.

4.2 NATIONAL INCIDENT COMMAND COMMITTEE (NICC)

The NICC is the highest policy decision-making committee for HPAI prevention and containment activities in the country under the guidance of NDMA. It shall be responsible for providing overall guidance in the implementation of the NIPPP both in the prevention as well as during the outbreak phase. The NICC shall liaise closely with the National Emergency Operations Centre (NEOC) for necessary support especially in the event of the Influenza Pandemic reaching Phase 4 and beyond. The National Centre for Animal Health (NCAH), DoL will be the secretariat to the NICC.

4.2.1 Members of NICC

The following members constitute the NICC:

- Secretary, Ministry of Agriculture and Forests - Chairperson
- Head, Department of Livestock, MoAF (Member Secretary)
- Head, Bhutan Agriculture and Food Regulatory Authority, MoAF
- Head, Department of Public Health, Ministry of Health
- Head, Department of Disaster Management, Ministry of Home & Cultural Affairs
- Head, Department of Forests and Park Services
- Head, Department of National Budget, Ministry of Finance
- Chief of Police, Royal Bhutan Police
- Head, Animal Health Division, DoL
- Head, Quality Control and Quarantine Division, BAFRA
- Head, National Centre for Animal Health

4.2.2 Roles and responsibilities of NICC during prevention phase (peace time):

- Authorize review of the NIPPP including SOPs from time to time.
- Keep the NDMA updated on new developments of the epidemic worldwide and nationally.

- Issue Executive Order for activation of Veterinary Vigilance Team (VVT) and Border Vigilance Team (BVT) based on the recommendation of the Technical Advisory Committee (TAC).
- Recommend ban on import/export of poultry, poultry products and other risk goods for prevention of incursion and/or spread of HPAI virus into and within the country. The recommendations should be science-based and be decided in collaboration with other relevant stakeholders such as the Ministry of Economic Affairs, BCCI, etc.
- Designate sectoral media spokespersons for risk communication, one each from MoH and MoAF in the event of outbreaks of HPAI in the neighbouring countries
- Identify the inter-sectoral lead agencies for the effective implementation of the NIPPP.
- Ensure the adequacy, timeliness and relevance of communications activities.

4.2.3 Roles and responsibilities in the event of an outbreak of HPAI in Bhutan:

- Declare the outbreak of HPAI and issue official/public notification on recommendation of TAC.
- Authorize issuance of notice to the OIE and other trading partners.
- Take policy decisions on response and control measures based on the advice and recommendation of the TAC.
- Command establishment of Incident Operation Centre and Rapid Response Teams (RRTs).
- Provide policy direction for response activities.
- Issue executive orders, public notifications and press release on disease outbreak and its containment activities.
- Facilitate and mobilize all the logistics required for responding to control HPAI outbreaks.
- Designate media spokesperson(s) for providing disease status update, response policies and strategies, press release, etc.
- Ensure the adequacy, timeliness and relevance of communications activities.
- Maintain liaison with relevant sectors such as the Department of Disaster Management (DDM), Ministry of Economic Affairs (MoEA), Ministry of Finance (MoF), Ministry of Foreign Affairs (MoFA), Gross National Happiness Commission (GNHC), Bhutan Chamber of Commerce and Industries (BCCI), Ministry of Education (MoE), International organizations, non-governmental organizations, etc.
- Provide updates to the NDMA.

4.2.4 Frequency of meetings and quorum:

The NICC meeting shall be convened within 24hours of laboratory confirmation of the outbreak by the NCAH. During normal times, NICC shall be convened at least once a year or as and when deemed necessary by the Chairperson. The committee will also meet as and when there are outbreaks in neighbouring/trading countries to decide on regulatory issues or when there are serious public health threats. A simple majority shall constitute the quorum and as far as possible all the members shall be present. In addition, technical experts shall be invited to the meeting.

4.3 NATIONAL ONE HEALTH TECHNICAL COMMITTEE (TECHNICAL ADVISORY COMMITTEE)

The National One Health Technical Committee (NOHTC) will also function as a technical advisory committee (TAC) for HPAI prevention and control (NIPPP) activities. The TAC members will comprise of experts from different sectors to advise and provide technical recommendations to NICC and IOC.

4.3.1 Members:

S.N.	Current Members	Designation	Responsibilities
1	Dr. RB Gurung	NCAH, DoL, MoAF	Chair
2	Dr. Thinley Yangzom	Clinician Representative, JDWNRH	Vice Chair
3	Dr. Sithar Dorjee	Representative, KGUMSB	Member
4	Dr. Sonam Wangchuk	Head, RCDC, DoPH, MoH	Member
5	Dr. Karma Rinzin	Head, Animal Health Division, DoL, MoAF	Member
6	Dr. Kinley Choden	Representative, WCD, DoFPS, MoAF	Member
7	Dr. Kinley Penjor	Representative, BAFRA, MoAF	Member
8	Ms. Jamyang Choden	Program Officer, IHR, DMS, MOH	Member
9	Dr. Kinley Penjor	Officer on Special Assignment (OSA)	Member
10	Yeshey Pelden	Program Officer, ZDCP & AI, CDD, MoH	Member Secretary

4.3.2 Terms of Reference for Technical Advisory Committee

- Review and provide technical advice to the NICC on the emergencies related to HPAI prevention and control activities;
- Conduct situational assessment and advice NICC on activation of Veterinary Vigilance and Border Vigilance Team when HPAI outbreaks are reported in the neighbouring countries and when the imminent threat of HPAI incursion is observed;
- Review and provide technical recommendations on HPAI outbreak containment including pre-emptive culling before the laboratory confirmation and advice on activation of NICC;
- Advise NICC and IOC on the scale of rapid containment activities including manpower requirement, timeline and allocation of resources depending on the magnitude of a disease outbreak;
- Review the IOC reports and provide timely advice to IOC on HPAI containment activities during HPAI outbreak;
- Review and revise NIPPP and SOPs for HPAI prevention and control activities;

- Facilitate mobilization of resources for HPAI prevention and control activities;
- Carry out any specific tasks assigned by NICC

4.3.3 Meeting and Procedures

The committee will meet bi-annually and as and when required.

5 NATIONAL INCIDENT COMMAND STRUCTURE FOR HPAI RESPONSE

Following an outbreak of HPAI H5N1 / H7N9 and other notifiable avian influenza viruses in the country, the NICC shall be activated within 24 hours of laboratory confirmation of the disease by NCAH.

The NICC shall authorize the activation of the Incident Operations Centre (IOC) and the formation of RRTs which will spearhead the containment activities of HPAI H5N1 control in the country. The flow of command will be as per Figure 3 for the effective containment of the outbreak.

Incident Command Structure & Rapid Response Teams

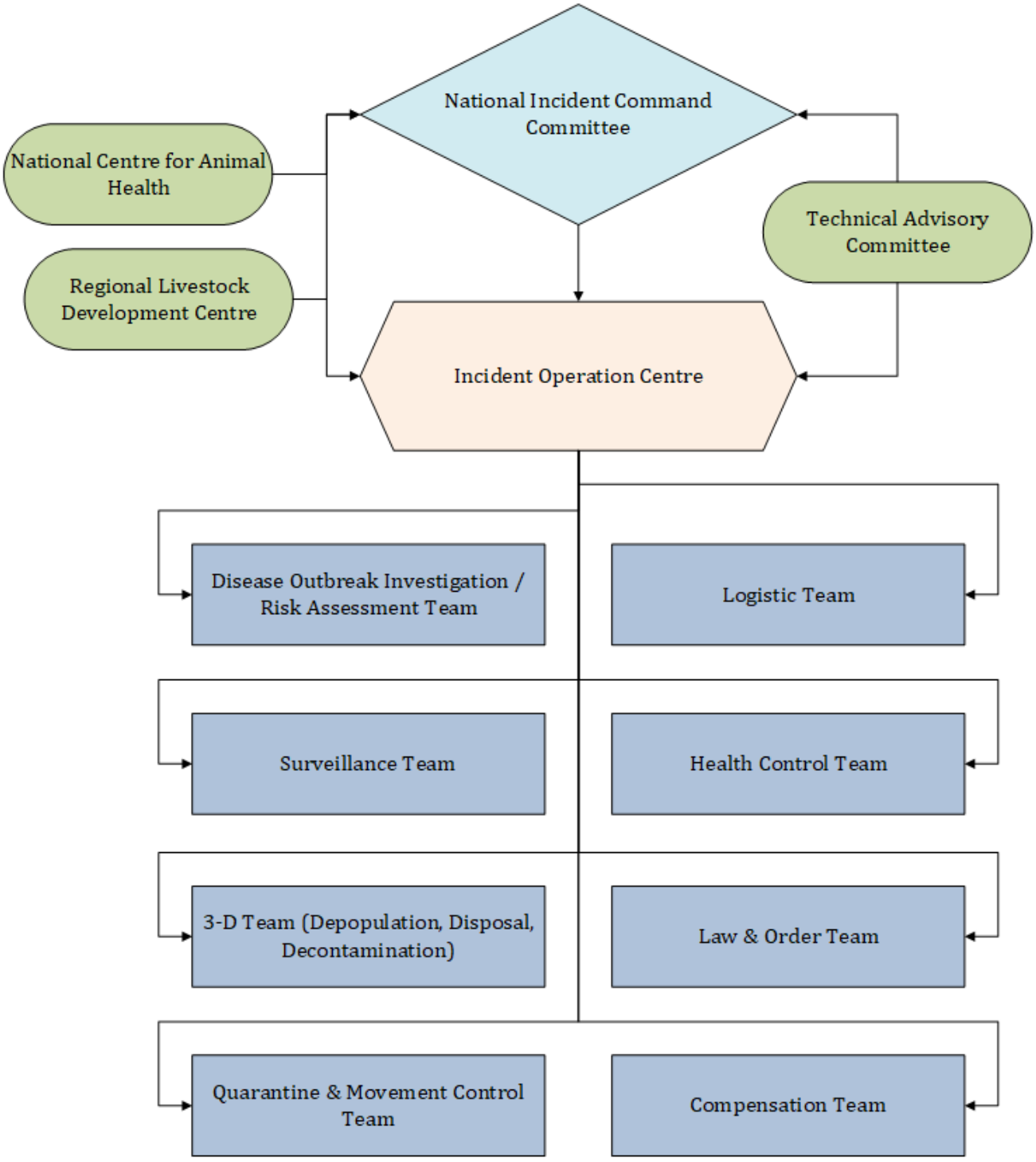


Figure 3 Incident Command Structure for the response to outbreaks of HPAI H5N1 / AI H7N9 / other notifiable avian influenza viruses

5.1 INCIDENT OPERATION CENTRE (IOC)

The IOC (Figure 3.) is the field level coordination and implementation body for rapid response and control measures. The IOC will be responsible for providing field level information and updates on the disease status, progress on response and control activities to the NICC. In addition, it will ensure that all policy decisions and directions for response and control activities are conveyed to the different RRTs.

Since the disease response operation demands a multi-sectoral approach, the IOC members shall be composed of *inter alia* all team leaders of the RRTs and include the following (Table 2):

Table 2 Members of the Incident Operation Centre

Sl no	Member	Agency	Main Tasks
1	Regional Director/ Head of Animal Health Section under RLDC	Concerned RLDC	Incident Commander/ overall coordination including reporting to higher authorities
2	Regulatory and Quarantine Officer (Livestock)	Concerned PLQO / Dzongkhag BAFRA	Deputy Incident Commander - Assist Incident Commander
3	Veterinary Epidemiologist	NCAH/ RLDC/ DVH	Investigation/ Epidemiological Surveillance
4	Veterinary Officer	DVH/ RLDC	Clinical and lab. Surveillance
5	Regulatory and Quarantine Officer	Concerned BAFRA Office	Oversee enforcement of quarantine and movement control
6	Regulatory and Quarantine Officer/Inspector	Concerned BAFRA Office	Oversee depopulation, disposal and decontamination measures (3D)
7	Medical Officer	Concerned Hospital	Prophylaxis & treatment (Vaccine/Drugs medication and health check-up of the front-line workers, screening of suspects)
8	Dzongkhag Livestock Officer/Dzongkhag Disaster Management Officer	Concerned Dzongkhag	Logistic support/ risk communication/ Member Secretary for the Compensation Committee
9	Dzongdag/ Dungpa/ Dzongrab/ Thrompon/ Gup	Concerned Dzongkhag (s)	Dzongkhag level logistics support (as Chief Disaster / Emergency Coordinator) Chair of the Compensation Committee
10	SP or OC or representative	RBP concerned Dzongkhag	Enforce Law and Order
11	Co-opt Members	Concerned relevant offices	Priority activities as deemed necessary (e.g.; Wild bird surveillance)

5.2 MODUS OPERANDI

Upon receiving Executive Order from the NICC, the IOC will be set up within 6hours and start implementing the control measures. The IOC will further activate the RRTs to implement disease control measures. The IOC will convene at least one meeting daily and accordingly update the information to the NICC. The Dzongdag as the Chairman of the Dzongkhag Disaster Management Committee will provide the necessary logistics and other support.

The response activities in the event of an outbreak of Avian Influenza H5N1/H7N9 will need to be undertaken as per the timeline given below.

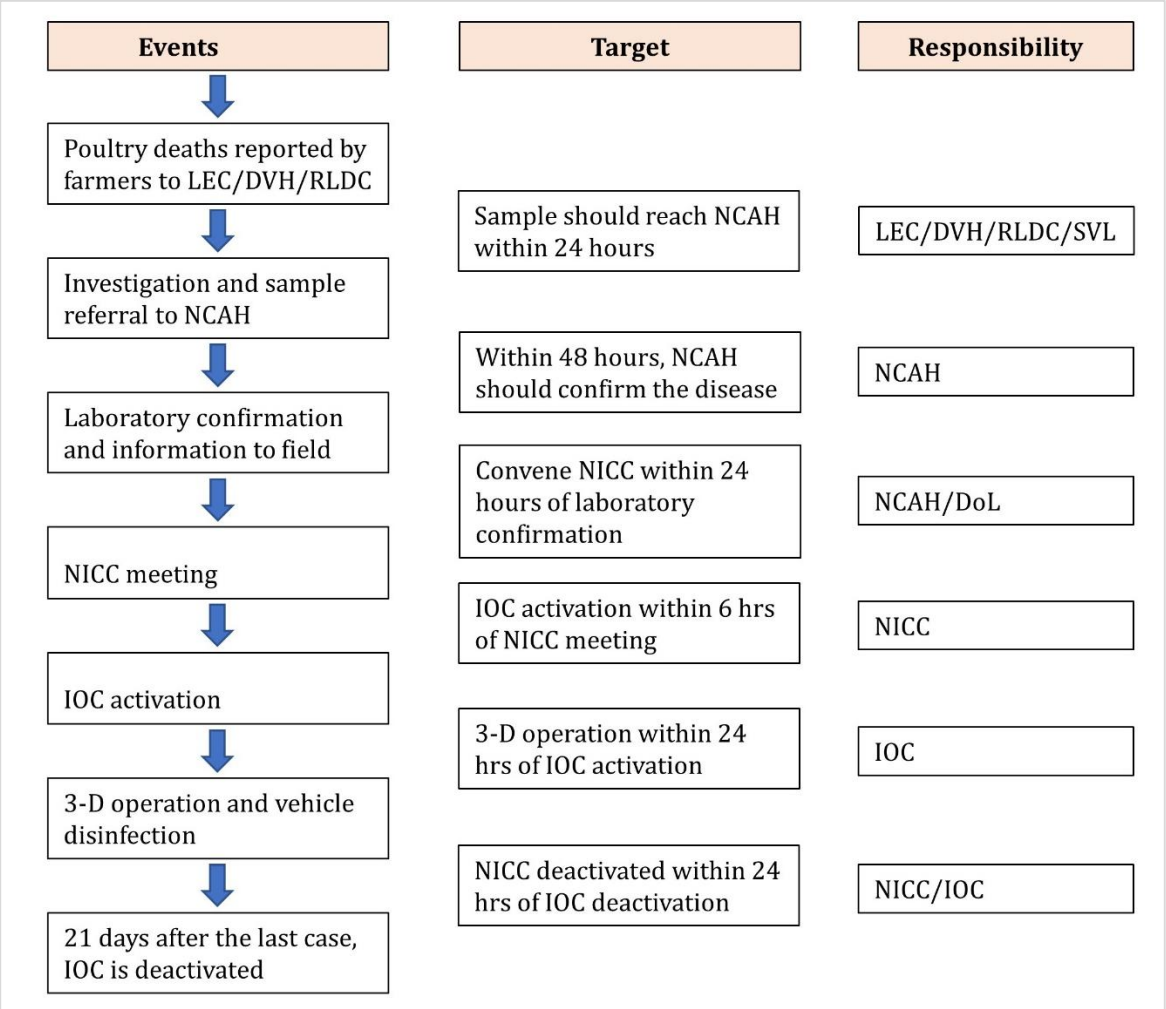


Figure 4 Timeline for major activities following an outbreak of HPAI H5N1/H7N9/other notifiable avian influenza viruses

Important Note:

In case of simultaneous outbreaks beyond the reach of the one IOC in the same region, a second IOC (Table 3) shall be constituted with members consisting of equivalent counterparts of the concerned region/Dzongkhag except for the technical experts from DoL and BAFRA. The DoL and BAFRA shall identify and mobilize appropriate technical experts to the second or more IOCs as given hereunder.

Table 3 Members of the second IOC

Sl No	Member	Agency	Main Tasks
1	Veterinary Epidemiologist (s)/ Veterinary Officer	DoL/ NCAH/ RLDC	Incident Commander Overall coordination including reporting to higher authorities
2	Officer In-charge/ Regulatory and Quarantine Officer, of concerned PLQO/Dzongkhag BAFRA office	Concerned PLQO/ Dzongkhag	Deputy Incident Commander – Assist Incident Commander
3	Veterinary Epidemiologist (s)/ Veterinary Officer	NCAH/RLDC/DVH	Investigation and surveillance
4	Veterinary Officer	DVH/ RLDC	Clinical and lab. Surveillance
5	Regulatory and Quarantine Officer	Concerned PLQO/ Dzongkhags	Oversee implementation of 3D operations
6	Regulatory and Quarantine Officer	Concerned PLQO/ Dzongkhags	Oversee Quarantine and movement control measures
7	Medical Specialist/Officer (relevant)	MoH	Prophylaxis & treatment (Vaccine/drug medication and health check-up of the front-line staff)
8	Dzongdag/Dungpa/Dzongrab/ Thrompon/Gup/Dzongkhag Disaster Management Officer	Concerned Dzongkhag	Dzongkhag level logistics support Chair of the Compensation Committee
9	SP or OC or representative	RBP of concerned Dzongkhag	Enforce Law and Order
11	Co-opt Member	Concerned relevant offices	Priority activities as deemed necessary (e.g; Wild bird surveillance)

5.2.1 Roles and responsibilities of Incident Commander

- Overall coordinator of HPAI containment activities
- Supervise and Monitor the activities of different RRTs on daily basis
- Chair IOC meeting on a daily basis
- Keep NICC, TAC and relevant agencies updated on progress of IOC
- Submit the issues raised by the RRTs with a proposed recommendation to TAC/ NICC
- Facilitate and mobilize all logistics and supplies required to RRTs
- Liaison with relevant agencies within Dzongkhag/ Dungkhag/ Thromde/ Gewog level
- Submit budget proposals and settle all the bills
- Fully responsible and accountable for expenditures incurred during the containment of HAPI outbreak and auditing of the expenditures
- Scaling of manpower based on the burden of the outbreak

- Any other tasks assigned by NICC and TAC

5.2.2 Roles and responsibilities of Deputy Incident Commander

- Assist Incident Commander in implementation of HPAI outbreak containment activities
- Coordinate risk communication activities with Team Leaders of RRT
- Overall coordinator of 3-D operation, Movement control and Quarantine
- Maintain daily records of all IOC and RRT activities and prepare minutes of the meeting on daily basis
- Work detail budget proposals for discussion before submission to Ministry
- Daily field monitoring and supervision of containment activities.
- Any other task assigned by Incident Commander.
- Coordinate HPAI containment activities in the absence of Incident Commander

5.3 ROLE OF NCAH AND RLDC

The National Centre for Animal Health shall be the National coordinating centre for all preparedness and response activities on Avian Influenza H5N1/H7N9 and other notifiable avian influenza viruses. The NCAH shall provide necessary technical, logistic and financial support to the IOC to enable the IOC to undertake disease control measures effectively. The NCAH shall also work as secretariat to NICC and TAC and facilitate the organization of TAC and NICC meetings as and when instructed by the Chair of NICC and TAC. The NCAH shall be the link between IOC/RLDC and NICC/ TAC and shall provide technical backstopping to the disease control measures implemented by the IOC/RLDCs.

5.4 RAPID RESPONSE TEAMS (RRT)

The RRTs will be divided into different groups as per the mandate of the respective technical sectors involved in the disease control measures (Figure 3). The DoL will be mainly responsible for disease outbreak investigation, surveillance and logistic supply. BAFRA will be responsible for the 3-D operations, quarantine and movement control. The Ministry of Health will be responsible for providing prophylactic and first aid services; RBP will be responsible for the maintenance of law and order and providing support for different operations; Dzongkhag Disaster Management Committee will be responsible for providing necessary logistic support.

5.4.1 Disease Outbreak Investigation Team (DOIT)

The DOIT shall be responsible for disease investigation and confirmation of the HPAI outbreak and they shall be responsible for the identification and establishment of infected premises, dangerous contact premises, suspected premises, protected and surveillance zones. For detail TORs, refer to the SOP for DOIT as given in Annexure 1. The DOIT shall also be responsible to undertake risk assessment as directed by the IOC to establish the zones and to decide on other disease control measures to be applied.

The DOIT shall consist of the following members:

- Epidemiologist and/ or Veterinary Officer (Team Leader).
- Laboratory technicians
- Para veterinarians from the field
- RQO/RQI Livestock, BAFRA

5.4.2 Surveillance Team

The surveillance team (clean team) shall be involved in carrying out all necessary surveillance activities both in the protection and the surveillance zones. The team will also provide risk communication on HPAI to the communities and give assurance to the general public.

The detailed SOP and guidelines for carrying out surveillance are provided in the SOP for Surveillance in the Annexure 2.

The Surveillance Team shall be composed of the following members:

- Veterinary Epidemiologist/Veterinary Officer, NCAH/ RLDCs/ DVH
- Laboratory Technicians
- Para-veterinarians
- Forestry official during wild bird surveillance (as and when required)

5.4.3 3-D Team (Depopulation, Disposal, Decontamination)

The 3-D team shall be responsible for carrying out culling, disposal and decontamination of birds in the infected premises, suspect and susceptible birds in the protection zones as per the SOP provided in Annexure 3. All birds in the infected premises will be subjected to stamping out once a clinical disease or evidence of active HPAI virus infection is confirmed. In addition, pre-emptive culling will be done in protected zone (on high-risk premises such as dangerous contact premises–DCPs, contiguous premises-CPs and suspect premises-SPs) established through epidemiological assessment.

The 3-D Team shall be composed of the following members:

- ***Team leader:*** Regulatory and Quarantine Inspector/ Officer (Livestock)
- ***Technical Assistants:*** Two BAFRA Livestock Inspectors. One of them in the team shall act as animal welfare Inspector
- ***Record keeper:*** Concerned Livestock Extension Agents

Cullers:

- Two hired and trained personnel as a culler
- Two bird catchers (hired labourers) team).
- Gas operators: One BAFRA Inspector for operating CO_2 gas when used as stunning (optional)
- Two hired labourers for disposal of culled birds.
- Five hired labourers for digging disposal pits at each disposal site
- One police personnel for the smooth functioning of the 3-D operation.

Note: The number of 3-D teams required shall be based on the size of the outbreak and protection zone which shall be decided by IOC.

5.4.4 Decontamination Team

The decontamination team shall be responsible for carrying out cleaning and disinfection of infected premises, suspect and susceptible birds in the protected zones and all other infected or potentially infected materials and equipment as per the SOP provided in the Annexure 4. When the 3-D operation is one time during the whole IOC operation, members from the 3-D team can be used for the Decontamination team.

The Decontamination Team shall be composed of the following members:

- ***Supervisor:*** Livestock Regulatory and Quarantine Officer/ Inspector concerned Dzongkhag BAFRA Office.
- ***Assistants:*** Two BAFRA Livestock Inspector Four Hired and trained personnel for cleaning and disinfection in the protected zone

Note: The number of Decontamination Team shall be decided by the IOC based on the size of the protection zone.

5.4.5 Quarantine and Movement Control Team

The Quarantine and Movement Control Team shall be responsible for enforcement of quarantine and movement control at protection and surveillance zones to control and prevent the spread of the HPAI disease and contain the as soon as possible. Detailed procedure on enforcement of quarantine and movement control measures are provided in the SOP provided in the Annexure 5.

The Quarantine and Movement Control Team shall be composed of the following members:

- ***Team leader:*** Regulatory and Quarantine Officer/Inspector (Livestock).
- ***Technical Assistants:*** BAFRA Livestock Inspectors (number to be determined based on the geographical location and size of outbreaks and entry and exit points in the protected and surveillance zones).
- ***Recordkeepers:*** BAFRA Livestock Inspector
- ***Hired labourer:*** Four labourers for spraying and disinfection of vehicles
- ***Law and Order Personnel:*** One police personnel to oversee the vehicle traffic at the disinfection point.

Note: Based on the risk assessment findings and recommendations of DOIT, the strategic point for vehicle disinfection shall be decided by IOC. The quarantine and movement control team shall function in two shifts (8 hours).

5.4.6 Health Control Team

The medical team shall be responsible for providing all prophylactic treatment and first aid services to all the personnel involved in the disease control operations including monitoring the health of all personnel involved in the disease control operations for signs of Avian Influenza. In addition, they shall also be responsible for monitoring of any suspect patients for human case of HPAI virus in the affected households/ farms and areas. The team will also provide risk communication on HPAI to the communities and give assurance to the general public.

Health Control Team shall be composed of the following members:

- Medical officer
- Health Assistant and nurses

5.4.7 Law and Order Team

The main roles and responsibilities of law and order team are to ensure compliance and smooth operation of all disease control measures. They shall support all technical RRTs involved in the disease control measures, such as compliance from farmers, traffic regulations, compliance for 3D operations, and quarantine and movement control.
The Law and Order Team shall be composed of the following members:

- SP/OIC
- Police personnel

5.4.8 Logistic Team

The main role and responsibility of the Logistic team is to ensure that all necessary logistical facilities like PPE, materials and equipment, food provisions and transport are made available to all RRTs and to reinforce all essential supplies.
The Logistic Team shall be composed of the following members:

- Procurement Officer/ Administrative Officer/Accounts officer RLDC– Team leader
- Dzongkhag representative
- DDMO focal

5.4.9 Compensation Committee

The main role and responsibility of the Compensation Committee is to ensure provision of compensation in a fair, transparent and timely manner to all eligible owners/farmers. The committee shall strictly adhere to compensation guidelines given in the document.
The Compensation Committee shall be composed of the following members:

A: Dzongkhag level:

- The Gup /Dzongdag/Dzongrab or representative – Chairperson
- Dzongkhag Livestock Officer – Member Secretary
- The Dzongkhag Disaster Management Officer - member
- BAFRA RQO/RQI Livestock– Member
- Representative from RLDC – Member

B: Thromde level:

- Thrompon – Chairman
- Dzongkhag Livestock Officer – Member Secretary
- Thromde Disaster Management Officer - member
- BAFRA RQO/RQI Livestock– Member
- Representative from RLDC – Member
- Thromde Thuemi (in Thromde areas) – member

5.5 COORDINATION BETWEEN IOC AND DDMC

It is crucial that the Incident Operations Centre (IOC) and the Dzongkhag Disaster Management Committee (DDMC) liaise closely with each other for effective implementation of the response activities during outbreak of AI H5N1 / H7N9. The Dzongkhag Disaster Management Officer (DDMO) shall apprise the members of the DDMC on the status of the outbreak and convene emergency meeting of the DDMC to provide logistic and financial support to the IOC.

5.6 ROLE OF OTHER RELEVANT AGENCIES/ORGANIZATIONS

5.6.1 Ministry of Home and Cultural Affairs

The Department of Disaster Management (DDM) under the MoHCA being the national coordinating agency for disaster management has been entrusted to support, coordinate and manage activities with regards to any disaster in the country as per the National Disaster Management Act 2013.

5.6.2 District administration

District administrations are entrusted with local governance and coordination of all activities, development or otherwise, within their district. As per the NDMA, the Dzongda (district administrator) will be the chairman of the Dzongkhag Disaster Management Committee (DDMC) which is responsible for coordination and managing all disaster management operations in the Dzongkhag.

5.6.3 Royal Bhutan Police

The Royal Bhutan Police (RBP) under the MoHCA as the custodian of the maintenance of law and order in the country will be responsible for this critical function and shall support MoAF and MoH in enforcement of disease control measures and regulations during an outbreak of HPAI in animals or during influenza pandemic. The RBP will support the IOC in the disease containment works including 3-D operations; quarantine and movement controls; decontamination; awareness; and compensation process.

5.6.4 Ministry of Finance

The Ministry of Finance will provide adequate budget for Bird Flu containment activities in order to prevent further spread into human population and development of human pandemic.

5.6.5 Ministry of Education

The Ministry of Education's support role will be to allow/facilitate awareness activities in schools for teachers and students alike, and if required to sanction school closures and/or mobilization of teachers and students for certain activities/programmes,

5.6.6 Department of Revenue & Custom, Ministry of Finance

The Department of Revenue & Customs will be responsible for clearing imported goods at entry points. The department shall support BAFRA in examination and inspection of imported livestock and livestock products including other risk goods to ensure their safety in the event of an outbreak of HPAI.

5.6.7 Role of other government agencies

In the event of an outbreak of HPAI, support will be sought from other relevant agencies such as Ministry of Works and Human Settlement (water and sanitation); Ministry of Information and Communications (transportation and telecommunication), Ministry of Economic Affairs (electricity supply); Department of Trade for close supervision with BAFRA on issuance of trade licenses for import of poultry and poultry products.

5.6.8 International Organizations

Technical and financial support will be sought from the international organizations such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), World Organization for Animal Health (OIE) and the other UN agencies (UNDP, UNICEF, WFP) for the prevention and control of HPAI in Bhutan.

5.6.9 Department of Forestry & Park Services (DoFPS)

The Department of Forests & Park Services shall assist in wild bird surveillance during preventive as well as in outbreak response phase. Further, DOFPS shall be engaged during any emergency response action involving wild birds

5.7 COORDINATION BETWEEN IOC AND DDMC

It is crucial that the Incident Operations Centre (IOC) and the Dzongkhag Disaster Management Committee (DDMC) liaise closely with each other for effective implementation of the response activities during an outbreak of AI H5N1 / H7N9. The Dzongkhag Disaster Management Officer (DDMO) shall appraise the members of the DDMC on the status of the outbreak and convene an emergency meeting of the DDMC to provide logistic and financial support to the IOC.

5.8 REPORTING AND MONITORING MECHANISMS

The IOC shall submit daily updates including the minutes of meeting to NICC and TAC about the status of HPAI outbreak and containment activities. The NICC shall share information with DDM/NEOC as and when deemed necessary (Figure 5). The IOC should also share information/updates with the Gewog Disaster Management Committee (GDMC), Dungkhag Disaster Management Committee (DuDMC) and DDMC wherever necessary. The TAC should monitor the functioning of IOC and appraise NICC either through a report or by convening the meeting. The IOC shall adopt appropriate means of communication channels (e.g., social media) to share information and for proper coordination of containment activities. The IOC shall request the DDMC to convene a meeting in order to resolve issues at Dzongkhag level and also to provide the necessary support to IOC. The NICC shall request the DDM for convening of the NDMA in the event of urgency and seek directives for resolving issues that could not be resolved by NICC.

