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## **EXPANDED AGRIBUSINESS AND TRADE PROMOTION (USAID E-ATP)**

*In fulfillment of the following deliverable under task 3.4.1:*

### **All best practices built into key poultry value chain operators' capacity building plans**

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Agribusiness and Trade Promotion Project  
USAID/WA  
Accra, Ghana



Abt Associates Inc. | 4550 Montgomery Avenue | Suite 800 North  
| Bethesda, Maryland 20814 | T. 301.347.5000 | F. 301.913.9061  
| [www.abtassociates.com](http://www.abtassociates.com)

*In collaboration with:*  
ACDI/VOCA  
CARANA Corporation  
ASVELIS

Global Cold Chain Alliance (GCCA)  
J.E. AUSTIN  
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# **GUIDE ON GOOD PRACTICES FOR POULTRY BREEDING FLOCKS AND HATCHERIES IN WEST AFRICA**

## **EXPANDED AGRIBUSINESS AND TRADE PROMOTION (E-ATP) PROJECT**

**January 2011**

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**EXPANDED AGRIBUSINESS AND TRADE  
PROMOTION (E-ATP) PROJECT**

## **DISCLAIMER**

The author's views expressed in this publication do not necessarily reflect the views of the United States Agency for International Development (USAID) or the United States Government.



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# ACRONYMS

<b>AI</b>	Avian influenza
<b>ARL</b>	Rapid agglutination
<b>CEE</b>	<i>Communauté Economique Européenne</i>
<b>DOC</b>	Day-old chick
<b>E-ATP</b>	Expanded Agribusiness and Trade Promotion project
<b>HE</b>	Hatching eggs
<b>HPAI</b>	Highly pathogenic avian influenza
<b>OIE</b>	World Organization for Animal Health
<b>PCR</b>	Polymerase chain reaction
<b>RPA</b>	Rapid plate agglutination
<b>SNA</b>	National Union of Hatcheries (France)
<b>USAID</b>	United States Agency for International Development



# I. INTRODUCTION

The drafting of this guide was undertaken for the United States Agency for International Development (USAID)-funded Expanded Agribusiness and Trade Promotion (E-ATP) project.

In some areas of West African, poultry producers encounter difficulties with supply of day-old-chicks (DOCs). Distribution channels for DOCs are sometimes complex and poorly controlled, leading to high health hazards (in particular highly pathogenic avian influenza, or HPAI), both for the recipient farms and the country at large.

This document proposes a set of good practices to be implemented in breeding flock farms and hatcheries in West African countries (specifically those involved in the E-ATP project: Senegal, Mali, Burkina Faso, Côte d'Ivoire, Ghana, Benin, Togo and Nigeria), in order to encourage cross-border trade of DOCs and hatching eggs (HE).

This document was prepared on the basis of field observations of parental farms and hatcheries in Côte d'Ivoire and Ghana, exchanges with several DOC producers and national veterinary services (Accra seminar in August 2010), and the documents listed below:

- Article 6.4.1 "Hygiene and Disease Security Procedures in Poultry Breeding Flocks and Hatcheries" of the Terrestrial Animal Health Code - 2010 - OIE (World Organization for Animal Health).
- Directive 90/539 / CEE of the Council of 15 October 1990, on health police conditions governing intra-Community trade and imports of poultry and HE from third countries. European Economic Community.
- Memo DGAL/SDSPA/N2000-8059 of 31 March 2000, on the conditions for the approval of establishments for the intra-Community trade of Gallus gallus. Ministry of Agriculture and Fisheries (France).
- *SNA Quality Charter in Hatcheries*. National Union of Hatcheries. France-2003.
- *The Guide on Good Practices for Poultry Breeding Farms* (France).

By complying with this guide for good practices, the hatcheries will provide a level of sanitary assurance to their customers, thus enabling greater control and traceability of traded chicks, and limiting the risks of spreading infectious agents such as AI, newcastle disease and salmonella and others.

Adherence to the *Guide on Good Practices for Poultry Breeding Flocks and Hatcheries* is primarily a voluntary decision, the benefits being to ensure good quality of traded products in conformity with international regulations, and potentially gaining new customers.

In addition to regular microbial controls carried out by accredited laboratories, the compliance of poultry farming practices with what is laid out in the guide may be certified by audits to be conducted by competent specialists in close collaboration with public and private veterinary services.

## **2. DESIGN OF PREMISES AND GENERAL OPERATION**

### **2.1 POULTRY BREEDING FLOCKS**

#### **2.1.1 DESIGN OF BUILDINGS**

##### **2.1.1.1 GEOGRAPHICAL LOCATION**

The choice of an adapted and isolated location, taking into account the direction of prevailing winds, facilitates the implementation of hygiene and prophylaxis measures. The farm must be surrounded by a fence and equipped with a gate that allows monitoring of on- and off-site traffic. Signage must be placed at the entrance to indicate that access is subject to authorization.