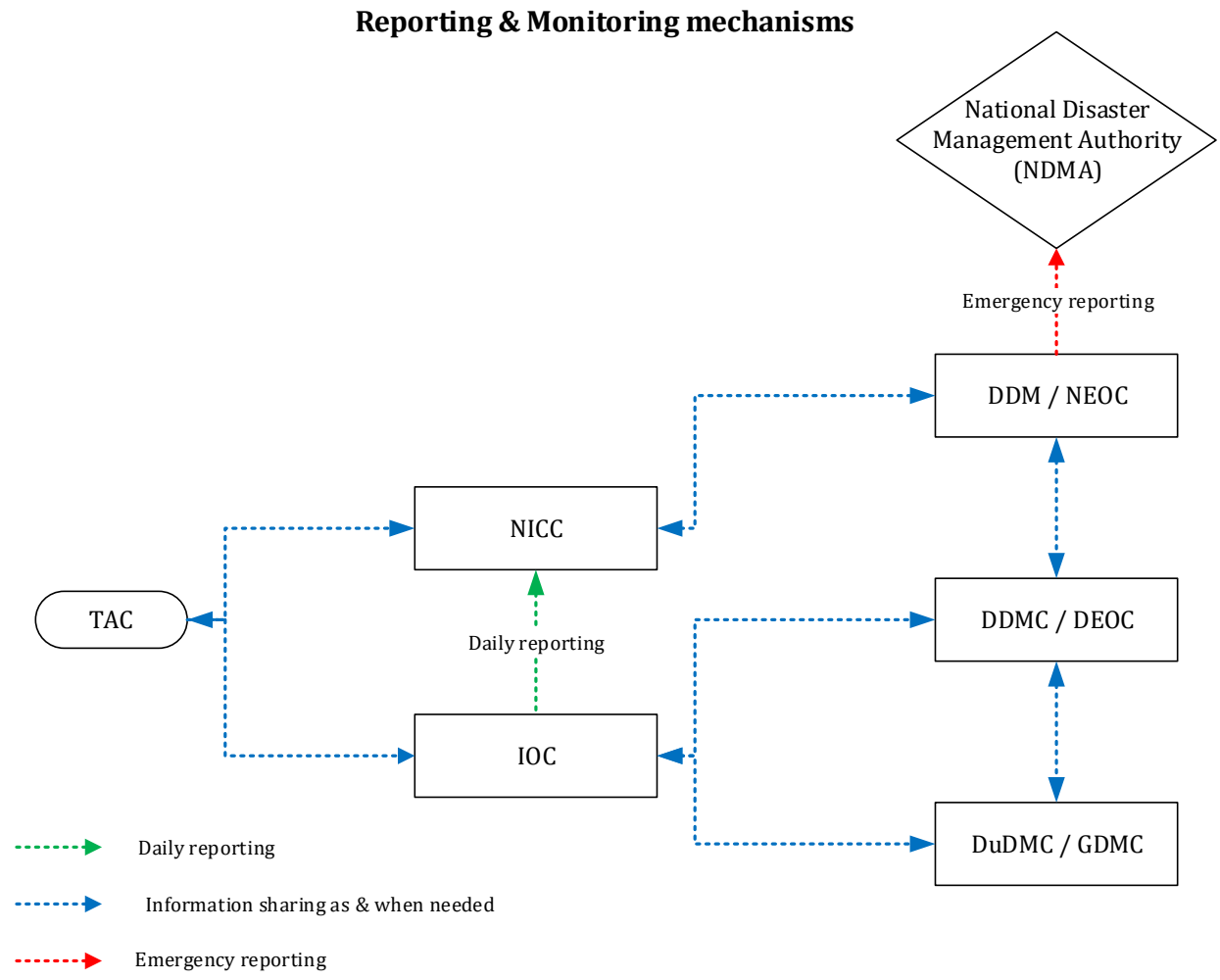


Figure 5 Reporting and Monitoring mechanism

5.9 FUND MOBILIZATION MECHANISM

The fund mobilization for the preparedness and response activities against HPAI H5N1/ AI H7N9 / other notifiable avian influenza will be undertaken as per provisions of the Disaster Management Act of Bhutan 2013. In normal times, concerned agencies (DoL, BAFRA, DoPH) will propose a budget during the annual budgeting exercise for prevention activities such as awareness, surveillance, BVT/ VVT activities, simulation exercise and capacity building activities in the concerned sectors.

In the event of an outbreak, concerned agencies will utilize the existing budget to undertake immediate disease containment activities as per the provisions of the Disaster Management Act of Bhutan 2013. The IOC will propose actual fund requirements in consultation with concerned agencies (DoL, BAFRA, and DoPH) to the NICC (Figure 6). The NICC will forward the budget requisition to the MoF for final approval. The MoF shall release the approved budget to the IOC through the concerned RLDCs.

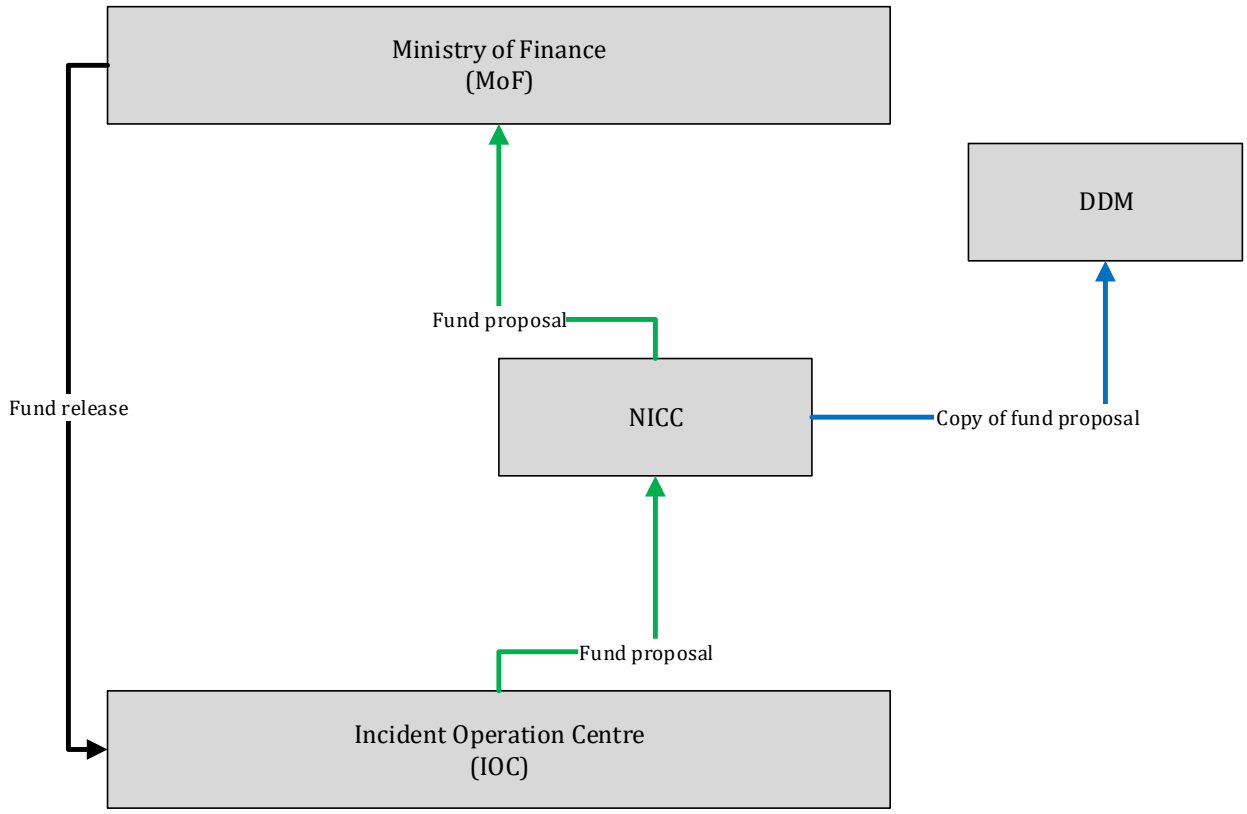


Figure 6 Fund mobilization mechanism in the event of an outbreak of HPAI H5N1 / AI H7N9

6 VETERINARY RESPONSE PLAN FOR HPAI H5N1, H7N9 /OTHER NOTIFIABLE AVIAN INFLUENZA

The objectives of the Veterinary Response Plan for HPAI are:

- To prevent any incursion of notifiable avian influenza into the country through effective surveillance system and import regulation;
- To rapidly control disease outbreak following an incursion of virus and regain HPAI freedom;
- To reduce the risk of human infection;
- To minimize morbidity, mortality and social disruption;

6.1 PHASE 1: PREVENTION OF HPAI H5N1/H7N9 OUTBREAK

The objective of Phase 1 is to prevent HPAI outbreak in the country through an effective surveillance and biosecurity system and maintain the country's freedom status from infection.

6.1.1 Surveillance for early detection of an incursion of HPAI

HPAI is listed under the Livestock Rules and Regulations of Bhutan 2017 as a notifiable disease in the country. Therefore, all individuals are bound by the law to report any suspected case of the disease to relevant authorities (DoL and BAFRA).

6.1.2 **Clinical and laboratory surveillance of the chicken population**

Clinical surveillance is aimed at the detection of clinical signs of HPAI at the flock level. Surveillance based on clinical inspection is particularly relevant for HPAI because the infection is characterized by a very high mortality rate in terrestrial poultry. Monitoring of production parameters such as drop in egg production and other vital signs such as increased mortality, reduced feed and water consumption, presence of clinical signs of a respiratory disease is important for the early detection of infection. Avian influenza H7N9 which was considered as low pathogenic avian influenza (LPAI) for poultry until 2018 is now categorized as HPAI H7N9 since it was found to be a potential threat to public health and poultry industry. However, this subtype can also exist as LPAI. In the case of LPAI infection the only indication may be a drop in feed consumption or egg production. In the case of AI H7N9, there will not be any clinical signs shown by affected poultry. Therefore, laboratory surveillance is important for this disease. Surveillance activities, both clinical and laboratory testing shall be carried out on a regular basis in the high-risk areas.

HPAI may be suspected if the following trigger signs are observed:

- In semi-commercial farms where biosecurity is medium and day-old chicks are imported, authorities should be alerted for investigation if the daily mortality rate is > 2% for two consecutive days;
- In village-based smallholder and backyard farms, authorities should be alerted for investigation if daily mortality rate is ≥ 5% for two consecutive days, or birds showing signs and symptoms consistent with HPAI on clinical and necropsy examination;

6.1.3 **Surveillance in domestic waterfowls**

Aquatic birds such as domestic ducks can act as reservoirs of infection for HPAI and should be examined whether these birds have been exposed. The duck population in the southern Dzongkhags, comprising approximately 10% of poultry population and those kept as pet shall be sampled and tested in sufficient numbers to give a 95% probability of detecting at least one sero-positive bird if infection is present above 20% (e.g., for a flock of 500 birds 14 random samples should be collected, assuming a test sensitivity of 100%).

If sero-positive, sufficient samples shall be collected for virus detection from cloacal or tracheal swabs (pools of five swabs per sample bottle) to give 95% probability of detecting at least one virus positive bird if 2% of the birds are excreting virus (e.g., 100 swabs for flocks of 500 birds).

6.1.4 **Surveillance in wild birds**

Investigation of unusual mortalities or die-backs in wild birds such as migratory birds (black-necked cranes in the Phobjikha and Bomdeling valleys), ruddy shelduck, egrets, black storks, fish eagles, white and grey bellied herons' cormorants, Indian Ibis, crows and pigeons throughout Bhutan shall be carried out. The investigation should conform to the protocols set out in the FAO manual "*Wild Bird HPAI Surveillance 2006*". The Nature Conservation Division of the Department of Forests and Park Services and Royal Society for Protection of Nature (RSPN) concerned with wildlife shall be involved in joint surveillance and monitoring of wild birds including the migratory waterbirds in the country.

There is a need to construct digital maps showing spatial and temporal distributions of migratory waterbirds, indigenous birds and their overlap with domestic poultry for developing risk-based surveillance of wild birds. It is estimated that about 40 species of birds migrate to Bhutan from various places.

Through the experiences of surveillance in the past, there were reported cases of rapid test positive in wild birds and ducks but negative by real-time RT-PCR. Such results may be attributed to the inherent characteristics of rapid tests that have low specificity. Positive rapid test results should not be the cause of panic for the general public. Therefore, any rapid test positive samples have to be confirmed by real-time RT-PCR. The roles and responsibilities of various institutions under the Department of Livestock for surveillance of avian influenza and monitoring are outlined below (Table 4).

Table 4 Responsibilities of different institutions for Avian Influenza surveillance

	Level	Responsible agency/Focal persons	Responsibilities
1	Gewog	Gewog Extension Centre /RNR-EC	- Carry out clinical surveillance in the existing poultry population in the Gewog. - Submit a flash report of any suspected cases to DLO/ RLDC or NCAH through the fastest means of communication. - Restriction on movement of poultry and poultry products from the affected/suspected and adjoining villages in collaboration with BAFRA. - Create awareness among farmers based on national guidelines. - Collect samples from suspected cases/dead birds in collaboration with the laboratory personnel and submit them to the laboratory. Also take samples from purposively selected apparently normal birds in the locality for sero-surveillance purposes. - Provide weekly follow up reports on any outbreak (suspected or confirmed) until further order.
2	Dzongkhag	Dzongkhag Livestock Sector	- Conduct disease investigation to validate the suspicion reported from gewog center and submit flash report to RLDC or NCAH. - Carry out rapid diagnostic tests for prompt diagnosis. - Recommend Dzongkhag Administration to issue ban order on the movement of poultry birds, their products including manure, egg tray and

			poultry feed etc from the affected zones based on the recommendation of TAC. - Contact RLDC or NCAH for further investigation of the suspected case(s) if required. - Create awareness among gewog extension staff and local village institutions through GT and DT. - Inform/liaise with the Dzongkhag Medical Officer and Dzongkhag Disaster Management Committee (DDMC). - Provide logistic support and assistance to the Gewog extension centre, investigation team and IOC - Monitor affected Gewogs and provide weekly follow-up reports to the RLDC/NCAH
3	Regional	Regional Livestock Development Center/ Satellite Veterinary Laboratory	- Conduct a thorough investigation of the reported outbreak. - Carry out rapid diagnostic tests for prompt diagnosis. - Provide appropriate technical recommendations. - Submit the investigation report and refer samples to NCAH for further confirmation. - Constantly monitor affected Dzongkhags and Gewogs. - Provide weekly follow-up reports on affected Dzongkhags and Gewogs. - Create awareness among DLOs and EAs. - Provide necessary logistic support and assistance to affected Dzongkhags. - Procurement of PPE and other essential items (region of HPAI endemic areas)
4	National	National Centre for Animal Health	- Laboratory testing of samples from suspected cases for confirmation. - Conduct regular surveillance in collaboration with RLDCs and Dzongkhags. - Refer samples to reference laboratories for further analysis and confirmation if required. - Liaise with relevant international organizations and intergovernmental agencies. - Overall coordination of the emergency preparedness plan implementation in the country.

			- Submit follow up reports and appraise DoL/BAFRA on latest situation/development in the country on a regular basis - Submit immediate notification and follow-up reports to OIE through WAHIS. - Generate extension materials for field staff. - Create awareness through mass media. - Provide logistic support to RLDCs and Dzongkhags. - Procurement of diagnostic kits and other essential laboratory items. - Procurement of PPE and other essential items

6.1.5 **Strengthen laboratory surveillance**

A well-equipped and well-functioning veterinary diagnostic laboratory is a vital component of the NIPPP. Laboratory diagnosis together with effective surveillance at international borders and high-risk locations is critical for pre-empting any detection of the virus in the country. A veterinary laboratory equipped to deal with avian influenza needs a full set of components comprising well trained technical laboratory personnel (veterinary virologist, technicians), infrastructure and equipment, PPE, testing protocols, diagnostic kits and reagents to meet the minimum standards for BSL-II plus criteria with a BSL-III hood available for use when required. In addition, the laboratory facility needs to be able to handle hazardous materials safely and protect its staff and the environment.

The objective of strengthening laboratory capacity is to build a laboratory with the following diagnostic capacities for carrying out effective surveillance and diagnosis of HPAI:

- Rapid Ag detection kit;
- ELISA;
- Virus isolation with HA typing for H5, H7, H9;
- Serology for H5 and H7 by HI;
- Real-time RT-PCR;
- Post-mortem facilities with BSC Class II and PPE;

The laboratory shall also have a capacity for rapid collection and transport of samples from suspect cases of avian influenza to a laboratory for HPAI diagnosis as described in the OIE Terrestrial Manual, and/or for sample referral abroad to the regional/world reference laboratories.

Identification of suspect flocks is vital to the identification of sources of HPAI and to enable the molecular, antigenic and other biological characteristics of the virus to be determined. It is essential that HPAI isolates are regularly sent to the regional or world Reference Laboratories for genetic and antigenic characterization.

Currently, all animal disease diagnostic facilities to a varying degree are provided by following laboratories in the country:

6.1.5.1 Laboratory Diagnostic capacity at NCAH

The Laboratory Services Unit (LSU) of NCAH located in Serbithang has been designated as a national focal laboratory for the diagnosis of avian Influenza in Bhutan. The LSU has real-time RT-PCR for type A and subtype H5, N1, H7, N8 and N9 in addition to rapid test for type A and subtype H5. For H7N9, LSU has HA/HI tests for serological investigation. The Centre has BSL2 plus containment facilities that can handle infectious viral materials without compromising the safety of the laboratory personnel and also the environment.

6.1.5.2 Laboratory diagnostic capacities at RLDCs

The four Regional Livestock Development Centres (RLDCs) have facilities for conducting postmortem examinations and are equipped with a rapid antigen detection tests, such as rapid antigen detection kit and PPE for the safety of personnel. Facilities for safe and proper collection of samples and their preservation for submitting to NCAH or to international reference laboratories are made available at these laboratories. In addition, BSC Class II, autoclave and incinerator for safety purposes are also available in these laboratories. Facilities for PCR diagnosis have been set up at RLDC, Kanglung which is expected to be used for routine surveillance and diagnosis of AI in the eastern region. Facility and capacity for AI H7N9 HA/HI serology have been set up in some of the RLDCs in the high-risk zone.

6.1.5.3Satellite/Thromde Vet Lab (S/TVL) and District Vet Lab (DVL)

The SVLs at Gelephu, Phuentsholing and Deothang and DVLs of high-risk Dzongkhags are equipped with rapid antigen detection tests and PPE. Besides the rapid tests, these laboratories in the field have facilities and capacity for safe and proper collection of samples and their preservation (including carcass) for submission to RLDC/NCAH.

6.1.6 **Surveillance strategies in the event of HPAI H5N1/H7N9 outbreak in the neighbouring countries and during high-risk season**

6.1.6.1Activation of Veterinary Vigilance Teams

The VVTs shall be activated vide executive order from the Chair of NICC based on the recommendations of TAC when there is an outbreak of a disease in the country or in adjoining neighbouring countries. The team will be deployed in the targeted high-risk areas. The main role of this team is for early detection of the disease so that the response and control measures can be implemented in time in order to prevent its spread to wider areas with minimal socio-economic impact.

The team is also responsible for coordinating the preparation of a report on activities implemented and submission to NCAH and DoL, HQ on a weekly basis.

The VVT shall be composed of the following members:

- RVO/DCVO/VO from RLDC/ DVH/ NCAH;
- Laboratory technician from NCAH/ RLDC/ DVH/ S/TVL;
- A representative from BAFRA;
- Dzongkhag livestock staff;

Roles and responsibilities

- Clinical and laboratory surveillance as per the protocol;
- Submit immediate report to the DoL and NCAH on any suspicious case;
- Submit weekly report to the DoL, HQ and NCAH;
- Submit or make weekly briefing to the concerned Dzongdags;
- Carry out advocacy, awareness and sensitization program to all relevant stakeholders;
- Carry out wild bird surveillance in collaboration with Nature Conservation Division;
- Keep vigilance on any HPAI outbreak-related events in the immediate border areas and report to DoL, NCAH, BAFRA and other stakeholders;
- Appoint "Tshogpa" or other local leaders/relevant person as the focal point to report any suspicious cases at the village level/area to any VVT member in collaboration with the local government;

6.1.6.2 Activation of Border Vigilance Teams (BVT)

Bhutan Agriculture and Food Regulatory Authority shall form BVTs for monitoring the entry of illegal poultry and poultry products for the prevention of entry of HPAI H5N1/H7N9 into the country. The BVT will be activated by BAFRA as and when there is a report of confirmed HPAI outbreak in the neighbouring countries with an executive order from NICC. The BVT members shall be stationed at various entry points along the Bhutan- India border and will be the first line of defence.

Team composition:

- OIC/RQO-Livestock of concerned PLQO/Dzongkhag BAFRA office;
- Livestock Regulatory and Quarantine Inspectors;
- Temporary recruits;

Roles and Responsibilities

- Maintaining stringent border vigilance and surveillance;
- Implement a ban order on the import of poultry and poultry related products from HPAI H5N1/H7N9 affected countries;
- Keep strict vigilance along the Bhutan-India border to curb illegal movements of poultry/poultry products and live bird market;
- Effective targeted surveillance at commercial and government poultry farms including backyard village chickens that import live bird;
- Strictly monitor and regulate the bio-security practices in the commercial farms in the high risk and educate poultry farmers about the importance of maintaining biosecurity in the farms;
- Discourage free-range rearing of poultry in waterfowl prevalent areas especially in the southern Dzongkhags;
- All transport vehicles carrying poultry at border check posts shall be inspected for sick or dead poultry. If sick or dead birds are detected, cloacal or tracheal swabs shall be collected and submitted to laboratories for testing and apply stringent quarantine measures;
- Collect samples from intercepted and quarantined birds that are suspected of HPAI and submit for testing locally as well as in referral laboratories;
- Disinfection of vehicles traveling into the country from HPAI affected areas across the border;

- Monitoring the use of disinfectant footbath for people moving into the country from HPAI affected places;

Regulatory officials in the country shall be regularly updated with the latest information about avian influenza and shall be kept on high alert so that the entry points are manned with extra diligence to ensure that no unauthorized imports of poultry and poultry products are allowed into the country.

6.1.6.3 Ban on import of Poultry & Poultry Products from HPAI Affected Countries

The TAC shall recommend to the NICC to impose a ban on the import of poultry and poultry products from HPAI H5N1 and H7N9 suspected or affected countries based on risk assessment. BAFRA and DoL shall be involved in risk assessments of farms from which poultry or poultry products are sourced and draw up import health conditions based on Animal Health Code for Livestock Import 2018 and Livestock Rules and Regulations 2017. Import risk analysis for poultry and poultry products needs to be carried out from time to time and at short notice based on changing disease situation with trading partners in order to develop contingency and risk management strategies for minimizing the risk of introduction of HPAI virus into the country.

The list of HPAI affected countries shall be regularly updated from OIE, WHO and other sources and a ban on import shall be updated accordingly. Similarly, the ban shall also be lifted upon declaration of freedom from the disease based on OIE and national requirement. Recognition of regionalization, compartmentalization of Bird Flu affected or free for trading shall be determined by NICC based on the recommendation of TAC. All actions and responses to be taken including the lifting of the ban must be based on a risk analysis report.

BAFRA shall ensure that all regulatory measures of inspection and monitoring of import regulation, and enforcement of ban imposition within the country can be put in place immediately as per the executive order of NICC. BAFRA shall enforce import ban on poultry and poultry products originating from HPAI suspected and affected countries and shall include all items that are in transit at the time the ban is implemented. BAFRA shall also implement inspection of all the imports of poultry and poultry products including other risk goods for any trigger sign to prevent the virus incursion.

A review of the national policies on veterinary services and regulations should be carried out to determine the adequacy of existing legislation and to make recommendations for updating regulations to address current needs**.**

6.1.6.4 Training & simulation exercise for staff on response to HPAI Outbreak

It is essential for all veterinarians, para-veterinarian, laboratory officials, and regulatory officials to be trained on prevention and response for HPAI H5N1/H7N9 outbreaks and on their roles and responsibilities outlined in the NIPPP. They should be updated on the current knowledge on HPAI H5N1and H7N9 and its situation around the world. In addition, they should be trained on how to carry out surveillance on avian influenza. They should also be trained properly on the use of PPE and other equipment for controlling HPAI outbreak. The laboratory staff need to be trained on diagnostic technologies including the real-time RT-PCR.

The concerned agencies shall coordinate and organize training and field simulation exercises on a rapid response on a regular basis during peacetime. The budget for such training shall be provisioned by concerned agencies on a regular basis.

6.1.6.5 Upgrading of Biosecurity of Poultry Farms

Biosecurity in all poultry farms, particularly those importing live poultry shall be upgraded to at least Sector 1 and preferably Sector 2 biosecurity standards as per FAO's recommendation. All government and major private poultry farms should be out of bounds for any unauthorized personnel and should have proper boundary fencing to avoid unauthorized entry of people and vehicles and also to prevent contact with wild or domestic water birds. All entry and exit points should be well guarded, foot-dips shall be made available for daily workers, and effective zoo-sanitary and other control measures must be put in place. This should include changing footwear, use of gloves and masks and overcoat while entering and exiting sheds. In high-risk areas, backyard poultry farming should be improved with proper biosecurity measures. All the semi-commercial and commercial poultry farms including the government farms should strictly follow the **Biosecurity Checklist for Commercial Poultry Farms (Appendix C) of the In-country Livestock Biosecurity Guideline 2015** and the standards set by the technical department including the infrastructure design**.** The farms should be mandatorily be registered with the BAFRA and DoL as required by **Section 27 & 28 of the Livestock Rules and Regulation 2017**. BARA shall ensure compliance of farm biosecurity measures and other standards by carrying out inspection and monitoring of farm on a periodic basis. Based on the degree of non-compliance, the BAFRA should issue improvement notice or suspend the operation of farms.

6.1.6.6 Strengthen Disease Reporting System

Any suspicious case of HPAI should be reported within 24 hours by the fastest means of communication. The Ministry of Agriculture and Forests maintains two toll-free hotlines; 1244 for reporting any suspected cases of avian influenza and inquiries about the disease and 1555 for inquiry and reporting of illegal trade of poultry and poultry products.

All poultry owners are mandated to report any suspected cases of HPAI. In addition, village *Tshogpa/Chipon* is identified as a focal person to report any suspected cases of avian influenza. These focal persons should be provided with a mobile recharge voucher during the peak time of outbreak/high-risk seasons. Farmers/poultry owners and the regulatory authorities are mandated to report any suspected cases/trigger signs of avian influenza to the animal health authorities (NCAH/RLDCs) for immediate investigation and response. Weekly reports for the whole country during the high-risk seasons shall be posted on the NCAH webpage at www.ncah.gov.bt

The Ministry of Health shall be informed of all suspected outbreaks and sharing of outbreak information shall take place at all administration levels; RNR Centres will share the information with the local BHU, district staff of Department of Livestock will share information with the District Medical Officer and the Department of Livestock and BAFRA will share information with the Department of Public Health. In addition, the Ministry of Health shall share information such as influenza-like illness (ILI) in humans following contact with poultry birds with the veterinary sectors for investigation.

6.1.6.7 Awareness Campaigns

Education of public and field staff on HPAI and its risk are essential components of the NIPPP. The general public shall be sensitized about the disease in order to obtain their full cooperation for prevention, early detection and effective response for HPAI outbreaks. Similarly, field extension agents and regulatory inspectors of BAFRA, meat vendors and poultry farmers shall be made conversant with the disease. The Dzongkhags should take responsibility for organizing local education campaigns and use resource personnel from NCAH or RLDCs and medical doctors from hospitals.

The most appropriate means of getting the message across to specific communities should be used, such as radio broadcasts and village meetings. The latter are particularly suitable since they give people the opportunity to ask questions and material (such as pamphlets and posters) can be disseminated that will reinforce the information given. Campaigns should inform people of the nature of the disease and what to do if they see suspect cases; what they can and cannot do during the outbreak and why; and the benefits of keeping away from HPAI outbreaks. Public awareness material that is targeted specifically at all stakeholders should be prepared. As well as the above groups, the material should be prepared for politicians, senior bureaucrats and the press. There may also need to be a publicity campaign directed at consumers to reduce unnecessary buyer resistance to animal products, based on perceived public health risks. Following are the awareness programs to be implemented during the prevention phase:

- Awareness on HPAI and its economic impact on producer
- Awareness through trainings of stakeholders (farmers, traders, meat vendors, livestock officials) on disease and control measures

Following are the awareness programs to be implemented during the outbreak phase:

- Awareness on the regulation of movement of livestock and livestock products
- Awareness on timely reporting of disease outbreak
- Awareness on safe disposal of HPAI that died of HPAI

Spokespersons from the MoAF and MoH shall be identified and given responsibility for the timely issue of the press release on the status of disease and other important risk communication messages. Their responsibilities shall also include dispelling the occasional unfounded rumours that could create panic among the public.

6.1.7 Specific surveillance strategies for HPAI /H7N9

Generally, the surveillance strategies given for HPAI H5N1 are also applicable to H7N9. However, the following additional strategies need to be considered. As the H7N9 do not produce frank clinical signs in poultry, clinical surveillance may not be useful unlike in H5N1. Therefore, laboratory-based surveillance should be emphasized. It is recommended to conduct serological surveillance in poultry for H7N9 to detect any ongoing infection in the flocks.

a) Species of poultry/birds

Surveillance for H7N9 should be prioritized in poultry, waterfowls, ducks, wild birds, pigeons in the decreasing order of priority.

b) Sampling

The following samples are appropriate for laboratory diagnosis.

- Oropharyngeal swab, cloacal swab and serum
- Oropharyngeal swab and cloacal swab samples should not be pooled.
- Currently, there are no rapid test available for HPAI H7N9 and therefore confirmatory diagnosis can be done through real-time RT-PCR at NCAH

c) Geographical areas

- High risk southern border areas
- Market chains-backyard and commercial farms
- Water basin and migratory areas

d) Frequency of surveillance activities

- Conduct surveillance as for H5N1
- Passive surveillance should be conducted throughout the year. However, targeted/heightened surveillance should be conducted during high-risk seasons (autumn and winter) and also based on the disease status or outbreaks in the neighbouring countries and high-risk countries

6.2 PHASE 2: VETERINARY RESPONSE TO HPAI OUTBREAK

The Department of Livestock will immediately investigate all suspected clinical signs of HPAI reported by the public and any incident notified by the VVTs and BVTs in pursuant to **section 9.7 and 9.8 of the livestock Act of Bhutan 2001**. If a suspect case is notified to RLDC then RLDC must immediately notify NCAH who in turn will notify BAFRA.

The RLDC and/or NCAH will deploy a Disease Outbreak Investigation Team to investigate the suspected case immediately. This team will undertake a comprehensive epidemiological assessment and conduct a rapid antigen detection test in the field to provisionally confirm the disease as well as collect appropriate samples.

The Department of Livestock will confirm the clinical diagnosis through laboratory tests at NCAH and refer samples to OIE designated reference laboratories abroad for further confirmation and typing of a virus.

6.2.1 Case Definition for HPAI

The case definition for HPAI is categorized into suspect, probable and confirmed cases, however, the need to respond quickly should always be an overriding principle when dealing with outbreaks of HPAI. The BAFRA and DoL will quarantine suspect and probable places and initiate response action under direction from NICC while awaiting HPAI laboratory confirmation.

Suspect: Sudden death of birds with very high mortality, severe depression, loss of appetite, nervous signs, watery diarrhea, severe respiratory signs and/or a drastic drop in egg production, with a production of abnormal eggs, presence of facial subcutaneous edema,

swollen and cyanotic combs and wattles. Young chickens, or those dying from the per-acute form of the disease, may not show any lesions

Probable: Detection of antigen for Influenza A on rapid field test and/ or detection of H5 antigen with consistent clinical signs suspecting AI.

Confirmed: A combination of symptoms consistent with HPAI; positive rapid test in affected birds; detection of H5N1/H7N9 by rRT PCR and /or; successful HPAI virus isolation.

6.2.2 Low Pathogenic Avian Influenza (LPAI)

Department of Livestock will apply the following LPAI case definition including AI H9N2, H10N8, H7N6 and H7N1 to the index case:

- Virus is isolated or
- Viral RNA specific for Influenza A is detected by real-time RT-PCR, and there is epidemiologically significant serological evidence of actively circulating virus, and
- LPAI confirmed by molecular methods or using an isolated virus, and the amino acid motif is not consistent with that reported for HPAI viruses

The intent of this definition is to have a high degree of scientific confidence in the presence of active infection. The DoL will have an initial case(s) of LPAI typed according to international standards that may include OIE reference laboratories.

6.2.3 Differential Diagnosis

Avian influenza and Newcastle disease (ND) of chickens and turkeys with various levels of pathogenicity are frequently indistinguishable on clinical and post-mortem examination from:

- Mycoplasmosis;
- Fowl cholera;
- Escherichia coli cellulitis of the head;
- Acute pasteurellosis;
- Infectious laryngotracheitis;
- Infectious coryza;
- Acute poisoning; or
- Misadventure causing high mortality (e.g. smothering, heat stress, dehydration).

The plan of action during an outbreak will generally follow the recommendations outlined in the Incident Command Structure of Veterinary Response to HPAI outbreak and as per specific SOPs.

6.2.4 Infected and Suspect Infected Place Procedures

Field staff must ensure that infection does not spread from Infected Premises. Investigating team vehicles must be left outside the Infected Premises and at a distance from the entrance to the premises. Demarcation for various zones is shown in Figure 7. The investigating team should take all the required materials as per the SOP for DO-IT with them. At least two sets of these kits should always be kept in readiness at each RLDC and NCAH.

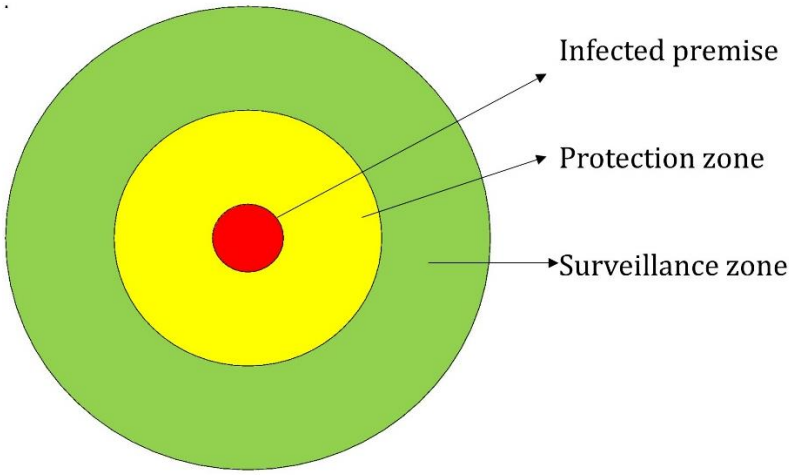


Figure 7 Demarcation of different zones

6.2.5 **Declaration of Provisional Protection Zone**

When HPAI is suspected, the respective field offices shall inform NCAH & BAFRA officials. Together they will immediately quarantine the Suspect Infected Place (farm or hatchery premises or a village) and declare a surrounding area or actual boundary to be demarcated based on an epidemiological assessment of risk from the point of suspected infected place as a Provisional Protection Zone. The Geographical limits of the Provisional Protection Zone should be determined after due consideration of the epidemiologic risk and natural Geographical settings. All places with poultry within the Provisional Protection Zone shall be considered at-risk and visited to establish their infection status. As soon as the disease is confirmed by the national laboratory and based on the advice of TAC, the pre-emptive culling of the birds in the affected flock/ farm and those farms/ flocks with the highest risk of spreading the disease will be done before the activation of NICC/ IOC.

6.2.6 **Detention of suspected birds/flock in the provisional protection zone**

Order for the detention of the suspected birds, avian products and in-contact materials within the declared Provisional Protection Zone is issued through a movement control ban, (Orders to be issued by Dzongkhag/BAFRA). Quarantine all birds, avian products and such materials in the suspected Infected Place while awaiting a confirmatory diagnosis,

Withdraw the movement control ban and declaration of free area upon the receipt of a negative confirmatory diagnosis from the laboratory.

6.2.7 **Declaration of Protection Zone**

If the case definition for HPAI is met, then a Protection Zone is declared as defined by the risk assessment team. Stamping out procedures, (a) depopulation by slaughter; (b) disinfection and (c) sanitary measures should be carried out on properties that have had direct and indirect contact with the Infected Place or Places. The decision to carry out the stamping out procedures should be done based on contract tracing and scientific risk assessment. The strict surveillance and movement control should be maintained on all other properties within the Protection Zone. It may not be necessary to use blanket stamping out on all properties within the Protection Zone. In the big commercial farms with very good

biosecurity practices the culling may only be initiated based on the test result and thorough risk assessment.

6.2.8 Declaration of Surveillance Zone

The area of the surveillance zone shall be determined by the risk assessment team and will be used for enhanced surveillance activities in this zone to monitor the possible spread of infection. Inspection, movement control, surveillance, screening and sanitary measures shall be the main tasks in the Surveillance Zone.

6.2.9 Control of HPAI Outbreak

Containment and eradication procedures will be implemented promptly following confirmation of a diagnosis of HPAI. A mobile disinfection unit should be positioned at all points of entry/exit to the Infected Place. The number of vehicles and staff involved in depopulation should be kept to a minimum. Any person who has been inside the Infected Place may only leave after a complete change in clothing and if possible, a shower. Staff involved in the depopulation of the farm must not have any contact with susceptible species for at least three days after the last contact with the Infected Place.

These general principles apply to the 3-D Team (depopulation, disposal & decontamination), vehicles for transportation of dead birds and disinfection crews.

6.2.9.16.2.9.1 3-D Operation (Depopulation, Disposal & Decontamination)

All birds, including those at high risk of infection, should be promptly culled and disposed of by incineration or burial following the detection of any disease positive birds in a village/farm. The BAFRA will enforce the implementation of 3-D operation and movement control measures once the outbreak has been confirmed.

Humane killing should be conducted with due consideration to religious sentiment and social obligation. Disinfectants must be used carefully with due regard to the nature of the material being used and possible adverse effects from environmental contamination.

6.2.9.26.2.9.2 Disposal of Birds

Burial Method

Burial may be the best means of disposal under certain conditions and a pit should be prepared as soon as the diagnosis is confirmed. A pit of 2x2x2 meter wide, breadth and height would accommodate 1800 birds. If the pit is made 1m deeper the capacity would increase up to 3000 birds. The dimension of the pit will be decided by the number of birds to be disposed and the area of culling including the Geographical terrain. The number of birds can be doubled, each meter deeper the pit is made.

Materials that cannot be disinfected such as wood and cardboard should be burnt. Carcasses should ideally be covered with a layer of soil and then a layer of calcium hydroxide, and then again with a layer of soil (at least 40 cm). The detailed description of the disposal procedure is given in the SOP of 3-D operation.

Incineration Method

Incineration may be used for the disposal of carcasses wherever feasible.

6.2.9.36.2.9.3 Disposal / Destruction of Infected Materials

All waste, organic and other materials that cannot be disinfected must be destroyed. All eggs, egg products, hay, animal feedstuffs, feathers and egg trays must be destroyed too. Litter and straw, depending on the amount present and on the characteristic of the farm can be either burnt or buried in a pit with the carcasses.

i. Eggs and egg products

May be buried in the pit with the animal carcasses or manure.

ii. Animal feed

Animal feed on the site must be decontaminated followed by burial and/or incineration method.

6.2.9.46.2.9.4 Disinfection of Infected Premises Checklist

– All units which are physically or functionally connected to the establishment (i.e., hatchery, egg storage rooms, packaging rooms, egg trolleys, egg product plants); vehicles, used for transporting live animals, eggs and animal feed should be disinfected with appropriate disinfectants. The vehicle may be allowed to stand, and disinfectant sprayed on wheels and any parts that have possibly come in contact with infected materials. The vehicle may be allowed to move half circle forward and again sprayed on wheels surfaces that have not come in contact with disinfectant during first spraying. Foot mats inside the car may also be disinfected;
– Washing and disinfection of walls, floors, and ceilings of the infected establishments must be performed to remove all organic material before disinfection;
– Metal structures such as cages may be decontaminated by heat treatment;
– All equipment inside the house such as drinkers and food hoppers must be washed and treated with a disinfectant for at least 48 hours;
– Water reservoirs must be emptied, washed and disinfected;
– Feed tanks (silos) need to be emptied, washed with a hot water-pressure pump and subsequently fumigated;
– After washing and disinfecting, all units must be fumigated twice with at least two weeks between fumigations.
– Drinking water source contaminated due to infected birds may be chlorinated at permissible dose by relevant authority
– A list of disinfectant which is active against avian influenza virus, their concentration, and recommended use is presented below:
– Virkon S (di-potassium peroxo-disulphate) for all purposes except on open skin/eyes.
– Sodium hypochlorite: 2% active chlorine solution for disinfection of equipment.
– Quaternary ammonium salts: 4% solution for the treatment of walls, floors, ceilings, and equipment.
– Calcium Hydroxide: 3% solution for the treatment of walls and floors.
– Cresol acid 2.2% solution for the treatment of floors.
– Synthetic phenols 2% solution for the treatment of floors.
– Formalin and permanganate for fumigation

A formula needs to be worked out for use of bleaching powder for vehicle disinfection owing to its corrosive nature in higher concentrations. For any new and effective compounds available shall be reviewed by the committee.

6.2.9.56.2.9.5 Withdrawal of disease control zones

Bans should be lifted 21 days after the last stamping out date and upon satisfactory completion of sanitary & biosecurity measures, restocking should be undertaken at 42 days by introducing a few numbers of poultry first and monitoring these daily for signs of disease. Strict surveillance for at least three weeks is recommended after restocking after which full repopulation can occur.

6.2.9.66.2.9.6 Enhance biosecurity at poultry farms and associated premises

As part of the implementation of routine biosecurity measures on Infected Places, associated premises, Protection and Surveillance zones, movement control from and into these zones will be strictly enforced by BAFRA. A sanitation policy for the rearing of ducks is also to be enforced to prohibit the grazing of domestic ducks in areas with nearby wildlife sanctuaries. Protection of ducks from wild birds using wire mesh or cyclone wire barriers should also be explored.

6.2.9.76.2.9.7 Compensation scheme

A compensation scheme has been incorporated in the HPAI control policy to encourage timely and positive reporting of any cases and also to compensate for losses due to disease or culling. The **Livestock Act of Bhutan 2001, under sub-para 9.3**, clearly states that the government has the authority to compulsorily destroy animals, animal products or feed or other risk goods that it considers to be risky and pays compensation as prescribed by the Ministry of Agriculture and Forests. The "Guidelines for compensation mechanism" outline the management of the compensation fund.

Eligibility for Compensation

Compensation payments will be made only:

- If mandatory culling measures have been announced and put into effect by the Government;
- For culled poultry, eggs, coops/sheds, feed and feed materials disposed under the supervision of the 3-D team and based on completion of all required documentation;
- After identifying those eligible for a compensation payment is made

Compensation will not be paid for poultry that has died as a result of HPAI and any disease other than HPAI. State-owned enterprises are not eligible for compensation. Compensation payments will be done only after acquiring approval from NICC.

6.2.9.86.2.9.8 Personnel Safety

Personnel engaged in disease control and eradication activities should be treated with antiviral prophylaxis for the duration of their exposure and for seven days after their last exposure or maybe vaccinated if available. The PPE should be worn at all times in infected or while handling diseased birds.

Personnel should be rotated off-site if the exposure period (culling and clean-up) is prolonged. Personnel may also be asked to take part in monitoring involving the collection of blood samples by the medical authorities to determine if they have been infected. Persons who do not agree to preventive and monitoring measures should not be engaged in activities resulting in contact with infected birds/materials.

6.2.10 Risk communication

Risk communication plays a key role in public health emergencies. An influenza pandemic is an epidemic that spreads on a worldwide scale and infects a large proportion of the human population. It is a major threat to public health worldwide because of its ability to spread rapidly through populations and to cause complications.

In response to the global outbreak of Avian and Human Influenza, the Royal Government of Bhutan, specifically the Ministry of Health and Ministry of Agriculture and Forests jointly developed the National Influenza Pandemic Preparedness Plan (NIPPP). A pandemic preparedness plan for the country was critical to build core capacities to diagnose and control influenza, identify resource needs, expertise and services are mobilized and deployed quickly to pre-empt the pandemic and reduce its impact.

One of the strategic action plans of the NIPPP is to implement a risk communication strategy for high-risk occupational groups, media and general community. This communication strategy intends to provide an overarching platform for all communication activities, with an emphasis on preparation. It seeks to bolster readiness through a series of strategies geared to all phases of a public health emergency. The communication goal is to lessen the impact of a public health emergency, enhance health outcomes, through promotion of positive behaviour and social change.

Although Bhutan has not detected human cases of avian influenza A (H5N1) and AI H7N9 so far, there is an on-going public health threat posed by the frequent outbreaks of A (H5N1) in poultry. Poultry farmers are ignorant of farming practices that put them at significant risk. Poultry are reared very near to the human dwellings and are free to roam around. Poultry deaths/sickness is not reported on time, due to fear of economic loss or lack of awareness on farm biosecurity. The poultry care providers do not practice healthy behaviour such as wearing protective gears while handling or feeding poultry and its products. Overall, there is very low level of awareness on poultry farm biosecurity aspects. In addition, there are illegal movements of poultry, poultry products and farm articles across the border and also within the country even during outbreak period.

Information and communication environment in Bhutan have undergone unprecedented changes in the past decade with the liberalization of the information and media markets, in addition to other forms of communication channels. The gap between urban and rural areas in terms of access to media and information has been narrowed to a great extent. New information and communication technologies (ICTs), particularly in the form of the internet and mobile telephony, have transformed information flow and communication patterns among all segments of the society. People use mobile phones both in urban and rural areas.

Given this situation, risk communication strategy for behaviour and social change is critical for the general public particularly the high-risk groups in enhancing their knowledge about

AI and promoting positive behaviour to avert respond to and recover from a possible pandemic situation. A document on Avian Influenza/Pandemic Influenza risk communication strategy has been developed by the Department of Livestock in consultation with relevant stakeholders. Based on WHO categorization of Pandemic phases and the NIPPP phases this communication strategy covers three phases of the epidemiologic situation. Viz. Preparedness phase, Response phase and Recovery phase.

The main objectives of this communication strategy are:

Preparedness Phase (Pandemic phases 1 -3):

- Enhance public knowledge and confidence on the value of Flu Wise etiquettes; biosecurity measures, reporting sick and dead birds, to reduce animal-to- animal transmission
- Increase Flu Wise and Flu Care etiquettes among general public to limit spread of seasonal human influenza

Response Phase (WHO Pandemic Phase 4 – 6)

- Increase Flu Wise and Flu Care etiquettes among the public to reduce human-to-human transmission.

Recovery Phase: (Post Pandemic)

- Enhanced Flu Wise etiquettes among the public to reduce animal-to-animal transmission for another outbreak

In the event of an outbreak of HPAI H5N1, in order to maintain coherence of information sharing thereby ensuring proper risk communication to the public, media spokespersons will be nominated from Ministry of Health and Ministry of Agriculture and Forests. These focal media spokespersons are authorized to provide updates and any other information about the ongoing avian influenza outbreak situation in the country to the public and media.

6.2.11 Routine HPAI Surveillance

Once the HPAI outbreak is controlled and declared freedom which is officially endorsed by World Animal Health Organization (OIE), the veterinary authority can return to conduct routine surveillance for early detection of repeat incursion of the virus. The routine surveillance should follow procedures described in the phase 1 for prevention of HPAI H5N1/ H7N9 outbreak.

7 ANNEXURE STANDARD OPERATING PROCEDURES (SOP)

7.1 SOP FOR DISEASE OUTBREAK INVESTIGATION

A disease outbreak has been defined as a short-term epidemic or a series of disease events clustered in time and space. The disease events are usually new cases of disease usually occurring at a higher frequency than that is normally expected.

An outbreak investigation is a systematic procedure to help identify causes and sources of epidemic with a view to control of an existing epidemic and prevention of possible future ones.

Purpose:

- To identify the causes and sources of disease outbreak
- To identify measures to prevent further transmission of the disease-causing agent (HPAI H5N1 virus).
- To control and contain the existing disease outbreak
- To prevent the possible future disease outbreaks.

Scope:

- This SOP outline the general principles and steps for investigation of Highly Pathogenic Avian Influenza (HPAI H5N1 and AI H7N9) in the field

Users or targets

- Veterinary Officers and para-veterinarians
- Veterinary Vigilance team
- Rapid Response Team

Team composition

- Veterinary Epidemiologist and/ or Regional Veterinary Officer (Team Leader)
- Veterinary Pathologist
- Senior laboratory technician
- Field para-veterinarians
- BAFRA officials

Materials and equipment

Items	**Units**	**Quantity**
Personal Protective Equipment (PPE)		
Disposable Glove	Pairs	50
N95 Masks	Sets	10
Disposable Overalls	Sets	10
Shoe cover	pairs	
Goggles	Pairs	10
Rubber Band	Box	1

Documents		
Outbreak Investigation Forms	Number	5
Laboratory Sample Submission Form	Number	10
Written Instructions/SOP Printouts	Number	2
Notebooks & pen	Number	5
Laboratory consumables		
Soap	Number	2
Alcohol Swabs pad	Number	50
Cotton Roll	Rolls	3
Syringes, 5ml	Number	30
Needles, 21 gauge	Number	30
Cold Box	Number	2
Rapid Antigen Detection diagnostic kits	Set	5
Eppendorf Tubes	Number	30
Waterproof Markers	Number	3
Ice Packs	Number	10
Leak proof plastic bags	Number	20
Leak proof autoclavable plastic bags	Number	3
Swabs	Number	50
Virus Transport Medium	Number	20
Tissue paper	Rolls	5
Sterile petri dish	number	10
Disinfectant - 5 litres jar	Number	3
Equipment		
GPS	Set	1
Spray Pack, Handheld 5l	Number	1
Plastic tub/ bowl - 15-20-liter capacity	Number	2
Brush for Boots	Number	2
First Aid Kit	Sets	2
Post-mortem kits	Sets	2
Disposable scalpels		20
Surgical scissors	Number	6
Forceps	number	6
Mobility	Number	2
Torch	Number	10
Communication set (mobile/ walkie talkie)		
Others		
Antiviral drug (Tamiflu)		
Fund		
Bottled Drinking Water		

Meals and refreshment		
Extension gears –tent, boots, sleeping bag, mat, rain coat		

Steps for Investigation

Pre-investigation preparation

- Formation of investigation team and planning the response among team members
- Bring the team together
- Discuss each person's roles and responsibilities
- Arrangement of materials and logistics (refer materials and equipment requirement)
- Epidemiological materials: Investigation form, notebook, laptop, GPS etc…)
- Medical: antiviral medication
- Laboratory: swabs, needles, cool box, viral transport medium
- Educational: SOPs, guidelines
- PPE
- Decontamination
- Mobility and refreshment
- Extension gears

Gather preliminary information: Following information needs to be collected by the team prior to their departure

- Farmers name and phone number (if available),
- Name of village, Gewog, Dzongkhag
- Type of enterprise and number of birds (commercial, semi commercial, backyard, village, chickens, duck, other birds or animals (specify), Date and time of report of outbreak from farmer to LEC/ DVH
- Date and time of report from LEC/ DVH to RLDC/NCAH
- Date and time of visit by veterinarian or field staff,
- Name of contact field staff, address and phone number
- Provide information about the team visit to outbreak area
- Date and time of visit

Field investigation

- Background information to collect
- Farm and village background information,
- Different bird categories and numbers (flock size,)
- Farm type and husbandry practices
- Whether any inter-mixing of birds and other animals such as cats, dogs and pigs
- General information regarding source of chicks/adults
- General information regarding buying and selling of poultry and poultry products
- General information about the affected village/ farm (no. of households; household rearing poultry, average flock size, farming system.
- Collect XY coordinates (using GPS), altitude, road network, Government offices, frequency of movement of people in an out of the outbreak area

Baseline mortality and clinical signs

- Determine baseline mortality for period (week or month) before the outbreak and in previous year, both generally, and more specifically for the same seasonal time period as the present outbreak in the previous year;
- General information of the present disease outbreak such as number of households affected, population at risk, poultry population in the surrounding villages etc.
- Record of the daily morbidity, mortality and case fatality figures in the farm/ village
- Record of the number of birds voluntarily killed (culled) if done;
- Record of the detailed clinical signs during these periods.

Bio-security arrangements

- Describe bio-security arrangement of the farm e.g. disinfectant foot wash, perimeter wall/fence, rodent and wild bird control, etc.;
- Mixing of different groups e.g. contact between free-ranging backyard chickens and commercial layers/broilers.
- Feed source
- Describe feed sources/s and assess visually for the possibility of wild bird/ waterfowl or any other toxin contamination.
- Water source
- Describe water source/s and assess visually its quality,
- Assess water source for possibility of wild bird or waterfowl contamination.

Wild birds

- Determine the presence of any migratory wild birds in the area
- Determine the seasonal fluctuation in wild bird numbers on open water areas in the vicinity,
- Assess contact with wild birds through sharing of common water areas (ponds, lakes, wetlands) or through water source/s.

Veterinary interventions

Record vaccination programs, drug use and other veterinary interventions

Laboratory investigation

Laboratory investigation in the field (refer specific SOP for sampling, packaging and transportation to the laboratory and rapid field test)

- Put on proper PPE
- Carry out rapid diagnostic test for influenza A on specimens of tracheal and lung fluid obtained from freshly killed, sick or dying birds.
- Collect cloacal swabs and blood samples from live birds and transport to the laboratory.
- Collect five to ten numbers of carcasses in a leak proof container and transport to the laboratory.
- Collect faecal sample and submit to the laboratory (refer SOP for faecal sample collection)
- Collect environmental samples (water, feeds etc) and submit to the laboratory (refer SOP)

Laboratory diagnosis

Refer specific SOPs for laboratory diagnosis. Following laboratory tests will be done at NCAH for confirmation.

- HA and HI tests
- Polymerase Chain Reaction tests
- Virus isolation (to be established at NCAH)
- Characterize the outbreak.
- Establish or verify the outbreak
- Provisional diagnosis made on clinical signs, epidemiological pattern, rapid field test and gross pathology.
- Interim immediate emergency disease control response should be in place before the confirmatory laboratory diagnosis is made (refer specific SOPs for disease outbreak response)

Establish the case definition for HPAI.

Suspect case: whenever sudden bird deaths with very high mortality occur with severe depression, loss of appetite, nervous signs, watery diarrhea, severe respiratory signs and/or a drastic drop in egg production, with a production of abnormal eggs, presence of facial subcutaneous oedema, swollen and cyanotic combs and wattles. Young chickens, or those dying from the per-acute form of the disease, may not show any lesions.

Probable case: Detection of antigen for Influenza on rapid field test and/ or detection of H5 antigen on HA/HI test with consistent clinical signs suspecting AI.

Confirmed case: A combination of symptoms consistent for HPAI; positive rapid test in affected birds; detection of H5N1 antigen on HA/HI test; successful HPAI virus isolation and PCR positives.

Differential diagnosis has to be made against NCD, Fowl cholera, IBD, heat stress and acute poisoning.

Describe the outbreak in terms of time, animal and place.

Time (draw epidemic curve by plotting cases against the time from available data-preferably time series)

- When was the index case?
- What is the exact period of the outbreak?
- Given the diagnosis what is the probable period of exposure?
- Is the outbreak most likely to be point source or propagated or both?

Animal (attack rates, risks etc)

- Any differences in the attack rates among different sizes of flocks.
- Which groups (layers, broilers, chicks, and duck) have the highest and which have the lowest attack rate?

- Any difference in the attack rate among different age group of birds?

Place (plot the location of outbreak on a map with physical characteristics such as road, water bodies, mountains, infrastructures etc)

- What are the Geographical distributions of the cases?
- What is the pattern of the cases among different poultry house or management system?
- Whether case farm is close to water bodies or other spatial risk factors?

Develop hypothesis based on the pattern of disease (animal, time and place).
- Source of disease outbreak- forward and backward contact tracing
- Mode of transmission
- Whether the outbreak is a common source or propagating
- If a common source, whether it is point or multiple exposure
- What are the risk factors associated with problem?

Control and Prevention Refer specific SOPS for RRT; surveillance; culling; disposal; decontamination etc. Interim immediate emergency disease control response should be in place before the confirmatory laboratory diagnosis is made (refer specific SOPs for disease outbreak response)

Declaration of provisional protection zone
When HPAI H5N1 is suspected, VVTs should inform the NCAH and immediately quarantine the suspect infected place (farm or hatchery premises or a village) and the surrounding area (a radius of about 3 km and/or based on risk assessment) as a Provisional protection zone. These provisional protection zones will include infected premises; suspected premises and dangerous contact premises. The Geographical limits of the provisional protection zone should be determined after due consideration of the epidemiologic risk and natural Geographical settings.

- All places with poultry within the Provisional Protection Zone shall be considered at-risk/ suspect and should be visited to establish their infection status.
- Quarantine and movement control on poultry and poultry products, farm workers; vehicles etc should be imposed (refer SOP for quarantine and movement control).
- Strict surveillance and movement control should be maintained on all other properties within the infected Zone.

Declaration of protection zone:
If the case definition of HPAI H5N1 is met, the area within a radius as decided by risk assessment team should be immediately declared as protected zone. The Geographical limits of the protected zone should be determined after due consideration of the epidemiologic risk and natural Geographical settings.

Once the outbreak is confirmed and based on NICC direction, RRTs should be immediately activated. Quarantine and movement control on poultry and poultry products, farm workers; vehicles etc should be imposed (refer SOP for quarantine and movement control).

Strict surveillance and movement control should be maintained on all other properties within the infected zone. RRT should carry out 3D operation on all the properties within the protection zone based on the direction of outbreak investigation/risk assessment team (refer specific SOPs).

Declaration of Surveillance Zone Refer SOP for surveillance

Rapid humane destruction of infected birds and flocks in the protection zone (Refer SOP for culling & disposal)

- Disposal of birds (Refer SOP for culling & disposal)
- Disposal / destruction of infected materials (Refer SOP for decontamination)
- Disinfection of infected premises (Refer SOP for decontamination)

Reporting

- Document the findings (Background; investigation procedures, epidemiological and laboratory findings; economic impact etc.
- Provide recommendations to all the relevant stakeholders (farmers/ producers; Managers; NICC, DoL, BAFRA and other agencies)
- Submit the final report

Surveillance and monitoring will be done by the surveillance team.

- Is the frequency of the disease remaining constant; increasing or decreasing?
- Is the control or eradication program effective?
- Does the disease have any impact on productivity or profitability?

DISEASE OUTBREAK INVESTIGATION FORM

Ref. No: …… Date: ……/……/……..

Farm name: …….. Owner name: ……..

Contact No: ……

Address

Village: …… Gewog: …….. Dzongkhag: ……..

Geo-coordinates:

Longitude (E): …… Latitude (N): ……..

Farm type: Commercial [ ] Semi-commercial [ ] Backyard [ ]

Number of birds:

Broiler: …….. Layer: …… Chicks: …… Pullets: ……. Ducks: ……

Others (specify): ……..

Feed details: Feed type: …… Feed source: ……..

Details of any supplement in feed/ water: ……..

Housing:

Permanent with CGI roof [ ] Temporary [ ] Coop [ ] Deep litter [ ]

Others [ ] *specify:* ……

Farming system:

Intensive [ ] Semi-intensive [ ] Free-range [ ]

Others *(specify):* ……

Source of birds:

Hatchery outside Bhutan [ ] Hatchery within Bhutan [ ] Government farm [ ]

Others *(specify)*: ……

Water source for birds: ……

Any contamination by domestic/wild water birds: Yes [ ]; No [ ]

Details of other animals present on the farm: ……..

Details of wild birds in the area: ……

Nearby water bodies: Pond [ ]; River [ ]; Stream [ ];

Others *(specify):* ……..

Details of disposal & management of manure: ……..

Farm biosecurity:
Footbath: [ ] Perimeter wall/fence: [ ] Rodent/wild bird control [ ]

Contact with free-range chicken [ ] Others *(specify):* ……..

Topography of outbreak area:
Road network [ ] Market [ ] School [ ] BHU [ ] RNR/LEC [ ]

Others (specify): ……..

Movement of birds:
Recently introduced birds from other places: Yes [ ] No [ ]

if yes give details: ……

Sale of birds/eggs/meat to other farms/places: Yes [ ] No [ ]

if yes give details: ……..

Movement of people/vehicle

Recent movement of people/vehicle from other places: Yes [ ] No[ ]

If yes give details: ……

Recent movement of people/vehicle to other places: Yes [ ] No[ ]

If yes give details: ……

Details of other bird population in the area:

No. of households: [ ] No. of farms: [ ] Proportion of HH owning poultry in percentage: [ ] Average No. of birds owned: [ ] Approximate population in the area: [ ] Other animal species in the area *(specify)*: ……..

Vaccination history of affected flock/ village:

Vaccine type	***Date of vaccination***	***Age***	***Vaccine details***
ND			
IBD			
Fowl pox			
Marek's			
Others *(specify):* ……..			

Information on disease outbreak:
Date reported from farmer to LEC/DVH: ……. /……. / Time: … : ...

Date reported to LEC/DVH: ……. /……. / Time: … : ...

Date of onset of clinical signs: ……. /……. / Time: … : ...

Date of onset mortality: ……. /……. / Time: … : ...

Details of affected poultry population

Date	Type of bird	No. affected	No. died	Pop at risk	No. destroyed	Remarks

Clinical details

Clinical signs observed: Diarrhoea [ ] Many deaths over past 2-3days [ ]
Oedema of comb/wattle [ ] Reduced feed & water consumption [ ] Reduced egg [ ]
Respiratory signs [ ] Congestion/cyanosis of comb, wattles or shanks [ ]

Others (*specify): ……*

Necropsy findings (if any):

Treatment given (if any):

Sample details:

Sample ID	Bird type	Specimen type	No. of specimens	Lab referred to:	Date of shipment	Test requested

Name & Designation:

Signature

7.2 SOP FOR HIGHLY PATHOGENIC AVIAN INFLUENZA SURVEILLANCE

Surveillance is a continuous and systematic process of collection, analysis, interpretation and dissemination of descriptive information for monitoring health problems. Surveillance is a key component in the emergency preparedness against this exotic disease, and plays a major role in an early warning system in case of its introduction to Bhutan. It also provides early information on the probable emergence of the HPAI virus in the country.