##### **2.1.1.2 SURROUNDINGS**

The immediate surroundings of the poultry buildings should be free of vegetation and detritus and, ideally, be in concrete or a similar material. If the implantation of trees is necessary to control the temperature this may be disregarded, but in such cases, fruit trees, which are likely to attract birds, should be avoided and low branches should be pruned.

##### **2.1.1.3 PROTECTION AGAINST WILD AND DOMESTIC ANIMALS**

Poultry buildings and feed and egg stores must be free of vermin and not accessible to wild birds. Domestic animals must not be allowed access to the premises.

#### **2.1.2 GENERAL OPERATION**

##### **2.1.2.1 INTRODUCTION OF CHICKS**

Animals used for restocking must be sourced exclusively from a poultry flock known to have an excellent health status and subject to regular monitoring of salmonella and other poultry pathogens.

##### **2.1.2.2 PRINCIPLE OF SINGLE PURPOSE FARMING**

Establishment of poultry parent flocks must be specialized, i.e. they must be devoted to a single species and ideally apply single batching principles. When several flocks are held on the same farm they should be treated as separate entities.

### **2.1.2.3 RESTING PERIOD**

When a farm or a building is emptied of its stock, all manure and equipment must be removed from the premises and cleaning and disinfection of premises and equipment carried out thoroughly.

Bacteriological monitoring of the efficiency of the disinfection is recommended in addition to visual inspection. If necessary, appropriate actions against rodents and insects must be implemented.

### **2.1.2.4 DRINKING WATER AND FEED**

All feed distributed in poultry farms must be from feed processors complying with good manufacturing practices. Samples should be collected and stored for three months. The use of pellet feed or feed that has been through another specific decontamination process for salmonella is recommended. Food must be stored in clean, closed containers. Bags and silos must be cleaned once per year. Troughs should be supplied with potable water of satisfactory quality. Two bacteriological controls should/must be carried out on drinking water per year.

### **2.1.2.5 MANAGEMENT OF DEAD BIRDS**

Sick or dead birds should be removed from poultry buildings as soon as possible and destroyed in an appropriate manner (e.g. incineration or burial with disinfectant away from poultry buildings and equipment).

### **2.1.2.6 REGISTRATION**

Comprehensive registers detailing the observed mortality, diseases diagnosed, treatments performed, and vaccinations implemented must be kept for each batch of animals.

## **2.2 HATCHERIES**

### **2.2.1 DESIGN OF PREMISES**

#### **2.2.1.1 "CLEAN AREA" AND "CONTAMINATED AREA"**

There is higher risk of increased microbe concentration during the hatching phase. The hatching area of the hatchery is more conducive to multiplication and spreading of potential contaminants. Hatcheries are thus to be divided into three zones:

- A "clean" area including the rooms where eggs are sorted, stored, preheated and incubated (also called the "egg shell" zone).
- A "transfer" area, progressing from clean to contaminated (because eggs start to hatch and microbes emerge from egg shells together with chicks), which plays the role of a buffer zone. Once the room becomes contaminated, it must be cleaned and disinfected before clean area-status can be reinstated.
- A "contaminated" area including hatching rooms, and spaces for chick sorting, shipping, washing and equipment disinfection (also called the "down" zone).

Hatchery waste must be disposed of in specific areas which ensure no possibility of contaminating chicks. The waste must be stored in an isolated room.

### **2.2.1.2 DESIGN OF SURFACES**

All surfaces (floors, walls and ceilings) must be made of materials which facilitate effective cleaning and disinfection. Floors are to be tiled or constructed of smooth-finished cement (e. g. quartz cement); walls must also be smooth-finished. Regular maintenance of these surfaces is essential. Floors should be sloped to stop pooling of stagnant water. A maintenance program for siphons and evacuation channels must be defined. Rounded joints between walls, floors and ceilings are recommended.

## **2.2.2 GENERAL OPERATION**

### **2.2.2.1 MOVEMENT OF EGGS AND “ONE WAY FLOW” PRINCIPLE**

Movement of eggs in the hatchery should be in a set direction from the clean area to the contaminated area; once in the contaminated area there should no means for an egg to return to the clean area. This principle also applies to:

- Equipment—to avoid any contact between contaminated items and washed and disinfected items.
- Staff—clothing should be changed before entering, and individuals should be allocated specific tasks in either the clean or contaminated zones. There should be no movement from contaminated to clean without washing, and change of clothing. Roles should be designed to limit the number of clothing changes in the course of operations. Staff compliance is vital; they constitute a major risk for contamination.
- Air—ventilation systems should follow clean-to-contaminated principles, or be isolated.
- Water—water should also flow from clean to contaminated areas, or isolated systems should be run in different hygiene status areas.