Purpose:

- The installation of an early warning system for HPAI.
- To understand the epidemiology and ecology of AI and its socioeconomic impact.
- To help design effective HPAI control programmes.
- To assess the temporal and spatial patterns and improve the effectiveness of HPAI control efforts.
- To ensure freedom from clinical disease and absence of infection in a country.
- To evaluate the existing disease control programmes.

Scope:

This SOP covers the surveillance guidelines during preventive, outbreak and post outbreak phase to demonstrate freedom from infection.

User/Target:

- Veterinarians, Laboratory technician and field para-veterinarians

Surveillance Team composition:

- Veterinary epidemiologist and/or RVOs of RLDCs (Supervisor)
- Veterinary Officers from NCAH, RLDCs, DVLs under DoL and BAFRA; Laboratory technicians; field staff.

Materials and Equipment

- Questionnaires/ survey forms
- Notepad and pen
- Mobility
- Communication facilities-mobile and hand set
- Sampling kits – swabs, needle, syringes, permanent marker pen, sample submission forms, Eppendorf tubes, faecal vials, transport media, cotton, antiseptics, face mask, gloves, soap, apron
- Diagnostic kits – rapid antigen diagnostic kits, HA/HI test kits
- GPS machine
- Poultry population figures
- Duck population
- Information on wild bird habitats
- Laptop with relevant statistical packages
- Extension gears –rain coat/ umbrella, cap, torch, walking boot

- Fund

Steps for surveillance:

Surveillance during the prevention phase

Surveillance during preventive phase comprises clinical and laboratory surveillance in all the Dzongkhags with intensive programs in high risk areas/ Dzongkhags and when there is imminent threat of HPAI H5N1 and AI H7N9 virus incursion. The high-risk Dzongkhags includes Samtse, Chukha, Sarpang, Samdrup-Jongkhar, Zhemgang, and Pemagatshel & Dagana that share open porous border with India.

1.1 Clinical disease surveillance:

Clinical surveillance is aimed at detection of clinical signs of HPAI at the flock level. Surveillance based on clinical inspection is particularly relevant for HPAI because the infection is characterized by very high mortality rates in terrestrial poultry.

The following trigger points may provide guidance in suspecting an AI infection (see Figures below).

- Sudden deaths of birds with severe depression, loss of appetite, nervous signs, watery diarrhea, severe respiratory signs and/or a drastic drop in egg production.
- Presence of facial subcutaneous oedema, swollen and cyanotic combs and wattles, subcutaneous haemorrhage on shank and body
- In organized farm if the daily mortality is >2% for 2 consecutive days;
- In village chicken and backyard farms, if daily mortality is ≥5% for 2 consecutive days,
- A reduction of food and water consumption by 20% for three consecutive days

Reporting system

The field staff should report the clinical surveillance report to the DLO/RLDC/ NCAH on a weekly basis (every Tuesday). Focal persons in each village will report any suspected cases to the field staff and act as contact point for reporting and dissemination of information to the farmers in the villages.

Detail investigation will be carried out by VVT/RLDC/SVL/DVH and LEC if there are any unusual mortality of poultry and wild birds in the farms/villages. In any suspicious situation, it is important that not only HPAI infection is ruled out but also a definite diagnosis is made of the cause of the problem.

Any rumour or report of any suspicious death of a poultry or wild birds by the media or general public should be investigated to verify authenticity of the rumour (rumour surveillance)

1.2 Laboratory surveillance

- Surveillance activities, both clinical and laboratory testing have to be carried out on a regular basis including routine and purposive sampling.
- When the outbreak occurs in neighbouring countries targeted laboratory surveillance in high risk Dzongkhags should be done on monthly basis

- Differential diagnosis should be made against the following diseases: Newcastle disease, IBD; fowl cholera; acute poisoning; or Misadventure causing high mortality (e.g. smothering, heat stress, dehydration, etc).

- Cloacal and/ or tracheal swabs from sick and dead birds should be subjected to rapid antigen detection tests at the field level. In case of positive cases on rapid antigen detection test samples should be referred to NCAH for HA/HI tests. In case of high clinical suspicion, even the negative samples should be referred to the NCAH and/ or other international reference laboratory *(refer SOPs for Rapid Antigen Detection tests).*
- Environmental samples like faecal droppings should also be examined for presence for AI virus (refer SOP)

1.3 Targeted surveillance

The targeted surveillance in domestic waterfowls, migratory wild birds, and backyard poultry in the vicinity of migratory bird habitats, breeding farms, imported poultry stocks, border check posts, live bird markets and processing establishments should be done as the probability of detecting HPAI virus is high. Targeted surveillance is recommended in view of the cost and resources in the country.

1.4 Surveillance in domestic waterfowls

Ducks play an important role in maintaining HPAI infection and transmitting it to other poultry species and therefore these birds must be serologically screened for H5, H7 or H9 virus.

Item	Details
Locations	Domestic waterfowl rearing areas in the country
Type of bird	Domestic water bird like duck, geese etc
Type of sample	Tracheal/Cloacal swabs/ wet droppings
Collection by	VVT, RLDC, NCAH, Field Staff
Period of collection	Regularly (based on risk assessment and disease situation in the neighbouring countries)
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent
Laboratory testing	Rapid antigen detection test/ followed by HA/HI/ and PCR

Backyard poultry in the vicinity of migratory bird locations

Backyard poultry reared in the vicinity of migratory bird locations can easily come in contact with exotic birds. Contaminated water reservoirs could play a vital role in transmitting the infection to local birds.

Item	Details
Locations	All areas in the vicinity of Migratory Bird locations (Phobjikha, Bomdeling, Bumthang, River basins (Chamkharchu; Puna Tshangchu; Sunkosh, fish ponds, national parks etc)
Type of bird	All poultry species
Type of sample	Tracheal/Cloacal swabs/ wet droppings

Collection by	VVT, RLDC, SVL, NCAH, field staff
Period of collection	During the time of bird migration (winter season)
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent.
Laboratory testing	Rapid antigen detection test in the field; HA/HI and PCR

1.5 Surveillance of wild birds/migratory birds

The role of migratory birds in spreading HPAI has now become important. Currently available epidemiological data suggest that wild migratory waterfowl are most likely to play a role in the HPAI cycle and could be the initial source of the AI virus into a country. Surveillance shall involve collection of faecal materials, dead carcasses and investigation of dead wild birds.

Item	Details
Locations	Habitats of the migratory wild
Type of bird	Black necked cranes in the Phobjikha and Bomdeling valleys, ruddy shell duck throughout Bhutan, egrets, black storks, fish eagles, white and grey bellied herons and, cormorants in southern parts of the country, bustards/hornbills and crows and pigeons.
Type of sample	Tracheal/Cloacal swabs/ wet droppings
Collection by	VVT, RLDC, SVL, NCAH, Field staff
Period of collection	During the time of bird migration (winter season)
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent
Laboratory testing	HA/HI and PCR

1.6 Surveillance of domestic chickens in high risk places

Threat is more in those Dzongkhags that share international borders in the south, east and the west. As such disease surveillance should be carried out in commercial, semi-commercial, back yard farms and village chickens in these vulnerable Dzongkhags. Particular attention should be given to the free-ranging poultry reared by national work force (along the highways); birds reared by employees of Thromde/municipal; and all establishments where free-ranging birds are reared with very poor biosecurity.

Item	Details
Locations	High risk Dzongkhags (Samtse, Chukha, Sarpang, Samdrup-Jongkhar; parts of Zhemgang, Pemagatshel & Dagana)
Type of bird	Layers and broilers, pullets, chicks
Type of sample	Tracheal, Cloacal swabs
Collection by	VVT, RLDC, SVL, NCAH, Field Staff
Period of collection	Once a year and based on risk assessment and disease situation in the neighbouring countries
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent

Laboratory testing	HA/HI and PCR

1.7 Poultry breeding farms and imported stock

There are only few Government run and private breeding farms in operation in the country. Though disease monitoring and surveillance are carried out effectively in these farms, the routine surveillance should be carried out regularly.

Testing of breeding stock in the farm

Item	Details
Locations	Government and private breeding farms
Type of bird	Parent stock, pullets, chicks etc
Type of sample	Tracheal, Cloacal swabs, wet droppings
Collection by	VVT, RLDC, SVL, NCAH, BAFRA
Period of collection	Once a year and during import (based on risk assessment and disease situation in the neighbouring countries
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent
Laboratory testing	HA/HI and PCR

Testing of day-old parent/grand-parent chicks on arrival and on- farm quarantine In addition to surveillance in the breeding farms wet droppings have to be collected by the animal quarantine staff (BAFRA) from the imported parent/ grandparent chicks and tested for the presence HPAI virus.

Item	Details
Locations	Designated farm or quarantine station
Type of bird	Day old parent/grand-parent chicks on arrival
Type of sample	Wet droppings
Collection by	Animal quarantine staff-BAFRA
Period of collection	One a year and based on risk assessment and disease situation in the neighbouring countries
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent
Laboratory testing	HA/HI and PCR

Day old birds imported from non-infected countries where vaccination against HPAI is practiced will be serologically screened before they reach the age of four weeks.

Item	Details
Locations	Designated farm or quarantine station
Type of bird	Day old chicks
Type of sample	Wet droppings
Collection by	Animal quarantine staff (BAFRA)

Period of collection	As and when DOCs are imported
Sample size	Sample size will be calculated to give 95% probability of detecting infection at expected prevalence of one percent
Laboratory testing	HA/HI and PCR

Testing of specified consignments (DOCs) during on-farm quarantine

- Surveillance at poultry processing establishments
- Surveillance is to be undertaken in the entire large-scale and selected medium-scale processing establishment.
- Cloacal swab/wet droppings from broilers should be collected randomly and tested for AI virus.

1.8 Surveillance at border check post

Since Bhutan share long open border with India in the south and with free movement of people, vehicle etc; it is important to be highly vigilant at the border check post. BAFRA Border Control Team should carry out the following activities to prevent introduction of HPAI virus into the country.

- Stringent vigilance at the border by BAFRA officials;
- Inspection of all transport vehicles carrying live poultry or poultry products, equipment including egg trays (if dead birds are detected, collect cloacal or tracheal swabs for laboratory testing);
- Inspection and quarantine of imported birds, pet or game birds;
- Disinfection of vehicle and people coming from suspected infected places.
- Feed and ingredients

1.9 Inspection of live bird markets

Live bird markets play an important role in the spread of the disease. All live markets at the border towns in Bhutan have been officially banned.

- Cage swabs (swabs of fresh faecal material from cages used to hold birds in markets) should be collected and tested if live markets start up again.
- Sample from pet/game birds should be collected and screened for HPAI virus.
- Surveillance during outbreak and post outbreak phase

2.0 Surveillance during outbreak in the Protection and Surveillance Zone Surveillance zone should be declared within 10 km radius or based on risk assessment from infected foci based on epidemiological risk assessment and Geographical settings. Intensive surveillance should be carried out to prevent further spread of the disease from infected premises and prepare for demonstration of freedom from infection.

The main goal of surveillance in the surveillance zone is to sample enough premises to produce a 95 % certainty (confidence) that at least one positive premises will be detected in the sample population, if at least 1 % of the premises in the surveillance area have a bird(s) shedding HPAI virus at the time of sampling (that is, 95 % confidence at a 1 % prevalence). Purpose:

- To assess the spread of disease from the infected premises and protection zone
- To evaluate the disease control measures in the protection zone.
- To demonstrate freedom from clinical disease and absence of infection in a country.

Scope:

This SOP covers the surveillance guidelines during the outbreak and post outbreak phase in the surveillance zone

User/Target:

- Veterinarians, Laboratory technician and field para-veterinarians

Surveillance Team composition:

- Veterinary epidemiologist and/or RVOs of RLDCs (Supervisor)
- Veterinary Officers from NCAH, RLDCs, DVLs under DoL & BAFRA; Laboratory technicians and field staff.

Materials and Equipment

- Questionnaires/ survey forms
- Notepad and pen
- Mobility
- Communication facilities-mobile and hand set
- Sampling kits – swabs, needle, syringes, permanent marker pen, sample submission forms, Eppendorf tubes, faecal vials, transport media, cotton, antiseptics, face mask, gloves, soap, apron.
- Diagnostic kits – rapid antigen diagnostic kits, HA/HI test kits.
- GPS
- Village and Gewog coordinates.
- Poultry population figures.
- Duck population
- Information on wild bird habitats.
- Laptop with relevant statistical packages.
- Extension gears – rain coat/ umbrella, cap, torch, walking boot
- Fund

2.1 Steps and activities in the protection and surveillance zone after HPAI outbreak

Active clinical and laboratory (virological and serological) surveillance should be conducted in the protection and surveillance zone to prevent further spread of the disease/infection and to maintain freedom status after the outbreak. It is appropriate to target clinical *surveillance* at particular species likely to exhibit clear clinical signs (e.g. chickens). Similarly, virological and serological testing could be targeted to species that may not show clinical signs (e.g. ducks).

Intensive surveillance should be carried out until 6 weeks of the last stamping out date and satisfactory completion of sanitary measures. In addition, routine surveillance should be carried out on regular basis there on.

- Formation of surveillance team and planning the response among team members
- Bring the team together
- Discuss each person's roles and responsibilities
- Arrangement of materials and logistics (refer materials and equipment requirement)
- Inventory of premises rearing poultry in the surveillance zone.
- This includes name of villages/premises, total number of households owning poultry, total number of commercial, semi-commercial, backyard and village chicken and duck/geese in the premises/locality, infrastructure facilities in the premises etc.

2.2 Number of premises to be sampled

The objective of surveillance in the surveillance zone is to sample enough villages or HHs to produce a 95 % certainty (confidence) that at least one positive premises or HHs will be detected in the sample population, if at least 1 percent of the premises or HHs in the surveillance zone have a bird(s) shedding HPAI virus at the time of sampling (that is, 95 % confidence at a one percent prevalence).

2.3 Selection of Premises for Sampling

Premises at high risk of having or spreading HPAI virus should be specifically targeted for the highest level of active surveillance. Factors that contribute to a premise being considered high risk may include:

- high likelihood, or a reported history, of birds moving onto or off of the infected premises or protection zone;
- presence of large number of loose chickens in the village;
- history of having sick birds, even if HPAI virus has never previously been detected
- Premises that were previously depopulated but later repopulated etc.

2.4 Selecting birds for Sampling

The following "priority categories" of birds should be selected for sampling in the order given below. Whenever possible, at least one sample should be taken from every category of bird present on premises or from each HHs in premises.

Priority 1: Sick birds – all birds on the premises should be visually examined; any birds that appear ill or lethargic should be sampled.

Priority 2: Other chickens

Priority 3: Other poultry (doves, pigeons, ducks, geese, swans)

Priority 4: Birds exposed to poultry – includes pet birds or other birds on premises where poultry is present.

When sampling birds within the above priority categories, the following "high priority types" of birds should be selected first.

- Newly introduced birds – select birds moved onto the premises most recently, especially those introduced in the last six weeks.

- Free grazing birds – select loose birds prior to caged birds or other birds whose movements are restricted in some way.
- Young birds – select younger birds prior to older birds, but all age groups should be sampled to some extent.

2.5 Sample Collection Procedure

For each bird selected for sampling, a sterile swab should be used to swab the cloaca. If birds on a premises show signs of HPAI, such as severe depression, in-appetence, drastic decline in egg production, facial oedema with swollen and cyanotic combs and wattles petechial haemorrhages on internal membrane surfaces, sudden death, it may be prudent to forego cloacal swabbing and submit either recently dead and/or euthanized birds directly to the laboratory.

2.6 Testing of Surveillance Samples

Virological *surveillance* using rapid antigen detection test (at the field level), virus isolation followed by HA/HI test and RT-PCR tests should be conducted:

- to monitor at risk populations;
- to confirm clinically suspect cases;
- to follow up positive serological results;
- to test 'normal' daily mortality, to ensure early detection of infection in establishments epidemiologically linked to an outbreak.

Surveillance to demonstrate freedom from HPAI H5N1 virus infection

All the member states of OIE have a responsibility to notify an outbreak of HPAI to OIE through an immediate notification system. Declaration of 'Disease Free Status' will be after 90 days on non-occurrence of disease through intensive surveillance. In order to demonstrate absence of infection in preceding 12 months in susceptible poultry population requires the support of a *laboratory to* undertake identification of HPAI infection through virus detection and antibody tests.

Case definition:

Absence of antibody and virus through antibody tests and virus detection tests

Materials and equipment required

- Swabs (tracheal, cloacal)
- Faecal swabs
- Serum
- Lab. Submission forms
- Laboratory techniques
- Rapid Antigen Detection diagnostic kits
- HA/ HI
- ELISA

- RT-PCR
- Statistical software

Team composition:

- Laboratory technicians
- Veterinarians, para-veterinarians

Laboratory analysis

Laboratory technologist at NCAH

Data Management

Epidemiology Unit, NCAH

Data analysis

National Epidemiologist with the help of Statistician

3.1 Surveillance area:

Targeted or prevalence directed sampling will be done in following areas:

- Poultry farm/birds in previously infected places
- Sentinel birds/Restocked birds
- Poultry farms/bird in the high-risk areas (Samdrup Jongkhar, Sarpang, Chukha, Samtse, bordering Gewogs of Dagana, Pemagatshel, districts bordering India).
- Farms with poor biosecurity measures in place.
- Roosting ground of migratory birds.
- Places with high water bird population
- Farms/premises that utilize contaminated water

3.2 Surveillance period:

Intensive surveillance should be carried out to prevent further spread of the disease from infected premises and prepare for demonstration of freedom from infection. Declaration of 'Disease Free Status' will be after 90 days on non-occurrence of disease. This should be followed by an active surveillance to demonstrate freedom from infection in preceding 12 months.

3.3 Sampling methods.

- Multi-stage sampling
- First sampling frame is villages (simple random sampling).
- Second sampling frame is the household/ farms (SRS).
- Stratified random sampling of birds
- Water fowls/ Ducks; domestic chicken; commercial flocks & wild migratory waterfowls).
- Proportion of samples for each stratum will be decided after more consultation.

3.4 Sample size

Sample size will be calculated to detect 1% of positive flocks at 95 % CI at 5% significance level.

Sample type

Cloacal/tracheal/wet droppings/serum will be collected from different species of birds in different location as described above

Laboratory testing:

Rapid antigen detection test in the field; Virus isolation followed by HA/HI test and RT-PCR, ELISA test shall be done to prove absence of infection.

Report

Reports will be submitted to OIE to gain disease freedom status.

7.3 SOP FOR CULLING AND DISPOSAL

Purpose

The purpose of this SOP is to ensure the implementation of culling and disposal for the control of HPAI H5N1 and AI H7N9 outbreak that can be carried out smoothly and successfully within the shortest possible time and re-establish Bhutan's HPAI-free status.

Stamping out method of the disease control strategy is to be adopted for the HPAI outbreak as it is the most acceptable and effective control method for eradication. This control measure needs to be accompanied by strict quarantine and control measures, decontamination of infectious material on infected premises (IPs), targeted tracing and surveillance, and enhanced bio-security by all levels of the poultry production and processing farms.

Scope

This SOP covers the guidelines and steps for humane culling and safe disposal of poultry, poultry products, feeds, litters and dismountable poultry sheds including other infected materials by the culling and disposal team.

Target/User: Culling and Disposal Team

Composition of the team

The team shall be composed of the following members:

- ***Team leader:*** Regulatory and Quarantine Inspector/ Officer (Livestock)
- ***Technical Assistants:*** Two BAFRA Livestock Inspectors. One of them in the team shall act as animal welfare Inspector
- ***Record keeper:*** Concerned Livestock Extension Agents

Cullers:

- Two hired and trained personnel as a culler
- Two bird catchers (hired labourers) team).
- Gas operators: One BAFRA Inspector for operating CO_2 gas when used as stunning (optional)
- Two hired labourers for disposal of culled birds.
- Five hired labourers for digging disposal pits at each disposal site
- One police personnel for the smooth functioning of the 3-D operation.

Note: The number of 3-D teams required shall be based on the size of the outbreak and protection zone which shall be decided by IOC.

Materials and Equipment Required

A. Personal Protective Equipment

Each culling member must be provided with a set of a Personal Protective Equipment (PPE) as protective measures to prevent infection which include:

- A coverall (with hood and boots)
- An N-95 respirator

- Goggles
- Outer glove– (Nitrile)
- Inner gloves – (Vinyl)
- Shoe covers
- A plastic apron that comes in a pouch
- A Respirator Fit Test Kit
- Additional good quality gloves for the coop dismantling and culling persons.

Each person should be provided with an adequate number of PPE sets depending upon the area of operation based on the Geographical terrain. These items should be worn at all times when they are in the infected birds or on the infected premises.

B. Disinfectants

Each culling group should be provided with each set of following disinfectants:

– A 5 kg container of Virkon® S disinfectant
– Sanitary Cloth Disinfectant Wipes (160-count canister of PDI HB Sani Cloth) or an antiseptic wash shall be used if it is not available.

C. Personal cleaning and disinfection supplies

– A scrub brushes (2 each for each group) for removing dirt and other particles before using disinfectants.
– Two sprayers (10 liters capacity) meant for dispensing Virkon® S or other disinfectant
– Four bars of soap that you can use to wash your hands and face.
– A plastic basin each for a foot bath.
– A large bucket that can hold approximately 20 liters – use to mix the Virkon® disinfectant powder with water.

D. Biohazard control materials

– A few alcohol cotton pad, 70% ethanol - these are generally used to wipe hands after removing PPE
– A red biohazard bag (two numbers each) for placing used PPE in as you remove it
– PDI HB Sani Cloth viricidal wipe (one packets each)
– Eye wash
– First aid kit.
– Flash light

E. Culling equipment

– Each culling group should have the following set of equipment:
– Long-handled fishing nets;
– Heavy-duty trash bags;
– Small plastic bags;
– Roll of paper towels;
– Ziplock bags;
– Clipboard, water-proof notebook and pen;
– Roll of duct tape.

F. Disposal materials and equipment

The following general equipment and supplies are required:

- Spade, crowbar, pickaxe and Shovels;
- Calcium hydroxide;
- Waste Containers bag;
- Roll of black plastic (2 rolls)
- Heavy-duty trash bags;
- Small plastic bags;
- A Roll of duct tape;
- Roll of paper towels;
- Ziplock bags;
- Fire extinguisher (portable size – 1 no.).
- Disposal pit

Culling Procedures

A. General consideration

All birds in the infected premises will be subjected to stamping out once a clinical disease or evidence of active HPAI virus infection is confirmed. In addition, pre-emptive culling will be done in protected zones (on high risk premises such as dangerous contact premises–DCPs, contiguous premises-CPs and suspect premises-SPs) established through epidemiological assessment.

Plan for culling should be established based on the information and situation of the infected premises by the team leader. The culling team must be led by the team leader and shall determine the site for the culling and disposal of poultry. To minimize the handling and reduce stress on the poultry, culling should be done at the affected farm, or as close as possible where they are housed. The welfare inspector should ensure the welfare aspect of the poultry during culling. The team leader must make sure that the area chosen for culling is not in the view of neighbours or other crowds, and that only individuals involved in culling operations are in the area. Clearing the culling area of unnecessary bystander not only makes the process more efficient, but also limits the number of people exposed to blood, feathers, other poultry parts, and potentially contaminated equipment and surface areas.

B. Culling procedure

Identify and establish a proper site outside and close to periphery of the culling and decontamination line for putting on PPE, unloading materials and equipment required for culling and decontamination.

Where the infected area is accessible by road, a culling and disposal crew vehicle shall be parked at this site. Take off all materials and equipment from the vehicle.

Before entering the infected premises

- Assemble the team and organize into groups as per the specific tasks to be performed in the orderly manner and distribute the materials and equipment to each member.

- The Team Leader shall then provide necessary briefing to all culling and disposal groups.
- Put on PPE as per the SOP for use of PPE before crossing the culling, cleaning and disinfection line (protected zone).

- Culling team shall be divided into groups – the first group should start culling in the infected farms and other group(s) shall start culling from the periphery of protected zones and move towards the centre of the infected area.
- Once personnel have entered premises, they may not cross back over the culling and decontamination line for any reason without removing and properly disposing of all PPE and proper personal disinfection.
- Groups identified for culling the infected farms shall only come out after completing the culling and disposal.
- In the infected premises it is preferable to cull the infected birds first followed by birds in contact with infected birds, and finally remaining birds in the flock.

Catching birds:

- Chicks are easily caught under the heaters and are killed by neck dislocation and put in the plastic garbage bins. If they are to be culled by CO2 then they are to be transferred into gassing bins.
- Broiler chickens on the ground are driven, using movable Hessian wall to the catching area where they are caught.
- In case of caged birds remove one bird at a time and kill by neck dislocation as described earlier. If the CO2 method is to be used remove 3 or 4 birds from cages and carry them by legs to the gassing bins.

Culling methods – decide on the appropriate method to be used and follow the procedures described below.

Culling Method

The method chosen for slaughter of poultry must be safe, humane and efficient. Any of the following two methods will be used for culling of birds depending on the population size.

A. Neck Dislocation

This method will be adopted for culling poultry in small size backyard farms and village chickens (approximately below 1000 birds). Neck dislocation is considered a humane method of poultry euthanasia and is the most common method for killing birds. The neck dislocation can effectively carry out using hands or with burdizzos, forceps, or pliers.

Following steps to should be followed for a neck dislocation:

- Place the bird breast-down on a flat surface (or hold the bird against your hip).
- Use one hand to hold both wings behind the bird's back.
- Using your other hand to hold the head between your middle and ring fingers, with the middle finger on the back of the chicken's head.
- 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.
- Hold the bird in this position until the flapping stops.

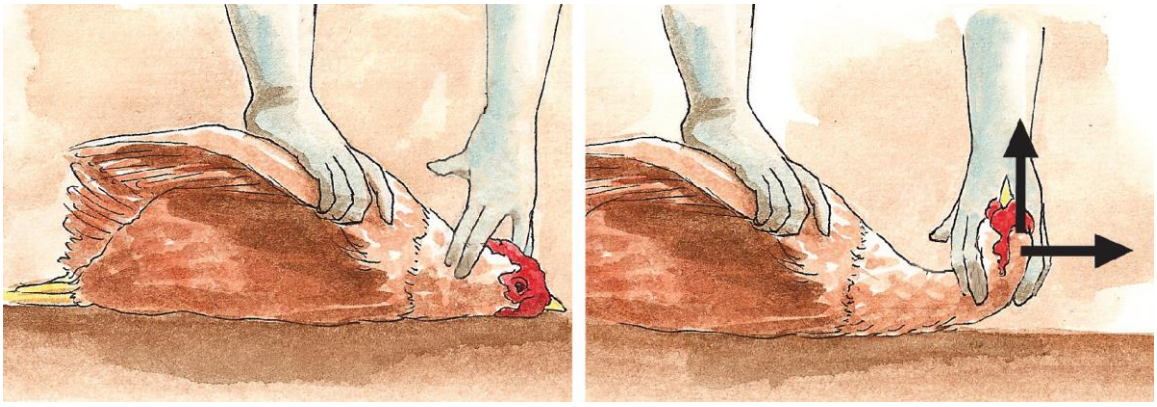


Figure 1: Demonstrates the neck dislocation method

Others might be more comfortable using this grip:

- Direct the bird's head toward you. Grasp the bird's head with a handshake grip.
- Place your thumb behind the head at the base of the skull, allowing the remaining fingers to extend under the throat.
- Hold the bird's feet with the other hand.
- 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.
- Be careful to stop pulling when the spine separates or the head may be pulled off.
- The bird dies immediately when the spine separates.

Figure 2: Demonstrates another way of neck dislocation.

Please keep in mind that the neck dislocation is preferred for water birds instead of CO2.

Disposal of Birds

Safety, biosecurity, and compliance with environmental regulations are the primary issues to be kept in mind for disposal of large volumes of HPAI-affected material. Burial is the primary method of disposal for birds, eggs, litter, refuse from cleaning and disinfection activities, and for other potentially contaminated material.

Ideally, birds should be disposed of on site by burial. Alternatively, if no approved site is identified, they can be transported and disposed of elsewhere. The dead birds have to be buried within 24 hours of death.

A. General Procedures:

- To prevent virus spread, you must seal the containers (disposal or gassing containers) so they do not leak liquids or release debris such as feathers, faeces or litter materials. The packed birds are further put into the large containers like gunny bags or biohazard materials for easy and faster transport.
- Carefully inspect the container for any breaches, holes, large cracks or sharp edges.
- Avoid puncturing any plastic bags with your feet or tools. Always inspect the plastic bags to ensure it is not damaged. Small plastic holes can be repaired easily with tape.
- Plastic openings must be sealed using duct tape. Similarly, container opening must be sealed with plastic and duct tape from the outside of the containers.
- When the container is full, or meets the maximum weight limit, thoroughly wet the birds with Virkon® S. This will decrease virus shed and also minimize feathers from flying.

B. Burial

The first choice, by far, would be on-site burial. Identify the site for burial such that wild animals or dogs cannot access the birds once they are buried. Dig one or more pits to bury all the birds on the property. Considerations include the amount to bury, site availability, soil type, water table, nearby wells or ponds and digging equipment available.

Burial site selection

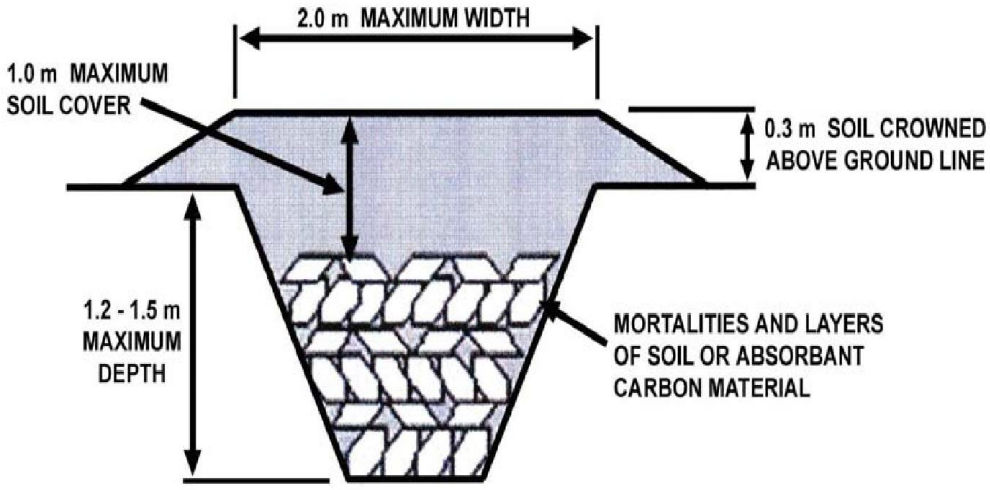
Important considerations for burial site selection include:

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 neighbours 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 for construction of diversion banks if required. Similar banks should be constructed to prevent any liquids escaping from the burial site.

Fencing is necessary to exclude animals until the site is safe for use. If government land is not available leasing/compensation for private land for the disposal pit for the next 3 years should be considered after negotiation depending on the emergency situation. In city area if the disposals of the birds are undertaken, the selection of the site should be done jointly with the city corporation and the necessary approval sought from the competent authority.



Burial pit construction

- The dimensions of the burial pit will be determined by the equipment used, site considerations and the volume of material to be buried.
- 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. The dimension of the pit will be decided by the number of birds to be disposed and the area of culling including the Geographical terrain.
- The number of birds can be doubled, each meter deeper the pit is made (3-6 meters)
- 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 and 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 odours and assists in absorbing the fluids of decomposition. After pit subsidence it will be necessary to replace any topsoil not utilized during pit closure. The refilling of the pit should be undertaken as and when required when the compression of the soil leads to space in the pit.
- Disinfectants are needed to be sprayed on equipment used and, on the pathway, used to take carcasses to the pit. The way to dispose off PPE, tools and bird carcasses and bird parts may be different in each situation or location.
- The burial pit should be located away from human and animal living areas and water–including wells, lakes, ponds or rivers.
- The burial pit 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.

C. Burning of carcass and infected materials

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. If you are burning carcasses or used PPE or other contaminated tools, keep the following in mind:

- Carcasses may be burn 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 always watches the fire . To make sure that enough fuel is used and that any carcasses or bird parts that fall off the fire are replaced again.
- The ashes can be buried as described in the section on burial above.
- In case of low-quality poultry sheds/coops where the scrapping of the litter materials is difficult, the coops should be dismantled and burnt on the site on the same day along with the litter materials in the infected areas. The compensation for the coops should be provided based on the assessment of the cost by the culling team and compensation committee.
- The bamboo baskets for enclosing and keeping the brooders poultry in the infected areas should be burnt and those in the buffer areas should be disinfected

In order to reduce the cost for the construction of pit and the disposal of the carcass alternative measures on the use of carcass incineration using the portable incinerator should be explored depending on the situation.

D. Protection of disposal pit

All the disposal pit should be properly fenced using the iron poles and barbed wires depending on the field situation. In case of remote areas, the use of wooden poles and barbed wires may be explored to reduce the cost of fencing.

Disposal of Infected Material

- Eggs and contaminated feed shall be buried along with other infectious materials at the site.
- Similarly, manure, litter, feather and poultry feed have to be buried. Litter may also be burnt if quicker decontamination is desired.
- Equipment and items that cannot be disinfected effectively have to be collected in a disposable bag and have to be burnt/incinerated.

Steps to be followed after Culling and Disposal

1. Culling and disposal team members should remove PPE and place them in trash bag, which are to be placed in biohazard plastic bags before crossing over the culling and decontamination line.
2. By the end of each workday, culling and disposal team members shall dump all the used PPE and other infectious materials.
3. All shall disinfect shoes, thoroughly wash hands at the wash station and sanitize your hands.
4. All tools and other equipment must be cleaned and disinfected before being brought across the culling and decontamination line
5. All personnel must disinfect their feet by dipping them in the footbath before leaving the place.
6. Similarly, all parts of vehicles (especially tyres) must be disinfected at culling and decontamination line.
7. Once the personnel protective equipment has been removed, designated personnel must disinfect personal footwear.
8. Personnel may not re-enter the infected premises without following the requirement for entering the infected premises.

Personal Safety

1. All individuals involved in culling operations should be provided with appropriate PPE and training on how to properly use them.
2. All should be treated with appropriate antiviral drug before entering infected area.
3. It is recommended that, if possible, all people exposed to infected chickens should be monitored by local health authorities for at least 7 days.
4. If symptoms of avian influenza are detected, there should be a clear way to report this information to local health officials. These symptoms include:

- Fever over $38^{o}C$
- Sore throat or cough
- Respiratory distress or failure

7.4 SOP FOR DECONTAMINATION

Purpose

The purpose of developing this SOP is to ensure that all decontamination procedures are carried out smoothly, effectively and successfully post disease outbreak.

Decontamination means removal or neutralization of infectious agent (H5N1 virus) through process of cleaning and disinfection. The purpose of decontamination is to ensure that live HPAI virus does not remain and re-emerge on the premises after depopulation of birds. Thus, cleaning and disinfection is a vital component of the decontamination team.

Scope

This SOP describes guidelines and steps to be followed for effective decontamination by the team.

Users/Target: Decontamination team

Composition of the team

- Supervisor: Regulatory and Quarantine Officer (Livestock)
- Assistants: Two BAFRA Livestock Inspectors
- Hired and trained personnel for cleaning and disinfection = 2 in each decontamination group.

Materials and Equipment Required

A. Personal Protective Equipment

Each member shall be provided with adequate protective measures from infection by means of Personal Protective Equipment (PPE) which include:

- A coverall (Enviroguard with hood and boots)
- An N-95 respirator
- Goggles (chemical splash)
- Outer gloves – (Nitrile, Size 10, 11-mil)
- Inner gloves – (Vinyl, 4 mil)
- Shoe covers (DuPont Proshield III)
- A plastic apron that comes in a pouch
- A Respirator Fit Test Kit (with Bitrex solution)
- Hard hat with face shield
- Gumboots

Multiple sets of PPEs will be necessary to allow for workers to take breaks. All the 3D members involved in the operation should use gumboots and thorough disinfection while moving from one house to the other.

B. Disinfectants

Different disinfectant for various materials should be procured as provided in the Table I and II.

C. Decontamination supplies

- Hand-operated and power sprayer (3000 PSI) used to dispense Virkon®S or other disinfectant.
- Numbers of 5meter hoses
- Adequate number of sufficient capacity water tank (500 litre)
- 1 rake lawn and 1 rake gravel
- 1 barrier/security line tape – 50 meters.
- 1 shovel flat-long handle
- 1 scoop shovel
- wheelbarrow
- 2 trash containers
- 1 roll duct tape
- 2 10 ft. ropes
- 2 regular brooms and 2 whisk brooms and dustpans
- Scissors
- 2 rolls of paper towels
- Alcohol wipe
- 2 heavy tie down straps
- 1 box of large and 1 box of small plastic bags
- Plastic tie downs
- Foot bath with tray and mat
- Sharp's container

D. Other supplies required

- Maintenance tools (screwdrivers flat and Phillips, hammer, adjustable wrench, crowbar, and scrapers)
- Masking tape

E. Personal cleaning and disinfection supplies

- A scrub brush for removing dirt and other particles before using disinfectants.
- Four bars of soap that you can use to wash your hands and face.
- A plastic basin that you can use to create a foot bath.

F. Biohazard control materials

- A few alcohol pads, 70%ethanol - these are generally used to wipe your hands after removing your PPE
- A red biohazard bag for placing your used PPE in as you remove it
- A container with a sprayer nozzle.
- PDI HB Sani Cloth viricidal wipe (one large, individual wipe)
- Eye wash
- Ice chest First Aid Kit.
- Flashlight

These items should be worn at all times when they are near the infected birds or in the infected premises.

Method - Decontamination

Adequate cleaning and disinfection of infected premises requires planning before the depopulation occurs as well as work after depopulation to effectively remove the virus.

Particular attention should be paid to the decontamination of litter as the AI virus can survive up to 35 days at 4ºC in faecal material and 7 days at 25ºC. As such, it is necessary to quickly disinfect the surface of the litter and burn or bury them. Contaminated fomites, such as clothing, footwear, crates, feed sacks, egg fillers and other equipment should be decontaminated, if possible, or destroyed. People should undergo personal decontamination procedures. Decontamination should include standard insect vector and rodent control to minimize mechanical spread of the agent to nearby premises.

A. General consideration

- There should be a detailed property assessment starting with making a map and marking in the location of electrical and water lines, drains, effluent run off.
- Identification of a decontamination site - Cleaning and disinfecting activities of infected premise should be limited to areas inhabited by or exposed to poultry. The team leader should evaluate each premise with this objective in mind and make a reasonable determination as to whether materials can be effectively cleaned and disinfected or should be discarded. In scavenging birds all the surrounding premises of the house including the kitchen garden, where the birds have scavenged, should be disinfected adequately in the infected areas

Materials fall into three categories:

- Structures: Rooms and pens/cages;
- Clutter: Items that are not structures for housing birds and require judgment as to whether they can be cleaned and disinfected effectively or must be discarded; and
- Trash: Items that impede the cleaning process and should be discarded.

B. Decontamination procedures

Preparation for decontamination

Identify and establish a proper site outside and close to periphery of the culling and decontamination line for putting on PPE, unloading materials and equipment required for decontamination. Where the infected area is accessible by road, a decontamination crew vehicle shall be parked at this site. Take off all materials and equipment from the vehicle.

Before entering the infected premises

Assemble the team and organize into groups as per the specific tasks to be performed in the orderly manner and distribute the materials and equipment to each member. The Team Leader shall then provide necessary briefing to all decontamination groups. Put on PPE as per the SOP for use of PPE before crossing the culling and decontamination line (protected zone).

- Decontamination team shall be divided into groups – The first group should start decontamination in the infected farms and other group(s) shall start decontamination from the periphery of protected zones and move towards the centre of the infected area.
- Once personnel have entered premises, they may not cross back over the culling and decontamination line for any reason without removing and properly disposing of all PPE and proper personal disinfection.

- Groups identified for decontamination of the infected farms shall only come out after completing their task.
- The decontamination team should allow the culling and disposal team to complete their task and then only start their operation.
- Prepare the select appropriate disinfectants as recommended in Table I and II.
- It is important to wear PPE when mixing disinfectants like Virkon®S disinfectant because it can irritate the skin and eyes.
- The following steps should be taken in order and under site supervisor direction.

3. Preliminary disinfection

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 preliminary disinfection 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.

The important area, structures, materials and equipment for cleaning and disinfection *inter alia* include:

- Poultry sheds and around the houses and as soon as the birds removed
- Feed storage area
- Poultry carrying baskets and bags.
- Culling sites
- Disposal sites
- Hatcheries
- Poultry processing facilities
- Watering and feeding troughs including other fomites
- Access roads and pathways used for moving poultry and poultry products including other risk goods (fomites).
- Vehicles

Disinfection should be repeated up to two times a day (morning and evening) from zero day to 14 days. Disinfection Virkon® S is treated as the best disinfectant for HPAI virus but other locally available disinfectants are also effective against it. 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 disinfected with appropriate disinfectant allowing sufficient recommended contact time as per the Table I and II.

4. 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.

All structural surfaces must be cleaned of any litter, feathers, dirt, or other contaminated materials. Roof areas must also be cleaned.

For pens and cages on the ground, the team will remove all contaminated material and bring the surface as close to level as possible.

The next step is a wash down with a low-pressure sprayer using a detergent or bleaching powder.

Around and under trees, the roost area will be trimmed and perch areas that are contaminated with faeces or feathers will be cleaned.

Fences should be thoroughly cleaned and disinfected as the tops of fences may be used for roosting by free-roaming poultry and wild birds, fences and fencing material can be contaminated with feathers and faeces.

If the facility has significant evidence of rodent activity, extermination should be done prior to starting the cleaning and disinfection effort.

5. Full scale disinfection

- Disinfectant to be sprayed in the following order: roof, walls and finally the floor.
- Inspection must be carried out to ensure that everything has been completed- repeat clean -up and disinfection if there is doubt.
- Spray another round of full disinfection from 48 hours to 14 days later.
- Final disinfection before restocking should be carried out.

6. Decontamination of equipment used for decontamination

The other consideration is the decontamination of contaminated equipment used. The primary concern would be for anything used during stamping out. This would include items like:

- CO_2 tanks,
- Gassing containers,
- Excavators,
- Back hoes,
- Torch.

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, egg trays are on the contaminated site they must be decontaminated before leaving the premise. Particular attention needs to be paid to mats under the 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.

7. Personal decontamination

- The following procedures will apply to ALL personnel before leaving an infected area any quarantined area which is grossly contaminated with the disease organism.
- Culling and disposal team members walk to the cleaning and disinfection line and remove PPE and place it in a trash bag, which are to be placed in a biohazard plastic bag.
- Industrial hard hats must be scrubbed and set aside.
- Hands must be washed in disinfectant and scrubbed.
- Warm soapy water is recommended for washing face, hair, skin, etc. Alternatively, the pH of the washing solution can be raised (by adding sodium carbonate) or lowered (by adding citric acid) to enhance antiviral action.
- Hair should be washed/sponged down with a shampoo.
- Disposable gloves must be decontaminated before discarding and reusable gloves are to be decontaminated before reusing.
- Plastic overalls - use a sponge or low-pressure pump and wash the overalls from top to toe to remove gross material paying particular attention to the back, under the collar, zip and fastenings and the inside of pockets.
- Boots and shoes should be scrubbed down, particular attention being paid to the sole.
- The person then walks across the area, washes feet in a footbath, changes into clean overalls and street shoes and leaves directly without re-exposure to contaminated areas.
- The plastic bags containing used overalls and other articles are sealed and given a second wash down in disinfectant and then either buried/burnt or taken for cleaning. These garments should be autoclaved or treated as contaminated clothing in a hospital laundry.
- On returning to home or lodgings, the person should have a long hot bath or shower.

Personal Safety

All individuals involved in decontamination operations should be provided with appropriate PPE and training on how to properly use them. All should be treated with appropriate antiviral drug before entering infected area.

It is recommended that, if possible, all people exposed to infected chickens should be monitored by local health authorities for at least 7 days. If symptoms of avian influenza are detected, there should be a clear way to report this information to local health officials.

Protocol for Mixing Virkon® S

Safety or protective gear is required when mixing Virkon S. Assigned individuals must wear a face shield or safety goggles, a dust mask, and rubber gloves. Mix the solution in a separate, well ventilated room (if possible), or outside. Restrict the number of people in the mixing area. Follow the requirements for handling and storage of disinfectant.

A. Equipment and Supplies Needed for Virkon S

1. Safety equipment needed
- Face shield or safety goggles;
- Rubber gloves;
- Coveralls; and
- Dust mask.

2. Supplies needed:

- 1.0, 2.5, or 5gallon plastic container with locking lid;
- Funnel; and
- Plastic measuring spoon or scoop (a scoop is included with the Virkon S).

B. Procedure for Mixing Virkon® S

Reseal the container holding Virkon® S powder.

- Pour Virkon® S solution into the 1.0, 2.5, or 5gallon plastic container using a funnel. Close the container tightly.
- Dispose of solution after seven days or when it begins to change from yellow to clear.
- Wash hands and any other areas where the solution or powder may have come in contact with the skin. Clean the mixing area.

C. Procedures for Handling Virkon® S Disinfectant

1. Store powder tightly in a closed plastic container in a cool, dry place. Ensure that the area where Virkon® S is stored is secured and cannot be accessed by authorized persons.
2. Follow instructions on the label for disposal.

Table 1: Recommended Disinfectants and Concentrations

Disinfectant	Form and final concentration	Contact time and effectiveness
1. Soaps and detergents		Leave in contact 10 minutes.
2. Oxidizing agents		
2a. Sodium hypochlorite	Liquid (conc. liquid (10-12% available chlorine), dilute to final 2-3% available chlorine (1:5)	Not good for organic materials. 10-30 minutes contact.
2b. Calcium hypochlorite	Solid or powder, dilute 2-3% available chlorine (20 g/litre powder, 30g/l solid)	Not good for organic materials. 10-30 minutes contact.
2c. Virkon®S	2% (20 g/litre)	10 minutes. Excellent disinfectant
3. Alkalis		
3a. Sodium hydroxide (Caustic soda- NaOH). Do not use with aluminum and like alloys	2% (= 20 g/litre) 300ml per Sq. mtr.	10 minutes. Do not use in presence of aluminum
3b. Sodium carbonate anhydrous (washing soda) (Na2CO3. 10 H20)	4% (40 g/litre) from powder 100 g/l from crystals	10 minutes. Recommended for use in the presence of organic materials as above. 30 minutes
4. Acids		
4a. Hydrochloric	2% (20 ml/litre)	Corrosive, use only when better not available.
4b. Citric	0.2% (2 g/l)	30 minutes, safe for clothes and body

		decontamination
5. Formaldehyde gas	Special generation required	15-24 hrs. Toxic, only if others cannot be used.

Table 2: Recommended Disinfection

Particular	Disinfectant/chemical/procedure
Dead birds/Carcasses	Bury or burn
Animal housing/ equipment/ cages	Any of the followings: 1. Virkon® S 2. Sodium hypochlorite 3. Calcium hypochlorite
Humans	Soaps and detergents.
Electrical equipment	Formaldehyde gas
Water tanks	Sodium hypochlorite
Ponds used by poultry/ducks	Sodium hypochlorite
Feed	Burial or burning
Effluent, manure	1. Bury or burn, 2. Acids 3. Alkalis
Human housing	1. Soaps and detergents 2. Sodium hypochlorite 3. Calcium hypochlorite 4. Virkon® S
Machinery, vehicles	1. Soaps and detergents 2. Calcium hypochlorite
Clothing	1 Soaps and detergents 2. Sodium hypochlorite 3. Calcium hypochlorite 4. Virkon® S 5. Alkalis
Aircraft	1. Soaps and detergents 2. Virkon® S
Footbath	1. Dettol

7.5 SOP FOR VEHICLE DISINFECTION

Purpose

- To minimize the further spread of disease from the affected area
- Neutralize the HPAI virus through process of spraying the appropriate disinfectant/ chemical

Scope

- This SOP outlines the general principles and steps to be followed for effective disinfection of the vehicles moving out of the affected area by the team.
- Also outlines the general principles and steps to be followed for the setting up of foot dips

Users/target: Disinfection and movement control team

Composition of the team:

- RQO (livestock) team leader,
- BAFRA livestock inspector
- Hired and trained personnel for cleaning and disinfection

Materials and equipment required

A. Personal protective equipment

- Each member shall be provided with adequate protective measures.
- Respirator
- Gloves (latex and hard)
- Shoe cover
- Rubber boots
- Plastic apron

B. Disinfectants and detergents

Bleaching powder at formulated concentration (see annexure)

C. Disinfection supplies

- Hand operated/electrically operated power sprayer used to dispense the disinfectant.
- Adequate rolls of hoses
- Adequate number of sufficient capacity water tank
- Sieve
- Bucket
- Mug
- Shovel
- Crowbar
- Electrical wires and appliances
- Water pipe

- Tent
- Canopy umbrella
- Plastic chair and table
- Adequate fuel and lubricant
- Foot bath with tray and mats
- First aid kits
- Heating appliances
- Flash lights/torches

D. Other supplies

- Maintenance tools (screw drivers, wrench set, pliers)
- Masking tape

Methods

- Identify and establish **strategic** sites for disinfection of vehicles and setting up of foot dips
- Make sure all the prerequisites are made available, so not to compromise the working environment and quality of works.
- Set up the disinfection equipment on both sides of the road. A provision shall be made to cover underneath the vehicle as well.

The decision on setting of vehicle disinfection at exit points shall be based on the findings and recommendations of disease outbreak investigation team after conducting risk assessment. The decisions involve following:

- Selecting the strategic point for vehicle disinfection point (either One exit or two exit points)
- Duration of the vehicle disinfection
- Methods of vehicle disinfection (Vehicle dip or spraying)
- Number of men power for vehicle disinfection.
- Selection of disinfectant

General Considerations:

The method, including type of detergent/disinfectant applied, and level of decontamination required should be based on a risk assessment. For vehicle disinfection, either vehicle dip or power spraying methods shall be adopted based on the suitability, practicality and effectiveness as recommended by DO-IT. Vehicle dip method of disinfection is preferred over vehicle spraying method when there is high flow of vehicle (traffic).

Procedures for Vehicle Disinfection:

1. Preparation for vehicle disinfection point

The vehicle disinfection site should be strategically selected to cover all vehicles coming from potentially contaminated areas to prevent the spread of infection. For the selection of site, the team must consider level of disinfection required, scale/usage of the facility (for design and/or establish), chemical type (type of disinfectant), resources including personnel, PPE,

equipment, water supply, environmental impacts and mitigation (e.g. liquid and PPE waste disposal, chemical spill), and types and sizes of vehicles to be disinfected.

2. Disinfection Procedure:

- A sign board must be placed about 100 meters ahead of the vehicle disinfection point with the message "You are approaching vehicle disinfection point, drive slow".
- At the disinfection point, a signboard with the message "Stop vehicle for disinfection" must be erected.
- The personnel engaged in the vehicle disinfection must drone basic PPE
- The disinfection solution must be prepared with proper concentration with proper recording.
- All vehicles coming out of the protection zone must be stopped for disinfection.
- Start spraying from the top of the vehicle and work towards the ground.
- Moving parts of vehicles or equipment e.g. wheels, tracks, tipper tray, buckets have to be moved during decontamination to access all areas
- Sufficient disinfectant must be applied to the surface of the vehicle which has a high risk of picking infection (tyre, front and rare side of the vehicle) and leave for appropriate contact time.
- A knapsack sprayer must be used to disinfect the interior space of the vehicle
- When vehicle dip is used, the whole tyre of the vehicle must be dipped inside the disinfectant and proper contact time must be ensured.
- The vehicle disinfectant solution should be changed regularly.
- Foot dip: A foot dip for the commuters must be placed at the strategic point with appropriate signage. The solution should be changed for the effectiveness.
- Vehicle driver and passenger: The team must ensure that the driver and passenger must mandatorily complete foot dipping procedure.

3. Recording:

- Record chemical use and inventory
- Record disinfectant solution concentration
- Record vehicle disinfected/daily
- Record people foot dipped/daily

7.6 SOP FOR QUARANTINE & MOVEMENT CONTROL

Purpose

The purpose of this SOP is to ensure that the implementation of quarantine and movement control measures in protected and surveillance zones are carried out smoothly, effectively and successfully to prevent and minimize the spread of HPAI virus from infected areas.

Scope

This SOP describes the guidelines and steps for implementing quarantine and movement control measures following an outbreak of HPAI. This will not apply for routine movement monitoring at other entry and strategic check posts

Target/User: Quarantine and movement control team

Composition of the team

- Team leader: Concerned BAFRA office
- Technical Assistants: BAFRA Livestock Inspectors (number to be determined based on the place and size of outbreaks and entry and exit points in the protected and surveillance zones).
- Record keepers: BAFRA Livestock Inspector (one each in all entry and exit points of protected and surveillance zone).
- One police personnel

Materials and Equipment Required

A. Personal Protective Equipment

Each culling member must be provided with adequate protective measures from infection by means of a set of a Personal Protective Equipment (PPE) which include: Coverall (with hood and boots)

- An N-95 respirator
- Goggles
- Outer glove– (Nitrile)
- Inner gloves – (Vinyl)
- Gum boots/Shoe covers
- A plastic apron that comes in a pouch
- A Respirator Fit Test Kit (with Bitrex solution)

B. Disinfectants

Each quarantine and movement control team should be provided with adequate quantity of following disinfectants:

- Soaps and detergents - humans
- Sodium hypochlorite – vehicles and machinery
- A 5 kg container of Virkon® S disinfectant – vehicles and machinery
- Dettol - foot bath

- Petrol, Kerosene and other lubricants.
- Portable Generator at each of the vehicle disinfectant points where there is no electricity supply.

C. Personal cleaning and disinfection supplies

- A scrub brushes (2 each for each group) for removing dirt and other particles before using disinfectants.
- Four bars of soap that you can use to wash your hands and face.

D. Biohazard control materials

- A few alcohol cotton pad, 70% ethanol - these are generally used to wipe your hands after removing your PPE
- A red biohazard bag (two numbers each) for placing your used PPE in as you remove it
- PDI HB Sani Cloth viricidal wipe (one packets each)
- Eye wash
- First aid kit.
- Flash light

E. Quarantine and Movement control

Each quarantine and movement control group should have following set of equipment:

- Power sprayer (3000 PSI) used to dispense Virkon®S or other disinfectant.
- numbers of 5meter hoses
- Adequate number of sufficient capacity water tank (500 litre)
- Continuous water supply
- Barrier/security line tape – 5 rolls of 50 meters.
- 1 roll duct tape
- rolls of paper towels
- Foot bath with tray and mat
- A large bucket that can hold approximately 20 liters – you will use this to mix the Virkon® disinfectant powder with water.
- Heavy-duty trash bags (20 nos.);
- Small plastic bags (50 nos.);
- Clipboard, water-proof notebook and pen (2 sets each);

F. Transportation

Depending on the number of de-population, disinfection and disposal sites there will be huge requirements for mobility. The number of vehicles required will be based on the decision made by the IOC.

Quarantine and Movement Control

Determine all possible entry and exit points around the periphery of protected and surveillance zones based on the map of disease outbreak zones declared by Incident Operation Centre based on the recommendation of the disease investigation team.

Establish only one or two entry and exit points from the protected and surveillance zones and seal all other entry and exit points in collaboration with RBP. Place appropriate sign boards and notice boards to inform the public about the quarantine and movement control measures in place.

Identify and establish a proper site outside and close to periphery of protected and surveillance zones for putting on PPE, unloading materials and equipment required for enforcing quarantine and movement control measures.

Where the infected area is accessible by road, a culling and disposal crew vehicle shall be parked at this site. Take off all materials and equipment from the vehicle.

Assemble the team and divide the team into separate groups for enforcing quarantine and movement control measures in the protected zone and surveillance zone.

Distribute the materials and equipment to each group. The Team Leader shall then provide necessary briefing to all respective groups on protocols to be followed for enforcement.

Put on PPE as per the SOP for use of PPE.

Quarantine measures will be imposed on the infected premises such that no movement of domestic animals including other risk goods from the protected zone shall be allowed.

Movements of manure and litter off these premises will be prohibited. The access of wild birds to sheds and water supplies will be restricted. Farmers shall be advised to prevent other species of birds entering the premises. Pets will be confined.

Persons present on the infected premises will be encouraged to restrict their movement as far as possible and in any event will be prohibited from visiting any other premises where poultry are kept. The team shall restrict the movement of vehicles and people in and out of the infected premises and divert highway traffic as far as possible within the protected and surveillance zones as directed by the National Incident Command Centre and Incident Operation Centre.

The team shall set up and manned continuously all entry and exit points entering the protected and surveillance zones. No person, animal or vehicle shall get entry into the protected and surveillance zones without prior permission from the team leader or his representative of the Quarantine and Movement Control Team.

Oversee all personnel involved in disease investigation, stamping out, decontamination operation follows complete protocols for entering and exit from these zones as laid down in the respective SOPs. All people entering these zones must follow proper disinfection procedures.

Personnel involved in the quarantine and movement control enforcement shall as far as possible restrict their entry into infected and surveillance zones. If necessary, permission should be sought from the team leader for entry into these areas.

All quarantine and movement control measures shall be lifted upon declaration of freedom from the HPAI and upon receiving official notification from the NICC and/or Incident

Operation Centre. The use of foot dip in the main entry and exit point in the infected areas should be placed and changed on a daily basis based on the decision of IOC. The detailed guidelines to be followed for quarantine and movement control measures are provided in Table 1.

Steps to be followed for exiting the quarantine and movement control duty

- The team members should remove PPE and place them in trash bags, which are to be placed in biohazard plastic bags before exiting the area.
- By the end of each work day, the team members shall dump all the used PPE, other potentially infectious materials including those seized ones.
- All shall disinfect shoes, thoroughly wash hands at the wash station and sanitize your hands.
- All tools and other equipment used shall be cleaned and disinfected at the end of day's operation.
- All personnel must disinfect their feet by dipping them in the footbath before leaving the place.
- Similarly, all parts of vehicles (especially tyres) must be disinfected at culling and decontamination lines.

Personal Safety

All individuals involved in culling operations should be provided with appropriate PPE and training on how to properly use them. All should be treated with appropriate antiviral drugs before entering the infected area. It is recommended that, if possible, all people exposed to infected chickens should be monitored by local health authorities for at least 7 days.

If symptoms of avian influenza are detected, there should be a clear way to report this information to local health officials. These symptoms include:

- Fever over 38^0C
- Sore throat or cough
- Respiratory distress or failure

Procedures for Releasing Quarantines on Infected Premises (IPs)

In order for infected premises to be eligible for release, the following conditions must be met:

- Cleaning and disinfection must have been effective and the premises must have been empty for 30 days following the completion of cleaning and disinfection. The absence of birds on IPs should be confirmed through visual inspection of the premises.
- If cleaning and disinfection are considered inadequate, the team will evaluate the premises to determine if further action, such as additional cleaning and disinfection or a holding period, is required. If no further action is deemed necessary, the premises can be considered eligible for release. If further action is required, the epidemiologist should also determine if the surrounding premises should be held from quarantine release pending action on the IP.
- If cleaning and disinfection was not possible, the premises must have been empty for 60 days.

Procedures for Releasing Quarantines on Contact Premises (CPs)

In order for a contact premises to be eligible for release, the following conditions must be met: All infected premises and contact premises within a one-kilometre zone must also be eligible for release. One of the following three circumstances must apply:

- Cleaning and disinfection must have been effective and the premises must have been empty for 30 days following the completion of cleaning and disinfection. (Premises with pet bird(s) under compliance agreement will be considered "empty" for quarantine release purposes); or
- If cleaning and disinfection are considered inadequate, an epidemiologist will evaluate the premises to determine if further action, such as additional cleaning and disinfection or a holding period, is required. If no further action is deemed necessary, the premises can be considered eligible for release. If further action is required, the epidemiologist should also determine if the surrounding premises should be held from quarantine release pending action on the IP; or
- If cleaning and disinfection was not possible, the premises must have been empty for 60 days.

Quarantine/movement control	**Protection Zone**	**Surveillance zone**
Movement out of susceptible birds	Prohibited	Allowed by permit from flocks with negative sero-surveillance; Waste to approved disposal. Vehicles, equipment to be disinfected.
Movement in of susceptible birds	Prohibited	Allowed by permit, subject to surveillance
Movement out of non-susceptible species	Allowed by permit, subject to disinfection	Allowed by permit, subject to disinfection
Movement out of litter and manure	Prohibited.	Prohibited
Movement out of equipment and feed	Allowed by permit, unless feed has been in contact with infected birds; subject to disinfection.	Allowed by permit, subject to disinfection.
Movement in and out of people	Allowed by permit, subject to disinfection.	Allowed, subject to disinfection.
Movement in and out of vehicles	Allowed by permit, subject to disinfection.	Allowed by permit, subject to disinfection.
Movement of fertile eggs	Prohibited.	Prohibited
Movement of table eggs	Prohibited.	Allowed by permit, subject to sanitization. Vehicles, equipment to be disinfected.