### **2.2.2.2 VENTILATION**

Airflows are important vectors of pathogens. It is therefore necessary to verify that doors are airtight when they are closed, and to control air flows. The air intakes should be located in non-contaminated areas. Diffusion in specific rooms is based on requirements of the room and provision of differential pressure systems so that the air flows from clean to contaminated zones.

There are different modes of ventilation, including:

- Static ventilation: air does not flow through the buildings; airtight doors provide barriers to contamination.
- Dynamic ventilation: the extraction is installed in such a way so as to avoid stale air cycling in the air intake. An input air filtration system is desirable and should be inspected and maintained according to the instructions of the manufacturer. The equipment must be accessible and easy to clean.

Sheaths of distribution, heaters and air exchangers should be cleaned at least once per month. The extraction system and conduits that lead to hatching machines must be cleaned after each hatching.

- Dual ventilation (static admission and dynamic extraction): depression should be hierarchical.

## **3. EGG HYGIENE**

The first hygiene measure to be applied in a hatchery is the sourcing of HEs from properly controlled and healthy poultry breeding flock farms. However strict the health management of a hatchery may be, it will not be effective if the introduced eggs are pathogen carriers, or vectors. It is essential that the egg-supplying farm provide verification of its sanitary status. It must be certifiably free of the key infectious agents considered in this guide (mycoplasma, salmonella, newcastle disease virus and AI virus).

### **3.1 EGG HYGIENE DURING COLLECTION**

#### **3.1.1 NESTS**

Nests should be of sufficient numbers to facilitate access by hens and limit the laying of eggs on the henhouse floor.

#### **3.1.2 LITTER**

The litter of the laying room should be dry, free of mold, and well maintained. The litter of the nests must be clean, in suitable quantity, and replaced regularly.

#### **3.1.3 FREQUENCY OF EGG COLLECTION**

Eggs are collected at least once per day and more frequently if required. Eggs should be stored in an appropriate room that is separated from the henhouse and equipped for this function, and kept in clean and disinfected containers (disposable or disinfected plastic trays).

#### **3.1.4 EGG SORTING**

Eggs laid on the ground are separated; very dirty, deformed, white or cracked eggs are removed during the first sorting. Slightly dirty eggs (where surface dirt is not larger than the size of a large coin) are dry-cleaned with light rubbing using an abrasive cloth or sandpaper, or by cleaning and disinfection using an approved system such as a dilute anti-microbial solution (see section on egg disinfection).

#### **3.1.5 DISINFECTION OF EGGS AT THE FARM**

Eggs shall be disinfected on the farm before transportation to the hatchery (see below chapter on egg disinfection).

## **3.2 TRANSPORT**

### **3.2.1 CONTINUITY OF ENVIRONMENTAL CONDITIONS**

During the transfer of eggs from the farm's storage room to the hatchery's storage room, it is essential to ensure the continuity of storage conditions (especially temperature). Ensure that condensation does not form on the surface of the eggshell as condensation can facilitate the penetration of bacteria.

### **3.2.2 PACKAGING**

Eggs must be transported to the hatchery in new or well-cleaned and disinfected packaging (e.g. disposable cartons, disinfected plastic trays, new egg boxes or disinfected incubation racks). All transportation packaging should be fumigated or disinfected using an effective liquid disinfectant prior to use. Routine operations of the hatchery or parental flock farm must include the cleaning and disinfection of vehicles and equipment.

### **3.2.3 VEHICLES**

Transportation of eggs to the hatchery is carried-out using decontaminated trucks, which have been washed and disinfected after and/or prior to each delivery. The driver's cab is clean and without dust. Transport vehicles must not carry eggs and chicks simultaneously. It is essential to design and implement a systematic plan for comprehensive cleaning and disinfection of vehicles used for the collection of eggs and chicks, and a rigorous monitoring of trucks departing for the loading of hatching eggs (HE) and having previously delivered chicks. In fact, this practice is not recommended as it increases the risk of contamination of poultry breeding farms.

## **3.3 AT THE HATCHERY**

### **3.3.1 DISINFECTION OF EGGS: PRINCIPLE**

The disinfection of eggs and trolleys is one of the most effective means of preventing the introduction of contaminants. Disinfection should occur on the farm and/or at the hatchery depending on the hygiene plan adopted by the hatchery. Implementation should follow protocols which have been rigorously assessed in terms of their effectiveness. On-farm disinfection immediately after egg collection is most effective. The required concentrations, temperatures, humidity and duration are formalized in a written procedure and complied with.