Movement of fresh/frozen meat and offal from susceptible birds	Prohibited.	Prohibited
Movement in of feed	Prohibited	Allowed by permit. Vehicles to be disinfected
Movement of abattoir waste	Prohibited.	Prohibited.
Movement out of dead birds	Prohibited	Prohibited
Movement out of horticultural and agricultural crop	Allowed.	Allowed.
Areas	Restricted area/vaccination zone	Controlled area/vaccination zone
General	Premises to operate a very high level of bio-security.	Premises to operate a high level of bio-security.
Movement out of susceptible adult birds	Allowed by permit,[2] from flocks with negative sero-surveillance; birds subject to immediate slaughter in CA under supervision at approved abattoir. Product subject to heat treatment at approved premises. Waste to approved disposal.[7] Vehicles to be disinfected.	Allowed by permit,[2] from flocks with negative surveillance; birds subject to immediate slaughter under supervision at approved abattoir. Product subject to cooking at approved premises. Waste to approved disposal. Vehicles, equipment to be disinfected.
Movement in and within of susceptible adult birds	Allowed by permit to an abattoir for immediate slaughter. Allowed by permit for restocking.	Allowed by permit. Vehicles, equipment to be disinfected.
Movement through of susceptible birds of all types	Allowed by permit. Birds not to be unloaded within RA.	Allowed by permit. Birds not to be unloaded within CA.
Movement out of day-old chicks	Prohibited unless exceptional circumstances exist and permit is approved by CVO[1] (eg if eggs sourced from outside CA; destination flock subject to quarantine and surveillance).	Allowed by permit if eggs sourced from outside CA. Vehicles and equipment to be disinfected.
Movement out of replacement birds (pullets, breeders)	Prohibited unless exceptional circumstances exist and permit is approved by IOC.	Allowed by permit; subject to disinfection of equipment and transport; quarantine and a high level of sero-

		surveillance of source grower flock
Movement out of litter and manure	Prohibited unless exceptional circumstances exist and permit is approved by IOC.	Allowed by permit. Vehicles to be disinfected.
Movement out of feed and equipment	Prohibited unless exceptional circumstances exist and permit is approved by IOC (e.g. if exposed to infected birds). Allowed by permit if not exposed to infected birds.	Allowed. Vehicles to be disinfected.
Risk enterprises, e.g. private avian laboratories, cull hen collectors, dead bird pick-ups etc (not processing establishments)	Prohibited unless exceptional circumstances exist and permit is approved by IOC.	Allowed by permit. See restrictions on movements and disinfection of risk materials, vehicles and equipment.
Sales, shows, pigeon races etc	Prohibited unless exceptional circumstances exist and permit is approved by IOC.	Prohibited unless exceptional circumstances exist and permit is approved by IOC. Allowed by permit for non-susceptible species.
To and from processing plants	Prohibited unless exceptional circumstances exist and permit is approved by IOC. If possible, processing plants should be kept out of declared RAs.	Allowed by permit. Poultry from the CA can be processed following on-farm inspection within the previous 24 hours. Vehicles to be disinfected. Equipment to be cleaned and disinfected at the end of the day. Poultry from outside the CA can be slaughtered subject to vehicle disinfection before leaving the CA.
Movement of fresh/frozen meat, offal and waste from susceptible birds	Allowed into or within RA. Allowed out of RA subject to heat treatment at approved premises. Waste to approved disposal. Vehicles to be disinfected.	Allowed into or within CA. Allowed out of CA by permit. Vehicles to be disinfected.

Movement of table eggs	Allowed into or within RA. Vehicles to be disinfected. Allowed out of RA by permit; subject to sanitization. Vehicles to be disinfected.	Allowed into or within CA. Vehicles to be disinfected. Allowed out of CA by permit. Vehicles to be disinfected.
Movement of fertile eggs	Allowed into or within RA. Allowed out of RA by permit; subject to sanitization of eggs, disinfection of equipment and transport, quarantine and surveillance of destination flocks.	Allowed into or within CA. Allowed out of CA by permit, subject to sanitization of eggs, disinfection of equipment. Vehicles to be disinfected.
Movement of egg pulp from plants, including on-farm plants	Allowed into or within RA. Allowed out of RA by permit, subject to heat treatment Vehicles to be disinfected.	Allowed into or within CA. Allowed out of CA under permit. Vehicles to be disinfected.
Control of domestic pets and poultry	All pets and poultry are confined.	All pets and poultry are confined.

7.7 SOP FOR MOVEMENT OF POULTRY PRODUCTS DURING OUTBREAK & POST OUTBREAK PERIOD

Purposes:

The purpose of this SOP is to facilitate and regulate the movement of poultry products (eggs, chicken) during the period of outbreak and post outbreak phase until the case is resolved under following situation:

- From surveillance zone of an outbreak areas to the market or non-outbreak areas;
- From non-affected areas to the market or to other non-outbreak areas through protection zone if the route of transport of consignment is available only through the protection zone.

This SOP will be applied only when there is no other alternative means of disposing of the poultry product and shall be applicable to poultry meat, eggs and feeds based on the request/ application of the farmers/traders and on a case by case basis. Otherwise, the Livestock Act 2001, Livestock Rules and Regulation 2008 and SOP on quarantine and movement control of NIPPP would apply.

Scope:

This SOP describes the guidelines and steps for implementing movement of poultry products during an outbreak and post outbreak phase of HPAI.

Target/User:

BAFRA, Quarantine and movement control team, poultry farmers/traders

Composition of the team BAFRA

- Regulatory and Quarantine Officer (leader)
- Two Regulatory and Quarantine Inspectors
- One hired labour

Materials and Equipment Required

- Knapsack sprayer
- Formaldehyde and Potassium permanganate
- Virkon
- Gloves
- Apron
- Mask
- Cartoon, appropriate container (the traders has to procure these materials)
- Seal

Steps

The BAFRA officials shall visit the concerned farms and verify the stocks based on directions of NICC/IOC/risk assessment team. They would verify the origin of the products, quantity, and type at the farm.

Eggs: all eggs intended for transport/sale has to be sanitized/fumigated using formaldehyde and potassium permanganate gas, cleaned the egg dry and then packed in a clean box in the presence of BAFRA officials. The box (cartoon) should be properly sealed and then stamped with BAFRA seal.

- Formaldehyde gas is produced by mixing 0.6 gram of potassium permanganate ($KMNO_4$) with 1.2 cc of formalin (37.5 percent formaldehyde).
- The eggs can also be disinfected or sanitized by spraying the chemical onto the eggs or by dipping eggs into a container containing chemical

Poultry meat: the chicken meat should be packed in an appropriate sealed container in the presence of the BAFRA officials and sealed.

The BAFRA shall record the products details including quantity and type and issue certificate/permits of the consignments. The BAFRA shall also send the details of consignments through fax to another BAFRA office (place of destination of consignment for cross checking).

On arrival of the consignment at the place of destination, the consignment shall be opened in presence of the BAFRA officials. After downloading the consignment, the packing container shall be decontaminated by the BAFRA.

7.8 SOP FOR USE OF PERSONAL PROTECTIVE EQUIPMENT

Purpose:

Personal Protective Equipment consists of specialized clothing or equipment worn by personnel involved in disease control activities for protection against infectious materials. This is critical in the event of Highly Pathogenic Avian Influenza disease outbreak, in protecting personnel involved in carrying out disease control measures.

Scope:

The document gives the guidelines to the use of PPE in an appropriate manner. In the event of a pandemic, the availability and appropriate use of PPE is critical in protecting the personnel involved. Disposable PPE should be used whenever possible, because the virus can remain infectious on garments for long periods of time and once used PPE should not be reused.

User:

All personnel involved in active disease control measures.

Manpower:

- Supervisor: The Commander of Incident Operation Centre
- Implementer: All personnel involved in active disease control measures.

Materials/Equipment:

- Coveralls / aprons
- Shoe cover/boots
- Respirators / face masks
- Face shield/ Goggles
- Hood/cap/hair cover
- Apron
- Gloves
- Disposable bag
- Hand wipe/alcohol mop

Procedure:

Note: 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 infectious materials. Wash your hands with soap and water before you begin, and remove watches and other non-smooth jewellery like bracelets.

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.

Putting on coveralls

Shoe cover: Secondly, put on the shoe covers. They should fit over your coverall feet, giving you another layer of protection to protect your shoes from contamination.

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 mould the nose area to the shape of your nose by pushing inward while moving your fingertips down both sides of the nosepiece.

Putting on respirators

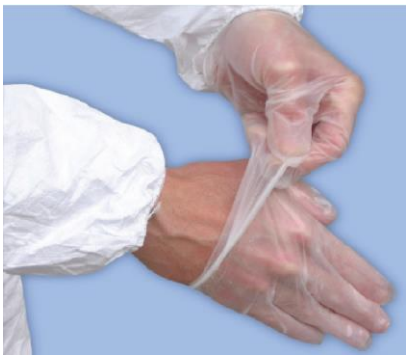
Goggles/Face shield: Put on the face shield/Goggles and then pull coverall hood/cap/hair cover over the head, the elastic should hold it in piece.

Wearing goggles and then pulling over the hood of the coveralls

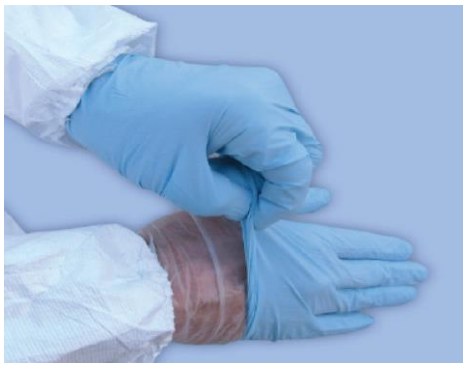
Aprons: Aprons are provided to fit over the coveralls. They are in a small packet that you will open up, place the apron over. Thee aprons will protect against splashes and prevents wetting your coverall.

Putting on aprons

Gloves: Put on the inner pair of gloves first (usually white or clear), and then the outer gloves which are usually be a different colour than the inner gloves and thicker. Pull them over the inner gloves. Pull the edge of the gloves over the cuff of your coveralls or gown, if possible.



Putting on inner gloves



Putting on the outer gloves

Now you are ready to enter the contaminated area

Procedure for Removing and disposing off PPE

- Open the germicidal wipe/alcohol mop and use it first on your outer gloves and then on your outer boots.
- Place it in the biohazard bag when done.
- Remove and dispose of your apron in the biohazard bag.
- Remove and dispose of your outer gloves in the biohazard bag.
- Unzip and roll down your coverall until it is inside-out, and then step out of it. Place the used coveralls into the biohazard bag.
- Remove and dispose of your outer shoe covers in the biohazard bag.
- Remove your goggles by pulling them up over your head. You should handle them by the headband or ear pieces. Place them in the biohazard bag.
- Remove your respirator by grabbing the top and then the bottom elastic bands and pulling them up over your head. Place the respirator in the biohazard bag.
- 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 biohazard bag.
- Close the biohazard bag by tying a knot at the top or otherwise tying it shut. The biohazard bag should be placed at a designated location so that it can be collected and burned or buried.
- Wash your hands and forearms with soap and water.

How to Wash Your Hands Correctly

- Wet your hands with water and apply soap. Use clean, running water.

- Rub hands together to make lather and scrub all surfaces.
- Continue rubbing hands for 20 seconds.
- Rinse hands well under running water
- Air dry your hands, or use a towel.

When to Wash Your Hands While Using PPE

- Before putting on your PPE
- Before putting on your gloves or respirator again after taking a work break
- Before and after changing your respirator
- After taking off your gloves and the rest of your PPE, and placing them in the waste bag
- Any other time your ungloved hands have come into contact with potentially infected animals, equipment or surfaces.

Important Facts to Remember when Using the PPEs

- All of the PPEs supplied are disposable and are designed for use one time only.
- None of the supplied PPEs should be reused or washed for reuse – reuse could result in infection of you or someone else.
- Do not use, or provide N-95 respirators to others, without instruction on the health risks associated with them. For example, workers with poor lung function may not be able to wear these respirators.
- If you can, do a fit test to make sure no particles can get through.
- N-95 respirators should not be hung around your neck when working, always wear them when working.
- PPEs must be changed immediately if torn or dirty.
- A designated area for putting on PPEs should be identified and all personnel should use this area to put on their PPEs. This should ideally be in a clean area away from birds or any other potentially contaminated equipment, such as cages, crates or farm tools.
- A designated area for removal of PPEs should be identified and all personnel should use this area to remove their PPEs. This should ideally be located away from the area that has recently been depopulated and/or decontaminated.
- Use of PPEs can sometimes make the job more difficult to accomplish because they can be cumbersome, hot, or uncomfortable. However, PPEs are necessary to prevent from becoming infected or from spreading the virus to other farms or people, especially the people they care most about.

Disposal

- All PPEs must be removed and discarded before taking breaks. A new set should be put on after the break.
- Used PPEs must be discarded immediately after use in an approved manner (such as burying or burning).
- Biohazard plastic bags should be sealed and disposed of properly. This means following the instructions of the local officials or person supervising the work on where to place biohazard bags when they are full.

- The way to dispose of PPE may be different in each situation or location. Local officials or those supervising the work will likely decide on how best to dispose of used PPE and other items that have come in contact with the virus.

7.9 SOP FOR HANDLING SPECIMENS SUSPECTED OF CONTAINING INFLUENZA A VIRUS

General recommendations

The possibility that an influenza infection in humans caused by avian influenza A viruses could occur following a laboratory accident is a risk to which it is crucial to be constantly alert. Compromised laboratory biosafety will increase the risk of transmission of infection in humans (Laboratory personnel). The responsibility of developing a comprehensive safety policy, including a safety manual and supporting programs for its implementation normally rest with the supervisor or head of an institute or laboratory.

However, laboratory biosafety is also the responsibility of all supervisors, laboratory employees and the individual workers. A good microbiological technique is fundamental to laboratory biosafety. The use of safety equipment, good procedures and practices, will help to reduce the risks involved in dealing with bio-safety hazards. The most important concepts are outlined below.

Standard precautions should always be followed; barrier protection (gowns, gloves) should be used whenever samples are obtained from suspect cases. In addition to these standard precautions, eyes should be protected and N95 masks should be used. Basic containment – Biosafety Level 2 (BSL2) – practices and procedures should be the minimum requirement for handling specimens.

Biosafety level 2 Special Practice

- All persons working in the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided appropriate immunizations for viruses handled or potentially present in the laboratory.
- Must have established policies and procedures describing the collection and storage of samples.
- Biosafety manual must be available and accessible.
- The laboratory head must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- Infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
– Spills involving infectious materials must be contained, decontaminated and cleaned up by trained staff properly and equipped to work with infectious material.
– Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated. All such incidents must be reported to the laboratory head
- All procedures that may generate an aerosol should be conducted within a **certified** BSC or other physical containment devices.

Biosafety level 3

Adopt BSL2 practice. In addition, the laboratory head must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents. Safety equipment include the following:

- All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
- Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
- Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. People who wear contact lenses in laboratories must also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:

● Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
● Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
● Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

- Eye, face, and respiratory protection must be used in rooms containing infected animals.
- All safety equipment practices adopted for BSL2 should be followed.

WHO strongly recommends that the BSL3 precautions described above are adopted and followed for work in BSL2 laboratories with influenza A/H5 virus specimens. Where laboratory facilities do not meet at least basic BSL2 containment conditions, specimens should be referred to suitably equipped central/reference laboratories for primary diagnostic tests.

Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day. A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine (0.1%). A stronger solution, containing 5 g/l available chlorine (0.5%), is recommended for dealing with bio-hazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solutions, as domestic bleach, contain 50g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l or 5 g/l, respectively. Bleach dilutions should be freshly prepared and allowed a contact time of at least 10 min. Chlorine is corrosive and cannot be used on all surfaces. Alternative compounds for disinfection and sterilization are available.

Caution: It is illegal to allow the laboratory staff to work with HPAI suspected materials in breach of above safety guidelines and facilities and should be avoided at all cost.

Note: More detailed information on all above issues is provided in the *WHO laboratory biosafety manual*.

7.10 SOP FOR COLLECTION OF SWAB SAMPLES (CLOACAL, TRACHEAL & ENV. SAMPLES)

Purpose:

For submission to the laboratories and to rapidly diagnose Highly Pathogenic Avian Influenza

Scope:

The document describes collection of swab samples from cloacae, tracheal and environment, either from live or dead birds. The environmental sample include collection of swabs from fresh faeces / droppings. For collection of necropsy samples, refer to SOP on post-mortem.

User:

Veterinarians, para-veterinarians and laboratory personnel

Manpower:

- Supervisor: The veterinarian / senior technician will supervise the collection of swab samples
- Implementer: Veterinarian, para-veterinarians and laboratory personnel

Materials/Equipment:

- Gloves
- Mask
- Sterile Dacron/rayon swabs
- Screw capped polystyrene vials with viral transport media (VTM)
- Scissors
- Self-sealing plastic bags
- Lab marker/sample labels
- Sample data sheet
- Ice packs
- Cool box
- Packing tape
- PPE – requirement is either upgraded or downgraded depending upon the bird flu situation in the country
- Biohazard bag

Procedure:

Collection of cloacal and tracheal swabs:

- Restrain the bird appropriately
- Insert the swab deep into the vent/trachea and swab the wall
- Avoid excess solid faecal material or visible blood in cloacal swabbing.
- Immediately place the swab in the transport medium.
- Place the specimen in the self-sealing plastic bag
- Label specimen identification
- Preserve the samples in cool box with ice packs

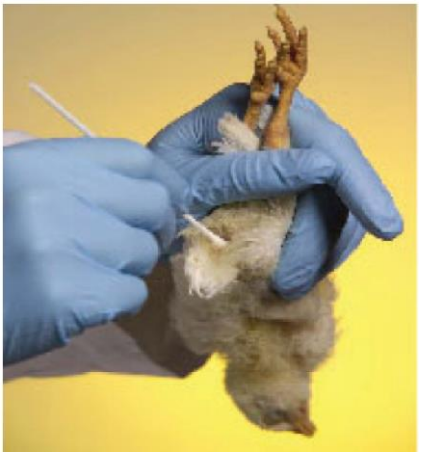


Illustration of cloacal and tracheal swabbing techniques

Collection of environmental swabs:

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

Transport media:

Swab samples (oropharyngeal and cloacal swabs or fresh faeces swabs) should be placed in:

- Isotonic phosphate buffered saline (PBS), pH 7.0–7.4 with antibiotics* or
- A solution containing protein and antibiotics*

***Note:** The antibiotics can be varied according to local conditions, but could be, for example, penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamicin (50 mg/ml) and mycostatin (1000 units/ml) for tissues and oropharyngeal swabs, but at five-fold higher concentrations for faeces and cloacal swabs. It is important to readjust the pH of the solution to pH 7.0–7.4 following the addition of the antibiotics. It is recommended that a solution for transport of the swabs should contain protein to stabilize the virus (e.g. brain–heart infusion, cattle serum up to 5% [v/v] or bovine albumin – 0.5% [w/v]). Faeces and finely minced tissues should be prepared as 10–20% (w/v) suspensions in the antibiotic solution. Suspensions should be processed as soon as possible after incubation for 1–2 hours at room temperature. When immediate processing is impracticable, samples may be stored at 4°C for up to 4 days. For prolonged storage, diagnostic samples and isolates should be kept at –80°C. Repeated freezing and thaw should be avoided.

7.11 SOP FOR COLLECTION OF BLOOD SAMPLES

Purpose:

For submission to the laboratories for sero-surveillance of Highly Pathogenic Avian Influenza H5N1 and AI H7N9

Scope:

The document describes collection of blood samples 2-3ml from live birds. The site for collection of blood is either from wing vein or directly from heart

User:

Veterinarians, para-veterinarians and laboratory personnel

Manpower:

- Supervisor: The veterinarian / senior technician will supervise the collection of blood samples
- Implementer: Veterinarian, para-veterinarians and laboratory personnel

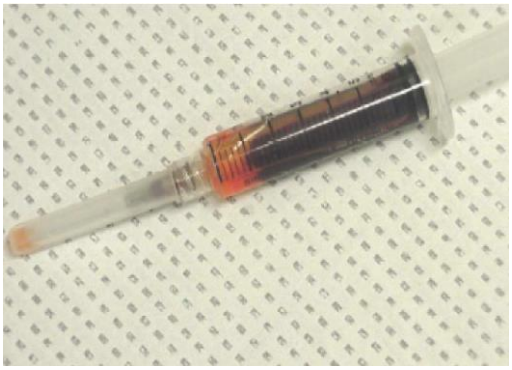
Materials/Equipment:

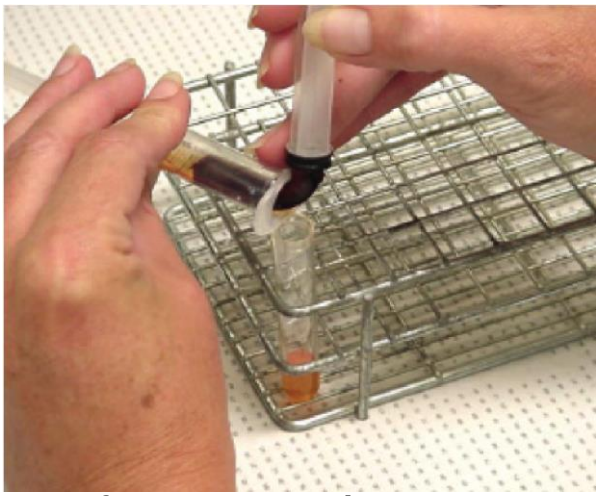
- Gloves
- Syringes 2.5ml
- Needles – 21 gauge
- Eppendorf / cryo-vials
- Scissors
- Cotton/tissue paper
- 70% ethanol
- Self-sealing plastic bags
- Lab marker/sample labels
- Sample data sheet
- Ice packs
- Cool box
- Packing tape
- PPE – requirement is either upgraded or downgraded depending upon the bird flu situation in the country
- Sharps disposal container
- Biohazard bags

Procedure:

- Restrain the bird appropriately
- Pluck the feathers near the wing vein
- Swap with 70% ethanol
- Collect the blood in the disposable plastic syringe
- Allow the blood to clot within the syringe. The syringe should be placed at 45-degree angle for better serum separation at room temperature or at 37ºC for 20-30 minutes.

- Separate the serum in Eppendorf tubes / cryo-vials for sending to the laboratory
- Label each tube with code number corresponding to that in the sample submission data sheet
- Pack properly in the plastic bags and keep in cool box





Blood collection and separation of serum in cryo-vial

7.12 SOP FOR COLLECTION OF DEAD BIRDS & NECROPSY SAMPLES

Purpose:

For submission to the laboratories for viral isolation and diagnosis of Avian Influenza

Scope:

The document describes collection of dead birds/tissues samples from dead domestic and wild birds for viral isolation and other diagnostic techniques.

User:

Veterinarians, para-veterinarians and laboratory personnel, however, necropsy shall be conducted by veterinarians only under strict bio-safety conditions, i.e. minimum requirement of class II biosafety cabinet.

Manpower:

- Supervisor: The veterinarian / senior technician will supervise the collection of dead birds. Senior veterinarian will supervise necropsy procedure
- Implementer: Veterinarian, para-veterinarians and laboratory personnel

Materials/Equipment:

- PPE – requirement is either upgraded or downgraded depending upon the bird flu situation in the country
- Gloves
- Self-sealing carcass collection bags (plastic)
- Lab marker/sample labels
- Data form
- Ice packs
- Cool box
- Packing tape
- Virkon/bleaching powder
- Biohazard bags

Procedure:

- Appropriate PPE to be worn before handling a dead bird (double gloves, gown, eye protection, face masks)
- Invert a plastic bag around your gloved hand and then surround the animal with the bag so that you do not directly touch the animal.
- Seal the bag tightly (double bag if required for strength and cleanliness)
- Clearly label the bag with an identification
- If more than one species has been affected, collect several specimens of each for diagnosis.
- When possible, fresh carcasses should be refrigerated (NOT frozen)
- Send the sample to veterinary laboratory for necropsy procedure in sealed cool box along with relevant sample data sheet

Note: Make sure you wear the appropriate level of personal protective equipment, based on the situation you are investigating. Try to minimize direct contact with dead birds and always keep animals away from your face.

In general, carcasses of birds that have been dead for less than 24 hours (fresh carcasses) are sufficiently adequate (moribund or viremic birds are best) for diagnostic purposes. In colder climates, carcasses may last in relatively good condition for longer periods of time; in warm climates, carcasses will decompose faster. A decomposing carcass is desiccated, bloated, green, foul smelling and has feathers that pull out easily.

Necropsy:

Necropsy is performed strictly by a veterinarian in the laboratory following a standard protocol; however, the necropsy will be performed wearing full set of personal protective equipment and under minimum of class II biosafety cabinet facility.

Tissue Samples for virus isolation: Samples to be collected from various organs should be as follows:

- Respiratory- Lungs, trachea, air sacs (posterior)
- Digestive- Liver, pancreas, small intestine, caeca, proventriculus, large intestine.
- Urinary- Kidney
- Lymphoreticular- Spleen, Bursa
- Cardiovascular - heart
- Reproductive – Ovary, oviduct

Note: Pool tissues from an organ system, digestive and nervous systems- collect separately. Heart and spleen can be pooled. Lungs and spleen can be pooled, Liver and kidney can be pooled.
Keep tissues cold (in ice)
Note: *Human exposure - special considerations for HPAI virus exposure:*

Anyone who handles birds suspected of being affected with avian influenza must use their best judgment and be aware of all possible routes of infection. Influenza may infect humans via contact with any mucous membrane (e.g. the entire respiratory and gastro-intestinal tracts and the eyes). Infection could occur by accidental stab with a needle or necropsy instrument contaminated with fresh moist tissue or fluids from infected animals and conceivably through contamination of a break in the skin. Thus, in short, infection occurs only as a result of direct exposure to live viruses in aerosol droplets or contaminated fluids. Trans-dermal infection (infection across intact skin) has not been described and the virus is not vector-borne. To date, with the exception of 1 case, all known human deaths resulting from H5N1 AI have been from exposure to poultry or areas where poultry are raised. Only 1 human case can be attributed to a person plucking the feathers of an infected swan. However, similar precautions should be taken when conducting a wild bird die-off investigation and depopulating a chicken farm.

7.13 SOP FOR RAPID ANTIGEN DETECTION TESTS (TYPE A)

Purpose:

To rapidly diagnose the HPAI virus that would further enable containment effectively and in the shortest possible time.

Scope:

This test needs to be done in the field itself for the detection of antigen type A Avian Influenza.

Users:

Veterinarians and laboratory staff of RLDCs, NCAH, DVH

Manpower:

- Supervisor: Veterinarian/ Laboratory Head
- Implementer: Laboratory staff.

Safety Consideration

- Use PPE for suspect cases
- Dispose the tests materials safely
- While doing post mortem, maximum precautions are to be taken (this is a technical discipline to be followed every time).

Materials

- 10×foil pouches, each containing one cassette, one pipette and a desiccant
- 10×assay buffer tubes (0.8 mL each)
- 10×swab sticks
- Product Manual

Test Procedure

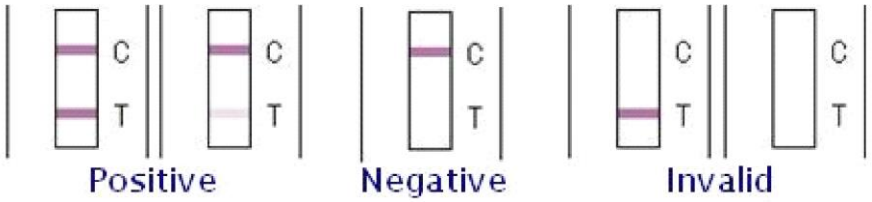
- Insert the swab stick into the bird's cloacal, oropharyngeal or dip the swab stick in the bird's eye to collect the secretions. Please make the swab wet sufficiently.
- Insert the wet swab into the provided assay buffer tube. Agitate it to assure good sample extraction.
- Take out the cassette from the foil pouch and place it horizontally.
- Gradually drip 3 drops of sample extraction into the sample loading well
- Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.
- Follow the test procedures as described in the guide protocol.

● Where ever possible do not pool unlike samples i.e. do not mix tracheal swab with cloacal swab for testing. This might give false positive results.
● In pooling samples, take approximately 5 samples to exercise economy.
● Timing is an important determining factor for reading accurate results. Use stopwatch to keep the timing while performing the test either in the lab or field.

- As far as possible perform the test in the field using this kit while the sample is still fresh.
- For testing in the laboratory, ensure proper refrigeration of the samples in the cool pack (8-10C). Use nutrient broth or viral transport media (VTM) as transport media for samples.

Result interpretation

- Positive: After the test is completed, the appearance of colour band on both C and T bands are observed, no matter if the T band is clear or vague.
- Negative: Only clear C band appears.
- Invalid: If the C band does not appear, irrespective of the appearance or non-appearance of T band the test is considered invalid.
- Results are reported as positive or negative
- All results should be considered in conjunction with other clinical information available from veterinarian. For further confirmation, it is suggested to choose other method such as polymerase chain reaction (PCR)
- Below is the pictorial representation of test result



Waste disposal

- Dispose the tests materials safely
- Ensure that the test materials (swabs, pipettes, vials, containers etc.) are properly packed and are brought back to the laboratory for proper disinfection by autoclaving and disposed of safely in the pit.
- Alternatively, the test materials and samples should be disinfected in 2% Virkon-S and autoclaved

Reporting

Results are reported as positive or negative

Quality Control

Test is invalid if no coloured band appears in the C zone, whether the T band appears or not.

7.14 SOP FOR RAPID ANTIGEN DETECTION (H5)

Scope

This test is for the detection of Avian Influenza A H 5 antigen during HPAI surveillance.

Test Principle

Avian Influenza Virus $H_5$5 Antigen Rapid Test is a sandwich lateral flow immuno-chromatographic assay for the qualitative detection of avian influenza virus H5 subtype (AIV H5 Ag) in avian secretions.

Safety Consideration

- Wear PPE
- Dispose the test materials accordingly
- Disinfectant the working bench with working solution

Materials

- 10×foil pouches, each containing one cassette, one pipette and a desiccant
- 10×assay buffer tubes (0.8 mL each)
- 10×swab sticks
- Product Manual

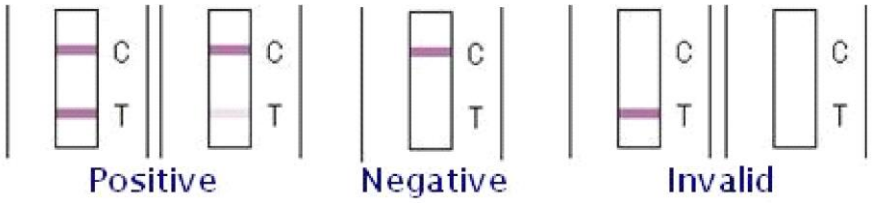
Test Procedure

- Insert the swab stick into bird's cloacae, oropharyngeal or dip the swab stick in bird's eyes to collect the secretions. Please make the swab wet sufficiently.
- Insert the wet swab into the provided assay buffer tube. Agitate it to assure good sample extraction.
- Take out the cassette from the foil pouch and place it horizontally.
- Gradually drip 3 drops of sample extraction into the sample hole "S".
- Interpret the result in 5-10 minutes. Result after 10 minutes is considered as invalid.

Result Interpretation

- Positive: After the test is completed, the appearance of colour band on both C and T bands are observed, no matter if the T band is clear or vague.
- Negative: Only clear C band appears.
- Invalid: If the C band does not appear, irrespective of the appearance or non-appearance of T band the test is considered invalid.
- Results are reported as positive or negative
- All results should be considered in conjunction with other clinical information available from veterinarian. For further confirmation, it is suggested to choose other method such as polymerase chain reaction (PCR)

Below is the pictorial representation of test result



Precaution/Limitation of procedure

- This test is to be used only if the samples have first tested positive against Influenza A
- Do not remove the test cassette from its pouch until immediately before use.
- Do not reuse the test kit.
- Do not use the test beyond its expiration date marked on the foil pouch.
- The components in this kit have been quality control tested as standard batch unit. Do not mix components from different lot numbers.

Reporting

Results are reported as positive or negative

Quality Control

Test Invalid: If no coloured band appears in C zone, whether T band appears or not.

7.15 SOP FOR ENZYME LINKED IMMUNOSORBENT ASSAY (SEROLOGY)

Purpose:

For sero-surveillance against avian influenza (AI) infection

Scope:

This test is applied for laboratory sero-surveillance to detect the past infections (antibodies) with AI and its scope is limited. Thus, it cannot be used in disease outbreak situations (phase II).

Users:

Veterinarians and laboratory staff of RLDCs & NCAH

Manpower:

- Supervisor: Veterinarian/ Laboratory Head
- Implementer: Laboratory staff.

Materials/Equipment:

- Commercial ELISA Kit (all-inclusive set) for testing by ELISA method
- PPE
- Clean laboratory work-bench.
- Pipettes
- Microtitre plates
- Microtiter plate sealer
- Multichannel pipettes
- PBS
- ELISA reader
- Disinfectants
- Plate lay-out
- Incubator
- Plate shaker
- Micropipette tips
- Fridge

Procedure:

Refer kit's insert/leaflet for standard procedure for ELISA and strictly follow it.

7.16 SOP FOR HAEMAGGLUTINATION-HAEMAGGLUTINATION INHIBITION TEST (HA-HI)

Purpose:

For sero-surveillance against avian influenza (AI) infection as well as for antigen detection in allantoic fluid

Scope:

This test is applied for laboratory sero-surveillance to detect the past infections with AI and detection of HPAI virus after replication in embryonated eggs. Thus, this test is not only versatile but simple too.

Users:

Veterinarians and laboratory staff of RLDCs & NCAH

Manpower:

- Supervisor: Veterinarian/ Laboratory Head
- Implementer: Laboratory staff.

Materials/Equipment:

- RBC from MDF chickens
- Killed Antigens and antibodies
- Pipettes
- Microtiter plates
- Microtiter plate sealer
- Multichannel pipettes
- PBS
- Reagent trough
- Plate lay-out
- Disinfectants
- Fridge
- Syringe 5 – 10ml
- Needles

Procedure

Hemagglutination (HA) test:

The HA-HI steps in flow-diagram are as follows:

Dispense 25 µl of PBS into each well of V-bottom 96 well Microtiter plate

↓

Place 25 µl of antigen/sample (allantoic) into first wells

↓

Make two-fold serial dilution of 25 µl of the samples across the plate

↓

Add 25 µl of 1% (v/v) chicken RBC to each well and mix gently

↓

Allow RBCs to settle for about 30 minutes at room temperature

↓

Interpretation

AT the end, estimate 4 HAU and verify 4 HAU by back titration

Hemagglutination Inhibition (HI) test:

The HA-HI steps in flow-diagram are as follows:

Dispense 25 µl of PBS into each well of V-bottom 96 well Microtiter plate

↓

Place 25 µl of serum into first wells

↓

Make two-fold serial dilution of 25 µl of the samples across the plate

↓

Add 4 HAU of virus/antigen in 25 µl volume to each of well and leave for a minimum of 30 minutes at room temperature or 60 minutes at 4^{o} C.

Add 25 µl of 1% (v/v) chicken RBC to each well and mix gently

↓

Allow RBCs to settle for about 40 minutes at room temperature or

60 minutes at 4^{o} C.

↓

Interpretation

The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (24 or $\log_2 4$) or more against 4 HAU of antigen

7.17 SOP FOR CONVENTIONAL & REAL-TIME RRT-PCR

Purpose:

Diagnosis and confirmation of Avian Influenza virus by identification of viral genome, viral subtyping, rapid viral genome identification by conventional and real time RT-PCR, characterization and sequencing

Scope:

The document describes the basic procedure for Reverse Transcriptase polymerase chain reaction using conventional as well as real time RT-PCR. Viral extraction, cDNA synthesis and PCR reaction may differ with different kits, thus specific literatures should be referred during the procedure.

User:

Veterinarians and laboratory personnel

Manpower:

- Supervisor: Laboratory head/technologist will supervise the procedure
- Implementer: Laboratory technologist and laboratory technicians

Materials/Equipment:

- Sterile 0.2 ml microcentrifuge tubes
- 10, 20 and 100 ul adjustable pipettes and tips
- Micro centrifuge machine
- Vortex
- Bio-safety cabinet
- Real time PCR machine
- Thermo-cycler machine
- Viral RNA extraction kit
- Ice making machine (portable)
- Deep freezers
- Cool box
- 70% ethanol
- Gel loading layout worksheet sheep
- PPE – Make sure you wear the appropriate level of personal protective equipment, based on the material you are handling.

Procedure:

Conventional RT-PCR:

Viral RNA extraction: Follow the viral RNA extraction kit for extraction of viral RNA
Store the final product (RNA extract) at -20^{o}C for short term storage or at -80^{o}C for long term storage.

Synthesis of cDNA

Mix and briefly centrifuge each of the following reagents before use. Combine the following in a 0.2 ml tube

Reagents	***Amount***
Viral RNA	8 ul
Primer 50 ng/ul random hexamers	1 ul
10 mM dNTP mix	1 ul
Final volume	10 ul

Incubate at 65^{o}C for 5 minutes and then place in ice for at least 1 minute. Prepare the following cDNA synthesis mix by adding each reagent in the indicated order

Reagent	***1 Rxn***	***10Rxns***
10x RT buffer	2 ul	20 ul
25 mM $MgCl_2$	4 ul	40 ul
0.1 M DTT	2 ul	20 ul
RNaseOUT™ (40 U/ul)	1 ul	10 ul
SuperScript™ III RT (200U/ul)	1 ul	10 ul

- Add 10 ul of cDNA synthesis mix to each RNA/primer mixture, mix gently and collect by brief centrifugation. Incubate Random hexamers primer for 10minute at 10^{o}C followed by 50 minutes at 50^{o}C.
- Terminate the reaction at 85^{o}C for 5 minutes and chill in ice. cDNA synthesized could be stored at -20^{o}C or used for PCR immediately

PCR reaction (Amplification of target cDNA)

Prepare a PCR mixture for each control reaction by adding the following to a 0.2 ml tube sitting on ice

Component	***Volume***
DEPC-treated water	30 ul
Gotaq Green Master mix 2X	50 ul
Multiplex 3 primers Mix (each 20 uM)	10 ul
Target cDNA from step (b) above	10 ul
Final volume	100 ul

Mix the contents of the tube and centrifuge and collect the reaction components. Place reaction mixture in preheated (94^{o}C) thermal cycler and perform initial denaturation step i.e. 94^{o}C for 2 minutes

Program 35-40 cycles of PCR as follows

Denature	94^{o}C for 30 seconds
Annealing	50^{o}C for 90 seconds
Extension	68^{o}C for 2 minutes
Final extension	68^{o}C for 15 minutes

Final product is maintained at 4^{o}C

Agar Gel electrophoresis

- Place 1.5% agarose gel into the electrophoresis chamber
- Remove 5 ul of PCR product from each reaction tube to a para-film sheet and mix with 1 ul of gel loading buffer
- Load 0.5 ul molecular weight marker mix with 1 ul gel loading buffer and 4.5 ul ultra-water to the first well of the gel
- Pipette 6 ul of PCR reaction, each sample to wells of gel separately
- Close the lid of the gel chamber and attach the electrodes
- Run the gel at 100V for 15 minutes.

DNA bands staining

- Put agarose gel into 0.5 ug/ml gel rad solution in water and let the gel stain for 20 minutes
- De-stain the gel in water for 20 minutes
- Expose the gel to UV light between 250-310 nm wavelength to visualize the bands
- Document gel with photograph and compare the PCR fragments with the marker.

Note: Ethidium bromide is a planar multi-ring compound and is a mutagen so it has been replaced with gel rad which is safer to handle in the laboratory. Always wear gloves while handling the gel and while performing PCR.

Real Time RT-PCR:

Note: The protocol for real time RT-PCR is optimized by different manufacturers depending upon the kits. Therefore, manufacturer specific protocols should be followed.

Avian Influenza (AI) viruses are members of the virus family Orthomyxoviridae. Influenza viruses in this family are designated type A, B or C depending on the antigenic properties of the matrix protein and the nucleoprotein. AI viruses belong to type A. The genome consists of eight single-stranded, negative sense RNA molecules, which encode ten proteins: PB2, PB1, PA, HA, NP, NA, M1, M2, NS1 and NS2. Sixteen serologically distinct haemagglutinin (H1-H16) and 9 neuraminidase (N1-N9) subtypes of type A influenza virus exist at present, and representatives of each have been isolated from avian species. To date, all highly pathogenic AI viruses that produce acute clinical disease in avian species have been associated only with H5 or H7 subtypes. Not all H5 and H7 subtypes, however, are virulent in poultry. Pathogenicity of AI viruses is associated with trypsin cleavage of haemagglutinin into two subunits (H1 & H2). Highly pathogenic strains of AI (HPAI) viruses contain several basic amino acid residues at the cleavage site.

Type A in humans, birds and some other mammals (swine, equine) can cause disease. Influenza type A has subtypes based on HA and NA proteins. 15 HA subtypes (H1 - H16) of which most can be found in avian, 9 NA subtypes, all are found in avian.

Newcastle disease is an infection of domestic poultry and many other bird species with virulent Newcastle disease virus. NDV synonymous with avian paramyxovirus serotype 1 (PMV-1), is an RNA virus and the most important of the 9 known PMV serotypes as a

pathogen for poultry. NDV can be isolated from oropharyngeal or cloacal swabs or tissues from infected birds by inoculation of the allantoic cavity of 9- to 11-day-old embryonated chicken eggs. Infection is confirmed by recovery of a hemagglutinating virus that is inhibited with NDV antiserum or by detection of NDV RNA by reverse transcriptase PCR.

Scope

One-step TaqMan reverse transcriptase PCR assay incorporating primers and probes specific for Influenza type A and Subtypes H5, N1 and NewCastle Disease Virus is for detection of Influenza A, H5, N1 and NDV viral RNA in nasal, cloacal and tissue swab samples.

Test principles

In RT-PCR, the RNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR. RT-PCR is currently the most sensitive method of RNA detection available. The quantification of mRNA using RT-PCR here is achieved in a one-step reaction. It can be used for both qualitative analysis and quantitative analysis. In Fluorescent End-Point PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in a reaction mixture after PCR. It allows detection of the accumulating product without reopening the reaction tubes after the PCR run.

Application

Detection of type A, H5 and N1specific genes of AIV by real time RT-PCR

Objective

This document is used to support diagnosis of AIV in chicken and other birds using real time RT-PCR test

Materials/Equipment

- Real-time PCR machine: QuantStudio-5
- MINI spin, Eppendorf, AG-22331, USA
- Mini spin, Spinwin
- Refrigerated centrifuge, PK-121R, Thermo Electron Corporation
- Bio-Safety Cabinet, Class –II, Esco

Reagents

- AgPath-ID One-Step RT-PCR Kit storage at -20°C
- QIAamp viral RNA mini Kit (250) Cat No. Qiagen 52906
- Primers

Influenza type A primers

- Forward primer IVA D161M 5'- AGATGAGYCTTCTAACCGAGGTCG-3'

- Reverse primer IVA D162M1 5'- TGCAAAAACATCYTCAAGTCTCTG-3'
- Reverse primer IVA D162M2 5'- TGCAAACACATCYTCAAGTCTCTG-3'
- Reverse primer IVA D162M3 5'- TGCAAAGACATCYTCAAGTCTCTG-3'
- Reverse primer IVA D162M4 5'- TGCAAATACATCYTCAAGTCTCTG-3'
- Probe: IVA MA 5'-FAM TCAGGCCCCCTCAAAGCCGA-TAMRA-3'

Influenza A, subtype H5 primers

- Forward primer IVA D204f 5'- ATGGCTCCTCGGRAACCC -3'
- Forward primer IVA D148 H5 5'- AAA CAG AGA GGA AAT AAG TGG AGT AAA ATT-3'
- Reverse primer IVA D205r 5'- TTYTCCACTATGTAAGACCATTCCG-3'
- Reverse primer IVA D149 H5 5'- AAA GAT AGA CCA GCT ACC ATG ATT GC-3'
- Probe: IVA H5a 5'- FAM- TCAACAGTGGCGAGTTCCCTAGCA-TAMRA
- Probe: IVA D215P 5'- FAM – ATG TGT GAC GAA TTC MT-MGBNFQ-3'

Influenza A, subtype N1 primers

- Forward primer AI_N1 1316F 5'- GYG GGA GCA GCA TAT CYTT-3'
- Reverse primer AI_N1 1379R 5'- CCG TCT GGC CAA GAC CAA-3'
- Probe AI_N1: 5' FAM -pdU-G-pdU-GG-pdU-G-pdU-AAAYAG-pdU-GA-pdC-A-pdC-BHQ-1-3'
- Sample: Swabs from Cloaca, Tracheal or Tissue
- Positive Control: known Influenza, H5 and N1 positive sample
- Negative control: nuclease free water

Procedure

Extraction of RNA (Template RNA)

- RNA is extracted and purified by using QIAamp viral RNA mini Kit (250) Cat No. Qiagen 52906 by following the manufacturer's instruction.

Master Mix preparation:

- Prepare the One step RT-PCR mix as shown below (type A). This will vary as this is based on the primer concentration:

Reaction component	**Volume per reaction (µL)**	**Volume for 20 Rxs (µL)**
Nuclease-Free Water	2.75	55
2X RT-PCR Buffer (Ambion P/N AM1005)	12.5	250
Kit lot No:		
25X RT-PCR Enzyme MIX	1	20
FAM-TAMARA PP MIX (Type A PPMIX)	3.75	75
Microstores No:		
Total Volume	20	400

Template RNA	5	
Final volume	25	

AgPath Thermal Cycling Parameter:

1X 45°C 10 min, 95°C 10 min

45X 95°C 15 sec, 60°C 45 sec

Note: Similar master mix will be prepared for H5 and N1 which will depend on the concentration of the primers.

Result interpretation and reporting

Qualitative analysis

Ct (Threshold cycle) value of each sample can be read as follows

Ct value > 45 Negative

≤ 40 Positive

40-45 Intermediate

Quantitative analysis

Assess the Ct value when amplification curve of Standard 1, 2, 3, 4 passes threshold line.

Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard 1, 2, 3, 4

Test validation

Each Ct value standard should be as follows.

Standard 1 > Standard 2 > Standard 3 > Standard 4

R-value of Standard curve should be 0.900~0.999.

The Standard result should be all positive

Precaution/Limitation of Procedure

- Remove the possibility of contamination of Nucleic acid and PCR products when processing PCR.
- It is recommended to work at the clean bench related to PCR.
- Use sterilized Filter tip.
- Do not make any bubbles from the test bottom when testing.
- Minimize the exposure of light when handling the probes
- Do not use expired kits expired validity.
- Read the result as Avian Influenza by following clinical symptoms and autopsy even if the kits show the positive result. You are required to ask for testing at the OIE reference laboratory or when the results are doubted.

– The detection limit of the kit is 10 copies. It may not detect less than 10 copies.
– The kit should be stored at -20℃. Under these conditions' reagents are stable through the expiration date printed on the label.
– Do not read result when Ct value is less than 5 or when there is non-significant curve

Reporting

Results are reported as positive or negative to Influenza A, H5 and N1 Subtypes.

Quality Control

Test validation

Each Ct value standard should be as follows.

Standard 1 > Standard 2 > Standard 3 > Standard 4

R-value of Standard curve should be 0.900~0.999.

The Standard result should be all positive.

7.18 SOP FOR SAMPLE PREPARATION, PRESERVATION & STORAGE

Purpose:

For further diagnosis, confirmation of Avian Influenza at Regional, National and International referral laboratories

Scope:

The document describes laboratory preparation, preservation and storage of samples for further diagnosis, confirmation of Avian Influenza at Regional, National and International referral laboratories

User:

Veterinarians and laboratory personnel

Manpower:

Supervisor: The veterinarian / senior technician will supervise the proper preservation of the samples

Implementer: Veterinarian and laboratory personnel

Materials/Equipment:

- Eppendorf tubes -2ml
- Vortex mixer
- Bio-safety cabinet class II
- Pipette
- Pipette tips
- Deep freezer (-20^{o}C, -70^{o}C)
- Refrigerated centrifuge
- Autoclave
- Autoclave bags
- 70% ethanol
- Mortar and pestle
- Syringe – 20ml
- Micro-filter 0.22um to 0.45um/ Gentamicin 50mg/ml
- Scissors
- Forceps
- Sterile sand
- Marker pen
- Tube rack
- Disposable autoclave bag
- Gloves (Make sure you wear the appropriate level of personal protective equipment, based on the material you are handling).

Procedure:

Swab samples:

As soon as the swabs are received by the testing laboratories, the samples are prepared in the biosafety cabinets as follows:

– Label 2 ml microcentrifuge tubes as per the sample number
– Mix the original sample pool by vortex and transfer approx. 2ml into the microcentrifuge tube
– Centrifuge the microcentrifuge tubes at 1800 rpm/10 minutes at 4^{o}C.
– Transfer the supernatant to another labelled microcentrifuge tube containing 60ul of gentamicin sulfate (50mg/ml).
– Incubate at room temperature for 30 minutes

The sample is ready for inoculation or for storage at -70^{o}C for a longer duration.

Tissue samples:

Tissue samples received at 4^{o}C in the laboratory are processed in the bio-safety cabinet as follows:

– Make a 10% tissue suspension in PBS by grinding approximately 1gm tissues with sterile sand in mortar and pestle and adding 9ml of PBS.
– Pipette out the suspension in centrifuge tubes.
– Centrifuge the suspension at 10,000rpm for 30 minutes at 4^{o}C.
– Collect the supernatant in sterile labelled tubes by filtering through 0.45um syringe filters.
– If filter is not available, add 60ul of 50mg/ml gentamicin sulfate.

The sample is ready for inoculation or for storage at -80^{o}C for a longer duration.

7.19 SOP FOR SPECIMEN TRANSPORT

Purpose:

For further diagnosis, confirmation of Avian Influenza at National and International referral laboratories

Scope:

The document describes laboratory packaging for quick and safe domestic and international transport of specimens for further laboratory investigation and confirmation.

User:

Veterinarians, para-veterinarians and laboratory personnel

Manpower:

Supervisor: IATA certified officer from National Referral Laboratory will supervise packaging of specimen for International transport. The veterinarian / senior technician will supervise the packaging of specimen for domestic transport

Implementer: Head of National Referral laboratory, Veterinarian, para-veterinarians and laboratory personnel

Materials/Equipment:

– Gloves (Make sure you wear the appropriate level of personal protective equipment, based on the material you are handling).
– Polystyrene screw capped vials
– Self-sealing plastic bags
– Sealing tape
– Lab marker / sample labels
– Absorbent cotton or tissue paper
– Laboratory request form
– Ice packs
– Cool box
– Biohazard label
– IATA approved shipping containers
– Waterproof envelopes
– Specimen category symbol

Procedure:

Domestic transport

– Place the specimen in a primary container (polystyrene screw capped vials) with identification number that must be leak-proof unbreakable and airtight.
– After tightening the cap, apply sealing tape (para-film) over the cap and top of the container and wrap in absorbent material (e.g. absorbent cotton or tissue paper) to absorb the accidental leakage.

– The sealed specimen container with a small amount of absorbent material must be placed in a suitably sized self-sealing plastic bag.
– Seal the bag. Two or more sealed specimens from the same source may be placed in a larger plastic bag and sealed. Specimens from a different source must not be placed in the same bag.
– Place the sealed bags containing the specimens inside a secondary self-sealing plastic container and seal it. Specimens from several sources may be packed inside the same secondary plastic container.
– Place additional absorbent material inside the secondary container to cushion and to absorb any leakage that may occur.
– Tape the laboratory request form sealed in a plastic bag to the outside of this secondary container.
– Place the secondary bag containing the specimen in a cool box containing ice/ice cubes.
– Seal the cool box properly with the help of brown tape running around full length and breadth of the box so that a plus or cross sign is made.
– Paste a biohazard label "Bird flu sample" outside of the cool.

International transport

– Transport of specimens should comply with the WHO guidelines for the safe transport of infectious substances and diagnostic specimens (WHO, 1997).
– The receiving laboratory should be notified before shipment of specimens in order to arrange for an import license for the specimens.
– Transport of specimens within national borders should comply with the procedures detailed within each country's regulations.
– International air transport of specimens from avian influenza infected animals must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations and Consignment of Diagnostic Specimens, 2003.
– The IATA Regulations, Consignment of Diagnostic Specimens, 2003 allow specimens known or suspected to contain the avian influenza agent to be transported as UN 3373 "*diagnostic specimens*" when they are transported for diagnostic or investigational purposes.
– Specimens transported for any other purposes, and cultures (as defined in the IATA Regulations) prepared for the deliberate generation of pathogens, must be transported as UN 2814 or UN 2900, as appropriate.
– All specimens to be transported (UN 3373, UN 2900, or UN 2814) must be packaged in triple packaging consisting of three packaging layers as indicated in the Dangerous Goods Index (refer diagram at the end of the chapter).
– UN 3373, Diagnostic Specimens, shall be packed in good quality packaging, which shall be strong enough to withstand the shocks and loads normally encountered during transport. Packaging shall 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.
– Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into

the secondary packaging. Secondary packaging shall be placed in a final outer package with suitable cushioning material. Any leakage of the contents shall not substantially impair the protective properties of the cushioning material or of the outer packaging.

For liquids

The primary receptacle(s) shall be leak-proof and shall not contain more than 500 ml. There shall be absorbent material placed between the primary receptacle and the secondary packaging; if several fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated so as to prevent contact between them.

The absorbent material shall be in sufficient quantity to absorb the entire contents of the primary receptacles and there shall be a secondary packaging that shall be leak-proof. The primary receptacle or the secondary packaging shall be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar). The outer packaging shall not contain more than 4 litres.

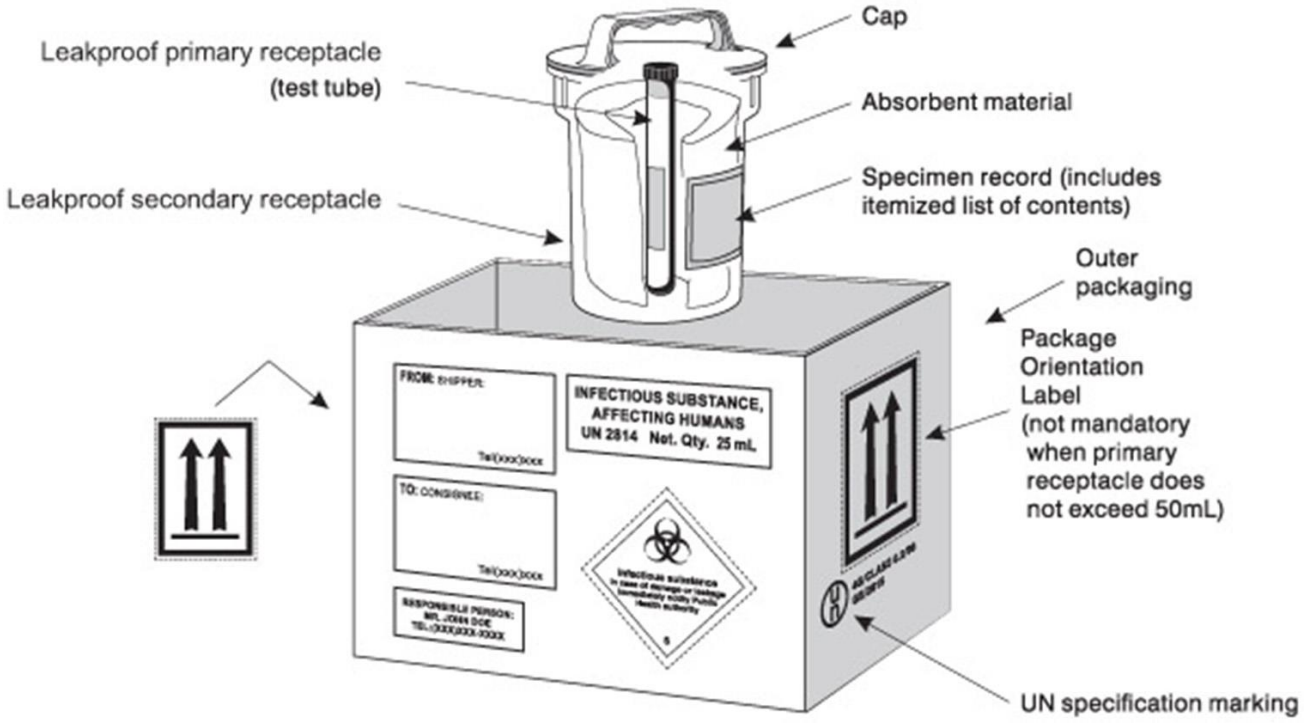
For solids

The primary receptacle(s) shall be sift-proof and shall not contain more than 500 g. If several fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated so as to prevent contact between them and there shall be a secondary packaging which shall be leak proof. The outer packaging shall not contain more than 4 kg.

For air transport, the smallest overall external dimension of a completed package must be at least 10 cm. Packaging must conform to certain performance standards.

For further information about definitions, packaging requirements, markings and labels, accompanying documentation, and refrigerants, please refer to the competent authority, current IATA shipping guidelines, commercial packaging suppliers, or available courier companies.

Note: Specimens should be collected and transported in a suitable transport medium on ice or in liquid nitrogen. Standard precautions should always be followed, and barrier protections applied whenever samples are obtained from patients. Specimens for influenza 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. Carbon dioxide can rapidly inactivate influenza viruses if it gains access to the specimens through shrinkage of tubes during freezing



IATA Approved triple packing system

LABORATORY SAMPLE DATA SHEET (Poultry)
Ministry of Agriculture
Department of Livestock

<table>
<tr><td colspan="3">Reference No.:</td><td colspan="3">Submitted to: NCAH/RLDC ( )</td></tr>
<tr><td colspan="3">Collection date:</td><td colspan="3">Submission date:</td></tr>
<tr><td colspan="6">Submitting veterinarian:</td></tr>
<tr><td colspan="6">Designation:</td></tr>
<tr><td colspan="6">Address:</td></tr>
<tr><td colspan="6">Contact phone number:</td></tr>
<tr><td colspan="6">Farm / Owner:</td></tr>
<tr><td colspan="6">Address of farm/village:</td></tr>
<tr><td colspan="6">Contact phone number:</td></tr>
<tr><td colspan="6">Means of sample shipment (Lab. Staff/paravets/Veterinarian/driver/others ( )</td></tr>
<tr><td>Species of bird</td><td>Type</td><td>Breed</td><td colspan="3">Age</td></tr>
<tr><td></td><td></td><td></td><td colspan="3"></td></tr>
<tr><td rowspan="2">No. of birds in affected flocks</td><td rowspan="2">No. affected</td><td rowspan="2">No. died</td><td colspan="3">No. of birds sampled</td></tr>
<tr><td>Sick</td><td colspan="2">Dead</td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="2"></td></tr>
<tr><td colspan="6">History of outbreak (including date of onset):</td></tr>
<tr><td colspan="6">Clinical signs:</td></tr>
<tr><td colspan="6">Necropsy findings (if done) *:</td></tr>
</table>

Treatment given:				
Preliminary diagnosis:				
Details of samples submitted:				
Specimen type	Specimen ID #	Preservative used	Tests requested	Remarks**
Date:			Signature:	

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

** Information on pooling of sample, storage condition before dispatch, other staff or teams involved (disease investigation/RRT), concerned agencies informed etc. should be given

LABORATORY SAMPLE DATA SHEET (Wild birds)
Ministry of Agriculture
Department of Livestock

Submitter information	Incident information
Submitter's name: ……..	Date of observation……
Dept/Organization: ……	Date of report: ……
Address: ……	Location (exact location – with GPS data if possible:
……	……..
Fax: ……..	Gewog: ……..
E-mail: ……	Phone: ……..
Signature:	Mobile: ……

Animal details:

Species affected (common name, genus and species):
……

Total of each species: ……Unaffected/Normal: ……Sick: …… Dead: ……..

Approximate ages of affected animals: Chick: ☐ Juvenile: ☐ Adult: ☐

Sex of affected animals: Unknown: ☐ Male: ☐ Female: ☐

Description of incident: ……..

……..

Environmental conditions: Weather, recent rainfall, changes in ground water levels, changes in domestic animal management:

……

Clinical signs in animals:

……

Gross pathology findings:
……..

Management actions taken: …… Please use additional pages as necessary for thorough descriptions and additional observations

Spp	Animal ID	Loc	Live/Dead Euthanized /Method	Carcass kept Fresh/ Frozen	Serum/ Plasma	Swabs collected Tracheal/ Cloacal	Tissues Fresh /Fixed	Photo Yes/ No	Collector
Specimen stored/sent where?									
Names of all people present during sample collection:									
Signature									

7.20 SOP FOR RESTOCKING OF POULTRY FARMS

Purpose

Poultry farms are depopulated after confirmation of HPAI outbreaks to stop further spread of this highly contagious infectious agent. Since HPAI virus can survive in the environment under certain circumstances, depopulation must be followed by secure disposal of all possibly infected materials. Before restocking 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.

Scope:

This SOP provides the guidelines for re-stocking of poultry birds after culling and disposal, and applying decontamination measures to re-populate the farms safely and smoothly.

User or target:

The SOP is intended for the Department of Livestock and BAFRA to facilitate quick and sound re-stocking of the farms affected by HPAI.

Restocking team members:

The Dzongkhag Livestock Sector will take lead to initiate restocking of poultry birds in the protected zone in close consultation with RLDC and technical experts from DoL and BAFRA. The team will be composed of veterinary epidemiologist and laboratory personnel and BAFRA Official to conduct risk assessment, laboratory testing, and surveillance.

- Epidemiologist/ Veterinary Officer, DoL
- Laboratory Personnel, DVH/ RLDC/ NCAH
- Livestock Production Officer, Dzongkhag Livestock Sector
- Regulatory and Quarantine Officer, BAFRA

Steps for restocking

Preconditions for restocking (refer SOP for culling, disposal, decontamination, etc)

- Complete culling and secure disposal of all poultry kept on the farm after the confirmed outbreak
- 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
- Disinfection after outbreak containment accomplished
- Intensified surveillance in the Surveillance Zone is in place
- No suspected case of HPAI in poultry or wild birds under current investigation within 10 km radius and or as determined by the risk assessment team.

Time for introduction of sentinel flock:

- At least 42 days of rest period after final culling and disposal;
- No confirmed outbreak of HPAI in poultry or wild birds within 10 km radius (Surveillance Zone) or as determined by the risk assessment team during the last 42 days;

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 to HPAI are the most suitable sentinel birds. Sentinel birds should be used prior to restocking in commercial farms. Their use in village chicken production should be targeted in those household(s) that have been affected by the disease. Introduction of sentinel birds can be used to shorten the period of rest prior to restocking.

Where to introduce sentinel birds?

– Commercial farms with confirmed outbreaks; and
– All commercial farms/select households within the Infected Zone (1 km radius)
– 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.

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 surveillance zone;
– Free of disease (no clinical symptoms of any disease);
– Flock of origin vaccinated against Newcastle Disease regularly;
– Complete records on productivity and mortality of the flock of origin are available; and
– Flock of origin checked clinically by a veterinarian 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:

How many sentinel birds per poultry house?

– One percent of the poultry house population, but a minimum of 5 birds per poultry house for commercial farms. For village conditions, 5 birds per household in 10% of the affected households

When to introduce sentinel birds?

– Sentinel birds may be introduced immediately after deactivation of IOC.

Premises can be proved to be free from HPAI through the sentinel birds at least 14 days after their introduction. For Commercial farms the owners will bear the cost of the birds and in the villages the Government will have to bear the cost.

Course of action

Step 1: check preconditions according to checklist (table 1)
All preconditions must be fulfilled before moving on to the next step.

Table 1: checklist for restocking of poultry in village/farm

Sl no	Precondition	Yes	No

1	Complete culling and secure disposal of all poultry kept on the Protected Zone after the confirmed outbreak.		
2	Disposal site secure, checked by veterinary authorities (RRT).		
3	Disinfection after outbreak containment accomplished at least 42 days before restocking, documented.		
4	No droppings or litter from possibly infected poultry remaining in the Infected/Protected Zone since the outbreak.		
5	No eggs, feathers or other poultry products from possibly infected poultry remaining in the Protected Zone since the outbreak.		
6	No poultry feed used for possibly infected poultry remaining in the Protected Zone since the outbreak.		
7	Intensified surveillance in the Surveillance Zone conducted.		
8	No confirmed outbreak of HPAI in poultry or wild birds within 10 km radius (Surveillance Zone) during the last 42 days.		
9	No suspected case of HPAI in poultry or wild birds under current investigation within 10 km radius (Surveillance Zone).		
10	Sentinel birds found healthy/ no sign of HPAI after 14 days of introduction		

7.21 COMPENSATION GUIDELINES

Background

Early detection and reporting as well as rapid response to outbreak of Highly Pathogenic Avian Influenza (HPAI) H5N1 and AI H7N9 depend critically on the incentives for poultry owners to report quickly any sick and at-risk poultry or other birds to the veterinary authority. In suspected and/or confirmed cases of AI, the Government decides mandatory culling. Without adequate compensation arrangements in place, poultry owners will have no incentive to report any sick and at-risk poultry that may result in the loss of all the flock and pose threat to human health. Therefore, it was felt essential to establish a fund within the Government to compensate the affected poultry owners from mandatory culling and inform them in advance about the availability of such funds.

The guideline intends to provide information on the operational aspects of the compensation fund with an aim to ensure quick and fair financial compensation to the affected poultry owners in the event of an enforced culling during the outbreak of HPAI H5N1 and H7N9. The Livestock Act of Bhutan 2001 under its sub-para 9.3 clearly states that the government has the authority to compulsorily destroy animals, animal products or feeds or any consignments that it considers to be risky and pay compensation as prescribed by the Ministry.

Objectives

The main objective of this guideline is to outline the operationalization and payment procedures for compensation modalities in the event of HPAI outbreak in the country.

Eligibility for Compensation

Compensation will be paid in case of mandatory culling of poultry birds approved by the government as part of containment procedures for HPAI outbreak. Compensation will not be paid for birds that have died as a result of any disease, including HPAI. State-owned poultry farms are not eligible for compensation payments. The DoL shall encourage the commercial and semi-commercial poultry entrepreneurs to insure their poultry birds and farms against poultry diseases including H5N1/H7N9 to reduce the risk. The Compensation Committee shall liaise with the insurance companies in expediting insurance claims wherever applicable.

Compensation payments will be made only:

– if mandatory culling measures have been announced and put into effect by Government/ NICC);
– for poultry culled, eggs, feed and feed materials disposed, and coops/ sheds destroyed under the supervision of 3-D team (Depopulation, Disposal and Decontamination);
– based on completion of all documentation as prescribed in this guideline;
– as proposed by IOC and after verification by compensation committee after identifying those eligible for compensation payments, ensuring that there will be no multiple claims;

– If the commercial/semi-commercial entrepreneurs have not received insurance claim from the concerned agency.
– Categories of poultry/materials to be compensated
– Adult Poultry (>4 months).
– Young Chicken (< 4 months).
– DOC
– Adult Ducks
– Ducklings
– Adult Turkey (> 4 months).
– Young Turkey (< 4 months)
– Coops, baskets and egg trays in the affected backyard and village chickens
– Eggs already existing in the farm (table and hatching)
– Feed and feed materials already existing in the farm
– Other species
– Other birds if any

Disbursement mechanisms of Compensation Fund

The NICC shall approve the compensation payments as and when proposed by the Compensation Committee through the Incident Operation Centre (IOC) as detailed out in Figure 5. Quarantining, culling, disposal, and disinfection will be undertaken by the IOC in line with provisions laid out in NIPPP 2019.

The IOC under directives from the NICC will implement the compensation procedures. A committee will be instituted to implement the actual compensation calculation and payments of compensation as per the rates approved by MoF. The compensation rates should be reviewed by TAC from time to time and revised rate proposal if required should be submitted to MoF through NICC.

Compensation Committee Members

The compensation committee will be formed as per the provisions outlined in NIPPP 2020 consisting of members as given below.

The Compensation Committee shall be composed of the following members:

A: Dzongkhag level:

– The Gup /Dzongdag/Dzongrab or representative – Chairperson
– Dzongkhag Livestock Officer – Member Secretary
– The Dzongkhag Disaster Management Officer - member
– BAFRA RQO/RQI Livestock– Member
– Representative from RLDC – Member

B: Thromde level:

– Thrompon – Chairman
– Dzongkhag Livestock Officer – Member Secretary
– Thromde Disaster Management Officer - member

– BAFRA RQO/RQI Livestock– Member
– Representative from RLDC – Member
– Thromde Thuemi (in Thromde areas) - member

In the event of an outbreak is away from Thromde or Dzongkhag Head Quarter, Gup will be Chairperson and Mangmi/ Tshogpa as committee member.

Responsibilities of compensation committee

The compensation committee shall ensure provision of compensation in a fair, transparent and timely manner to all eligible owners/farmers. The Compensation Committee will be responsible to:

– Verify and approve the list of poultry and other products eligible for compensation in the villages/farms
– To make payments to the eligible poultry farmers in a fair, transparent and timely manner.
– Compile daily records of the culled birds, properties/ materials destroyed and owner details (address, main occupation etc);
– Review the market value of the birds and eggs; and propose revision of compensation rates to TAC through IOC if required.
– Get proof of payment from the recipient of the compensation
– Submit completed documents to the IOC for payments made.

Role of IOC on compensation activities

The IOC will be responsible for the following tasks:

– Call for immediate meeting of the compensation committee in line with the provisions of the NIPPP 2019.
– Provide forms to the 3D teams and compensation committee for recording details of poultry culled, eggs/coops/feed destroyed etc.
– Create awareness to the owners on the compensation available for mandatory culling of poultry before the actual start of 3D operation.
– Review and approve the compensation rates proposed by the committee for further submission to the NICC through TAC.
– Maintain proper books of accounts for all compensation made for future auditing.
– Follow up with NICC for timely release of compensation fund for further disbursements.
– Compensation will be paid as soon as funds are received from the Ministry of Finance through the concerned RLDC.

Compensation procedures

Upon official declaration of outbreak of HPAI by the Government, the NICC will activate IOC at the outbreak area. The IOC will carry out 3-D Operations and movement control in the protection zone while the compensation committee will initiate processing of payment of

compensation to the eligible owners. Budgeting and disbursement of the compensation should be undertaken as per the process given below.

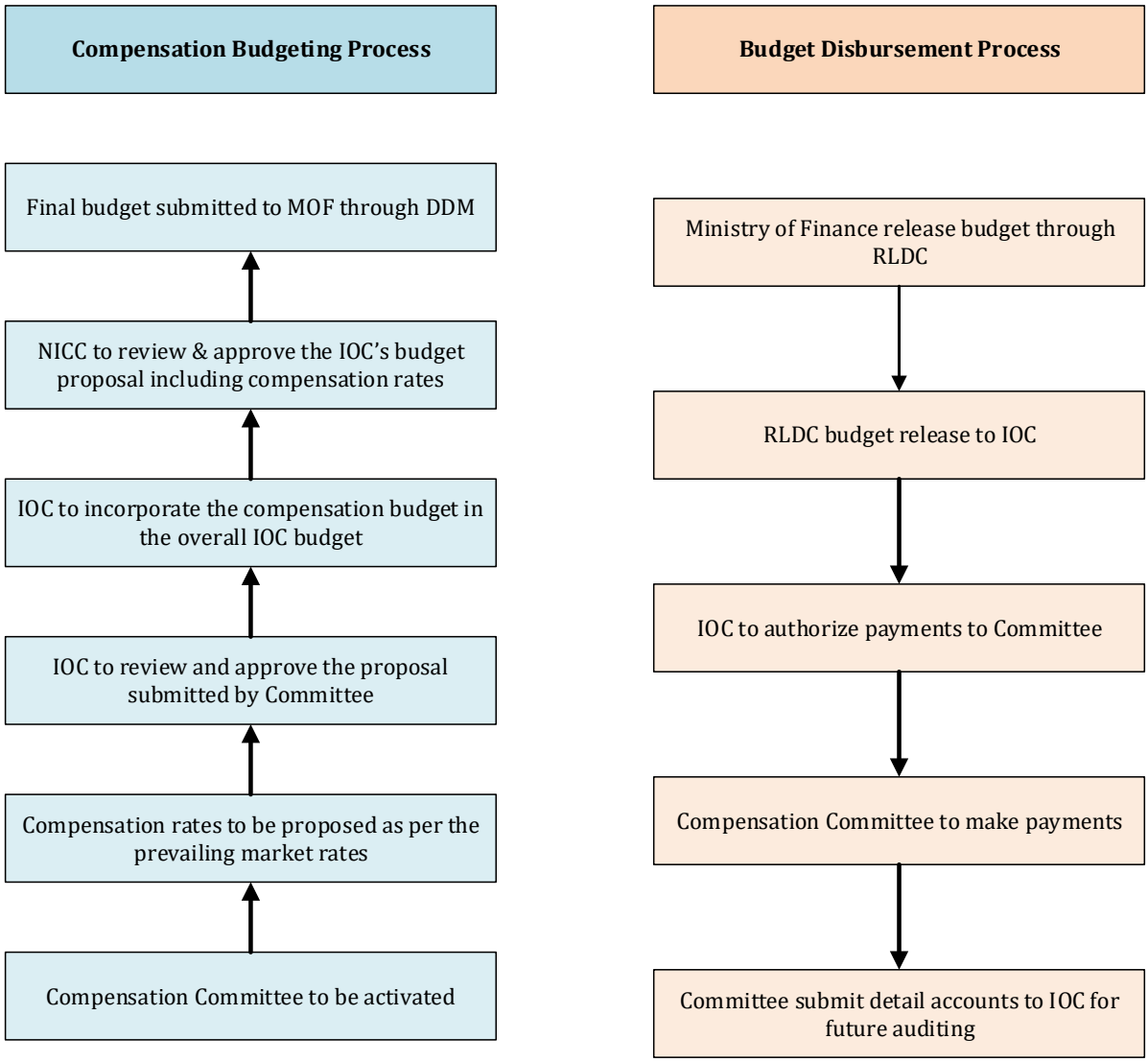


Figure 8: *Budgeting and disbursement process for compensation*

Compensation Calculation

For the purpose of the Compensation Fund, holders owning up to 500 birds are considered as "village/backyard farms" and the ones having more than 500 birds are considered as "commercial enterprises". However, compensation for backyard poultry owners for which poultry birds are reared for commercial purpose under improved housing and management conditions shall be based on the actual cost of production approach as laid down for commercial farms after considering the cost of reusable materials. The rates proposed by the Compensation committee will be further reviewed by TAC and submitted to MoF through NICC when revision is required.

Backyard farms

The backyard farms will be compensated according to "market value approach". Payment per bird will be at the rate of 75% of the market price of the poultry and eggs as reported by the district authority during the preceding weeks before culling took place. The list must be endorsed by the Compensation Committee and the IOC before it can be used. It should also be noted that the price of backyard local breeds tends to be higher than the prices of commercial breeds within the same locality. The rate for other consignments/ coops will be estimated by the Compensation Committee in consultation with the 3-D Team and upon final approval by the IOC.

Commercial farms

The commercial farms will be compensated according to what is called "Actual cost-of-production approach"

Layers:

Less than 1-month-old: at the cost of "Day-Old-Chicks" (the farm gate cost of the chicks if procured or the Compensation Committee will find out the actual cost of production from published studies or by actual calculation after visiting a similar farm).

Between 1 month to 4 months: at the cost of 1-month-old birds (the Compensation Committee will find out the actual cost of production from published studies or by actual calculation after visiting a similar farm)

Above 4 months (adult category): at the cost of 4 months (the Compensation Committee will find out the actual cost of production from published studies or by actual calculation after visiting a similar farm)

Spent Hens (above 72 weeks)

– 25% of cost of adult birds after 72 weeks of age

Broilers:

Less than 3-weeks-old: at the cost of "Day-Old-Chicks" (the farm gate cost of the chicks if procured or the Compensation Committee will find out the actual cost of production from published studies or by actual calculation after visiting a similar farm).

Above 3 weeks: at the cost of 3-weeks-old birds (the Compensation Committee will find out the actual cost of production from published studies or by actual calculation after visiting a similar farm).

For other birds (Ducks, Quails, Turkeys, Geese, Swan etc)

At the rate of 75% of the market price of the culled poultry (the Compensation Committee will establish the actual market price through enquiry)

It has to be specified that poultry categories and compensation rates will be implemented homogeneously across the nation so as to avoid movement of poultry and thereby enhancing

disease spread. For eggs, feed and feed materials farm gate prices or the prices established by the Compensation Committee will be applicable.

Mode of payment

The Compensation Committee will collect the copy of slips from the 3-D Team which should have details such as the owners name, location and what (birds, housing, eggs) had been destroyed. The details in the slip should be entered in the individual owner culling certificate (Form 1) and District summary culling record form (Form 2). The owner should produce the slips to the compensation committee and the payment will be processed after thorough cross checking by the compensation committee. The compensation rates for culled birds and other items will be based on rates approved by the Ministry of Finance which will be reviewed from time to time by the TAC based on the rates estimated by the Compensation Committee during the HPAI outbreaks. The payment of compensation to the owner of a commercial farm will be done in the form of cheque. For the backyard farmers, payment shall be made in cash. The compensation will be paid as soon as funds are released by the government.

Forms for Culling and compensation

The following forms need to be printed and kept ready at NCAH for use in case of an outbreak:

- Form 1 - Owner's Poultry Culling Certificate
- Form 2 - District Summary Culling Record

The forms for individual farmer/farm (Form 1) will be made in four copies (four different colours) and will be given each to the poultry owner and Compensation committee, one attached with payment voucher and one to be retained in the booklet.

The individual culling forms (form 1) will be completed for each poultry owner or enterprise. The form includes information such as personal and demographic data, number/type and age of poultry that are culled by category and the names and signatures of the Compensation Committee, and the poultry owner. A summary of the compensation made will be filled up in Form 2 by the committee for submission to IOC for records.

When a decision for mandatory culling is taken, the relevant forms will be sent to the Incident Operation Centre (IOC) by NCAH. The IOC will sign a "receipt of delivery" after checking that the forms: are intact, numbered correctly, in the amount agreed with the Fund Administrator.

The NCAH will maintain a database containing the numbers of all forms provided to each IOC. The IOC is required to return all unused forms, as well as any invalid or incorrectly completed forms to the NCAH when the culling records are submitted. The NCAH will also maintain a National level database for all the District summary records received. A comprehensive report will be submitted to the NICC by the NCAH.

FORM 1: Owner's Poultry Culling Certificate

Owner's Poultry Culling Certificate (backyard or commercial farms)
No: (pre-numbered)

Dzongkhag:	Gewog:	Village:	Culling Date:

Name of Poultry Owner / Enterprise		
Address: Contact no.:		I acknowledge the receipt of Nu.__ as full and final settlement of the compensation as calculated below.
Establishment date if for commercial farms:		
Citizen ID No. / Farm registration no.:		
Type of farm:		***Signature of poultry owner/farm owner or legal representative***

No.	Type	Qty	Unit Price	Amount (unit price x qty)	Comments
1	Adult Chicken > 3 mo.				
2	Young Chicken < 3 mo.				
3	DOC				
4	Adult Duck				
5	Ducklings				
6	Other species:				
7	Eggs				
8	Coops				
9	Feed & feed material				
10	Others:				
Total Compensation					

This Owner's Poultry Culling Certificate Form has been witnessed and verified by the Compensation Committee

Chairman (Name Signature)	***DOL/RLDC Rep. (Name Signature)***	***DLO/VO (Name Signature)***	***Gup/Mangmi (Name Signature)***	***BAFRA Rep. (Name Signature)***	***Dzongkhag Disaster Focal Person***

Instructions for Owner's Culling Certificate forms:

– Enter for either backyard farm owners or commercial farm owners
– Enter a new line for each different type of poultry.
– Running numbers must be included in the first column, continuing on each additional sheet.
– Lines not used must be crossed out across the entire sheet.
– Culling Certificate must be signed by all the members of the District culling committee and by the poultry owner.
– Each sheet must be completed in three copies for the following distribution:
– Red copy: poultry/enterprise owner
– Blue copy: Culling committee
– Green copy: IOC

FORM 2 – District Summary Culling Record

District: ……..

No: (pre-numbered)
Village: Gewog: Date:

SL	Owner	Type of avian qualifying for compensation										Total compensation
		Chicken				Duck		Other poultry		Feed/feed materials		
		<3 months		>3 months								
		Qty	Rate	Qty	Rate	Qty	Rate	Qty	Rate	Qty	Rate	
1												
2												
3												
4												
5												
6												

A. Total poultry this sheet													

B. Total compensation this sheet

C. Cumulative poultry culled or feed disposed in the District													
D. Cumulative compensation to be paid in District													

This District Summary Culling Form has been witnessed and verified by the below District Culling Committee

Chairman (Name &Signature)	DOL Rep. (Name & Signature)	DLO/District VO (Name & Signature)	Gup/Mangmi (Name & Signature)	BAFRA Rep. (Name & Signature)	Dzongkhag Disaster Focal Person

7.22 SOP FOR OSELTAMIVIR PHOSPHATE PROPHYLAXIS DURING HPAI OUTBREAK

Purpose

To give antiviral prophylaxis for high and moderate risk group during HPAI outbreak to prevent human transmission.

Scope

This procedure is applicable to Medical team for giving Oseltamivir prophylaxis during HAPI outbreak.

Users

Medical team during HPAI outbreak

Manpower

- Medical officer
- Pharmacist/Pharmacy Technician

Definitions

High risk group: Sharing household with or caring for a Patient. Unprotected close contact (<1 m) with patient

Moderate risk group: Persons handling sick animals or decontaminating environment without PPE. Direct exposure to sick/dead animals Infected with H5N1.Health-care worker in direct contact with patient without complete PPE laboratory personnel who might have unprotected exposure.

Low risk group: Health-care worker with PPE or contact >1 m with a patient, cullers of non-infected animals, persons with PPE handling sick/dead birds and dead human body or contaminated environment.

Materials required

- Gloves
- Mask
- Capsule Oseltamivir
- Hand sanitizer
- Weighing Scale
- Dispensing envelope
- SOPs and Guidelines
- Annexure form I

Procedure

- Identify high and moderate risk group amongst IOC members followed by in outbreak area
- Provide prophylaxis dose as per the guideline
- Document the details in the annexure Form I
- Follow up for compliance and ADR using Form I

7.23 SOP FOR HEALTH CONTROL TEAM AT THE SITE OF HPAI OUTBREAK

Purpose

To guide the Health Control team at the site of HPAI outbreak to effectively respond and prevent transmission of the virus from animal to human

Scope

This document describes the role of health control team at the site of outbreak

User

Health Control Team

Man Power

- DHO
- Medical officer
- Pharmacist/Pharmacy technician
- Laboratory personnel
- Nurses

Note: the number of health team members shall be determined by the nature of work on the particular day.

Materials/equipment required

- Forms annexure II
- Forms annexure I
- Note pads and pens
- RDT test kits
- Cold box/ice packs
- Swab sticks
- Virus transportation media
- First aid kits
- PPE set
- Antiviral medicines

Procedure

- Report to IOC
- Brief roles and responsibilities by the team leader and also activate triage team at the health centre.
- Provide antiviral prophylaxis to the risk groups of RRT members as per Oseltamivir SoP
- Enter the index zone after wearing PPE
- Screen index zone population and identify risk groups
- Provide prophylaxis to risk groups as per oseltamivir SoP
- Simultaneously screen for ILI signs and symptoms of all exposed population and document details using annexure form I

- Document all ILI suspicious cases on annexure form II
- Collect throat swabs from suspicious cases and do RDT at the site
- Follow sample collection and shipment procedure as per the existing guideline
- Do daily clinical surveillance for RRT members and population in the outbreak area
- Compile reports and submit to IOC on daily basis until deactivation of IOC
- Manage positive human cases as per the existing guidelines

POULTRY SURVEILLANCE FORM

Note: **Door to door survey form**

<table>
<tr><td colspan="2">Reference No.:</td><td colspan="2">Date:</td></tr>
<tr><td colspan="4">Name of the farm& farm owner:</td></tr>
<tr><td colspan="4">Contact telephone number:</td></tr>
<tr><td colspan="4">Address:</td></tr>
<tr><td colspan="2">Village:</td><td>Gewog:</td><td>Dzongkhag:</td></tr>
<tr><td>Geo coordinates</td><td colspan="2">Longitude (N)</td><td>Latitude (E)</td></tr>
<tr><td colspan="4">Information about the farm</td></tr>
<tr><td colspan="4">A. Type of farm:

Commercial [ ]; Semi-commercial [ ]; Backyard [ ]; Others [ ]</td></tr>
<tr><td colspan="4">B. Type of housing (tick or describe briefly)

Permanent shed with CGI roofing [ ]; Temporary shed [ ];
Coop [ ]; Others (specify)[ ]……</td></tr>
<tr><td colspan="4">C. Housing system

Deep litter [ ]; Others (specify)……..

Farming system
Free ranging system ( ) Intensive ( ) Semi-intensive ( ) Others ( )……</td></tr>
</table>

D. Bio-security arrangements in the farm

Disinfectant foot bath [ ]; perimeter wall/fence [ ];Rodent and wild bird control [ ];contact between free-ranging chickens [ ]; others (specify)……..

E. Presence of wild birds in area, give details

1. [Yes | No]

2. Species/Name

F. Poultry details

Bird type	Date introduced	Source of birds	No. of chicks (C)	No. of pullets (P)	Adults (A)	Total birds (C+P+A)	No of morbidity	No. of mortality	Remarks
Poultry Layers									
Poultry broilers									
Ducks									
Turkey									
Others									

G. Laboratory Details:

Sample Type	Bird type	No. of sample collected	Test type (field)	No. of test conducted	Total test positive	Total negative	Remarks

H. Participant information/Household information:

No. of child (C)	No of adults (A)	Guest/visitor (V)	Total member (C+A+V)	No. of people attended awareness on poultry disease	Date and time of visitor/from where?

I. Production details:

Bird Type	Total Chicken/Egg produced per week	Income generated per week (Nu.)	Market place (final outlet)	Remarks
Layer				
Broiler				

J. Contact Points

Chiwog/Tshogpa (focal point): Contact no. RNR-EC: Contact no.	
Disease outbreak Investigation Team Members	Signature
Name & Designation of team leader disease outbreak investigation	

ANNEXURE: COMPLIANCE AND ADR FORM

Name:					Age:			Sex:		
Contact No:					Weight:			Oseltamivir strength:		
Date issued:					Medication start date:					
ADR reported	Day									
	1	2	3	4	5	6	7	8	9	10	Remarks

Note:

1. Name and age of patient should be written on dispensing envelope/cover Physical counting of medicine is preferred for checking compliance
2. ADR reported should be noted down in detail
3. Risk status

ANNEXURE: HUMAN SURVEILLANCE FORM FOR ILI CASE

Sl no	Name	Age/Sex	Location	Contact no	Current ILI symptoms	Date of one set of symptoms	Result of rapid test	Suspected H5N1 case	Probable H5N1 case	Confirmed H5N1 case	RT PCR result	Geo-coordinates	Status of ILI patient

Note:

1. Any one leaving outbreak area with ILI case should be traced
2. Physical counting of medicine is preferred for checking compliance
3. ADR reported should be noted down in detail
4. RCDC to build capacity to detect N subtype of the virus

WILD BIRDS/MIGRATORY BIRDS SURVEILLANCE FORM

Sl	Species name/Id	Geo coordinates (N/E)	Total count	No. of specimen/ fresh droppings collected	Type of specimen	Morbidity	Mortality	Remarks

Team Members:

Signature

7.24 ANNEXURE: DIFFERENT INFLUENZA PANDEMIC PHASE AS PER WHO

	Phase	Transmission	Objectives	Major Strategic Actions
Pre-pandemic preparedness and Planning	1	Influenza virus subtype in animals only (risk to humans low)	Strengthen pandemic preparedness at all levels	Prepare Pandemic Preparedness Plan Establish surveillance in animals Establish collaboration between human and animal sector Establish Human influenza surveillance
	2	Influenza virus subtype in animals only (risk to humans substantial)	Minimize the risk of transmission to humans; Detect and report rapidly, if it occurs	Enhance animal surveillance and aggressive response to animal outbreaks Prevent importation of infection in unaffected countries Strengthen human surveillance Stockpile antiviral, PPE etc Collaborate between different sectors and WHO/OIE/FAO Develop & implement risk communication strategy Prepare health & essential service contingency plan
Emergency Response & pre-emptive	3	Human infection (Transmission in close contacts only)	Ensure rapid characterization of new virus Detect, notify and respond to additional cases	Enhance animal surveillance and aggressive animal outbreak containment Enhance human surveillance and aggressive outbreak management Identify all possible contacts quickly, early & strategic use of antivirals Strengthen infection control practices in health facilities Implement risk communication strategy & social distancing Issue alert for quick implementation of health
	4	Limited human-to-human spread; small clusters <25 cases lasting <2 weeks	Contain the virus or delay its spread	
	5	Localized H-to-H spread; Larger clusters 25-50 cases over 2-4 weeks	Maximum efforts to contain or delay the spread	

				& essential service contingency plan
Pandemic	6	Widespread in general population	Minimize the impact of pandemic	Implement health & essential service contingency plan Risk communication Treat cases with antivirals, if available Social distancing: close schools, ban gatherings Administer vaccine if available.

8 REREFENCES

1. Alexander, DJ. (1997). Newcastle disease and other avian Paramyxoviridae infections. In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM (ed), Disease of poultry, 10th edn. Iowa State University Press, Ames Iowa, pp 541–570.
2. Anon (1971). Methods for examining poultry biologics and for identifying and quantifying avian pathogens. Newcastle disease, p. 66. National Academy of Science, Washington, D. C.
3. Anon. Highly Pathogenic Avian Influenza (HPAI) outbreaks. Workbook for Upazilla and district officers of Bangladesh. Developed for the USAID Avian Influenza Program.
4. Anon (2005). National Technical Committee on AI, "National Influenza Pandemic Preparedness Plan", November 2005.
5. Anon (2005). Highly Pathogenic Avian Influenza Control Programme. Sri Lanka Exotic
6. Disease Emergency Plan SEDEP. Division of Animal Health, Department of Animal Production and Health, Sri Lanka.
7. http://www.epid.gov.lk/web/attachments/article/180/SEDEP.pdf
8. Anon (2007). FAO-OIE Global Strategy for the Progressive Control of Highly Pathogenic
9. Avian Influenza (HPAI). http://web.oie.int/eng/avian_influenza/Global_Strategy_fulldoc.pdf
10. Anon (2007). National Influenza Pandemic Preparedness and Response Project. World Bank Project Implementation Manual, December 2007.
11. Anon (2006). Prevention and Control of Avian flu in small scale poultry in Bhutan. A guide for veterinary paraprofessionals in Bhutan, 2006. National Centre for Animal Health.
12. Anon (2007). Department of Livestock Contingency Manual for Highly Pathogenic Avian Influenza, Version 1.2, 2007.
13. Anon (2006). Standard Operating Procedures- Highly Pathogenic Avian Influenza (HPAI) Task Force. AusVet AI Plan.
14. Anon (2006). Australian Veterinary Emergency Plan AUSVETPLAN. Disease Strategy Avian influenza Version 3.1, 2006
15. Anon (2011). Australian Veterinary Emergency Plan. AUSVETPLAN. Disease Strategy Avian influenza Version 3.4, 2011.
16. C. Baldock, A.R., Cameron and P. Black. (1999). Principles of Disease Investigation and Surveillance in Livestock Systems. In: Pramod Sharma and Chris Baldock (ed), Understanding Animal Health in Southeast Asia. Advances in the collection, management and use of Animal Health Information. http://aciar.gov.au/files/node/479/mn58-chapters1-6.pdf
17. FAO (2004). FAO Recommendations on the Prevention, Control and Eradication of Highly Pathogenic Avian Influenza (HPAI) in Asia (proposed with the support of the OIE), September 2004. http://web.oie.int/eng/AVIAN_INFLUENZA/FAO%20recommendations%20on%20HPAI.pdf
18. FAO (2004). Guiding principles for highly pathogenic avian influenza surveillance and diagnostic networks in Asian countries. FAO Expert meeting on surveillance and diagnosis of Avian Influenza in Asia, Bangkok, 21–23 July 2004.
http://www.fao.org/docs/eims/upload//210749/aj128e00.pdf
19. FAO (2006). Manual Wild Bird HPAI Surveillance, sample collection from healthy, sick and dead birds, **2006.** ISBN 92-5-000000-0.
20. Hadrill, D. (2010). Assessment of Response to Highly Pathogenic Avian Influenza. Crisis Management Centre, FAO, March 2010.
21. Second National Incident Command Centre Meeting for Highly Pathogenic Avian Influenza, March 2010.
22. Heine H, Trinidad L, Selleck P (2005). Australian Biosecurity CRC – Technical Report - Influenza virus type A and subtype H5-specific real-time reverse transcription (RRT)-PCR for detection of Asian H5N1 isolates.

23. Lee MS, Chang PC, Shien JH, Cheng MC, Shieh HK (2001). Identification and subtyping of avian influenza viruses by reverse-transcription PCR. *J. Virol. Meth*. 97:13-22.
24. Martin, V., Forman, A., and Lubroth, J. (2006). Preparing for Highly Pathogenic Avian Influenza. FAO Animal Production and Health. Food and Agriculture Organization of the United Nations. Rome, 2006. http://www.fao.org/docs/eims/upload/200354/HPAI_manual_en.pdf
25. OIE (2008). OIE Quality standard and Guidelines of Veterinary Laboratories: Infectious Diseases. ISBN 978-92-9044-706-1. http://www.oie.int/doc/en_document.php?numrec=3831803
26. OIE (2014). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2014. http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/
27. OIE (2014). Terrestrial Animal Health Code. Chapter 10.4. Infection with Avian Influenza Viruses. http://www.oie.int/international-standard-setting/terrestrial-code/access-online/
28. Spackman, E., Senne, DA., Myers, TJ., Bulaga, LL., Garber, LP., Perdue, ML., Lohman, K., Daum, LT., and Suarez, DL (2002). Development of a real-time RT PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *J. Clin. Microbiol*. 40(9):3256-60
29. Tshering, P; Chamling, SB; NCAH, Department of Livestock, "Risk Assessment of Avian Flu in Bhutan", March 2004.
30. USDA. (2013). Highly Pathogenic Avian Influenza Standard operating procedures. http://www.aphis.usda.gov/animal_health/emergency_management/downloads/sop/sop_hpai_biosecurity.pdf
31. WHO (2004). Laboratory Biosafety Manual, 3rd edition (revised) WHO Geneva, 2004.WHO/CDS/CSR/LYO/2003.4. http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf
32. Mai-Juan Ma, Yang-Yang, Li-Qun Fang (2018). Highly pathogenic avian H7N9 influenza viruses: recent challenges, Trends in Microbiology Vol 27 (2), p93-95
33. Park S, Park JY, Song Y, How SH, Jung KS (2019). Emerging respiratory infections threatening public health in the Asia-pacific region: A position paper of the Asian Pacific Society of Respirology, Asian Pacific Society of Respirology, https://doi.org/10.1111/resp.13558
34. Guidelines, Standard Operating Procedures and Protocols-Avian and Pandemic Influenza in Bhutan, Department of Public Health