### **3.3.2 DISINFECTION OF EGGS: METHODS**

The disinfection of eggs consists of:

- Formaldehyde fumigation.
- Application of a disinfectant for eggshells through spraying or immersion, in accordance with the instructions of the manufacturer.
- Application of other recommended sanitation procedures approved by the veterinary services.

The disinfection of eggs must be carried out using a product registered in accordance with national legislation. Manufacturer instructions must be respected and implemented scrupulously.

# 4. HATCHERY HYGIENE

## 4.1 GENERAL PRINCIPLES OF CLEANING / DISINFECTION

Ensuring the tidiness of the premises is an essential step in cleaning and disinfection operations. It reduces the risk of sheltering materials being contaminated by other objects, and facilitates access to surfaces.

### 4.1.1 CLEANING

#### 4.1.1.1 MECHANICAL CLEANING

A contaminant's adherence increases with time. Mechanical cleaning must be implemented as soon as possible after the work period. Sweeping of rooms should be administered on wet floors to avoid dispersing dust in the air. The use of a broom is not recommended; mops and scrapers are preferable. Collecting and removing as much superficial waste, dust and other detritus before using detergents and disinfectants is essential.

#### 4.1.1.2 CHEMICAL CLEANING

Chemical cleaning is realized through the administration of a water and detergent solution. This process is designed to remove organic and inorganic matter present on surfaces, where concentrations of contaminants are likely to be high; it does not, however, eliminate microorganisms. The concentration, temperature and duration of action are important for the effectiveness of detergents. Acid products will eliminate deposits of tartar and renovate stainless steel surfaces. Alkaline products are active on organic materials.

#### 4.1.1.3 RINSING

Rinsing removes the last traces of dirt and detergent. At the end of this operation, surfaces appear clean on visual inspection.

### 4.1.2 DISINFECTION

#### 4.1.2.1 DEFINITION

Disinfection is designed to destroy microorganisms remaining after cleaning and intermediate rinsing.

#### 4.1.2.2 USABLE DISINFECTANTS

The main categories of disinfectants used are:

- Halogens (chlorine, iodine).
- Quaternary ammonium.
- Aldehydes.
- Phenols.
- Peroxides.

Factors which may influence the effectiveness of a disinfectant include:

- The nature and condition of surfaces.
- The bacteriological and physico-chemical quality of the dilution water.
- Concentration.
- Temperature.
- The type of detergent used for preliminary cleaning.

The selected disinfectant should also meet a number of additional requirements. For example, it should be effective across a broad spectrum of microbial contaminants, effective at low concentrations, harmless to users, chemically stable, and non-corrosive.

These requirements should be defined in disinfection protocols.

Protocols should comply with manufacturer recommendations for use of their product.

The specific disinfectants and disinfection protocols must be approved by the veterinary authorities of the country of operation, and comply with international regulations.

## **4.2 SPECIFICITIES**

### **4.2.1 INCUBATORS/SETTERS**

The incubator cleaning program must be monitored. It is good to provide a periodic sanitary vacuum for the incubators, per incubation room and per turnover. This way, disinfection can be associated with a general revision of the machines. Dirt can quickly pile up on and behind the incubators. Regular cleaning of these parts is carried out.

### **4.2.2 HATCHING MACHINES/HATCHERS**

After each hatching, the interior and exterior of the hatching machines, the hatching room(s), and the dust- and down-evacuation channels must be thoroughly cleaned, washed and disinfected. It is preferable to have several hatching rooms, each corresponding to a different hatching day, to allow a complete break and effective cleaning and disinfection without affecting following batches. The hatching machines and trolleys must be dried before restocking.

### **4.2.3 ROOMS**

The floor of the incubation room must be cleaned, washed and disinfected at least once per week. The hatching rooms must be cleaned, washed and disinfected after each hatching. Transfer, sorting and

shipping, and equipment-washing rooms must be washed and disinfected after each work period (i.e. each time a batch is moved). This is also desirable for the egg-sorting room, which at minimum must undergo this treatment at least once a week. Egg-unloading and chick-loading platforms must be cleaned, washed and disinfected at the end of each day in use.

#### **4.2.4 EQUIPMENT**

All equipment used (trolleys, racks, etc.) must be washed and disinfected after each use and before storage.

# 5. STAFF HYGIENE

## 5.1 STAFF TRAINING

Hatchery staff must be actively involved in the sanitary operations of the hatchery. Staff must be rigorously trained, in theory and practice, on the following:

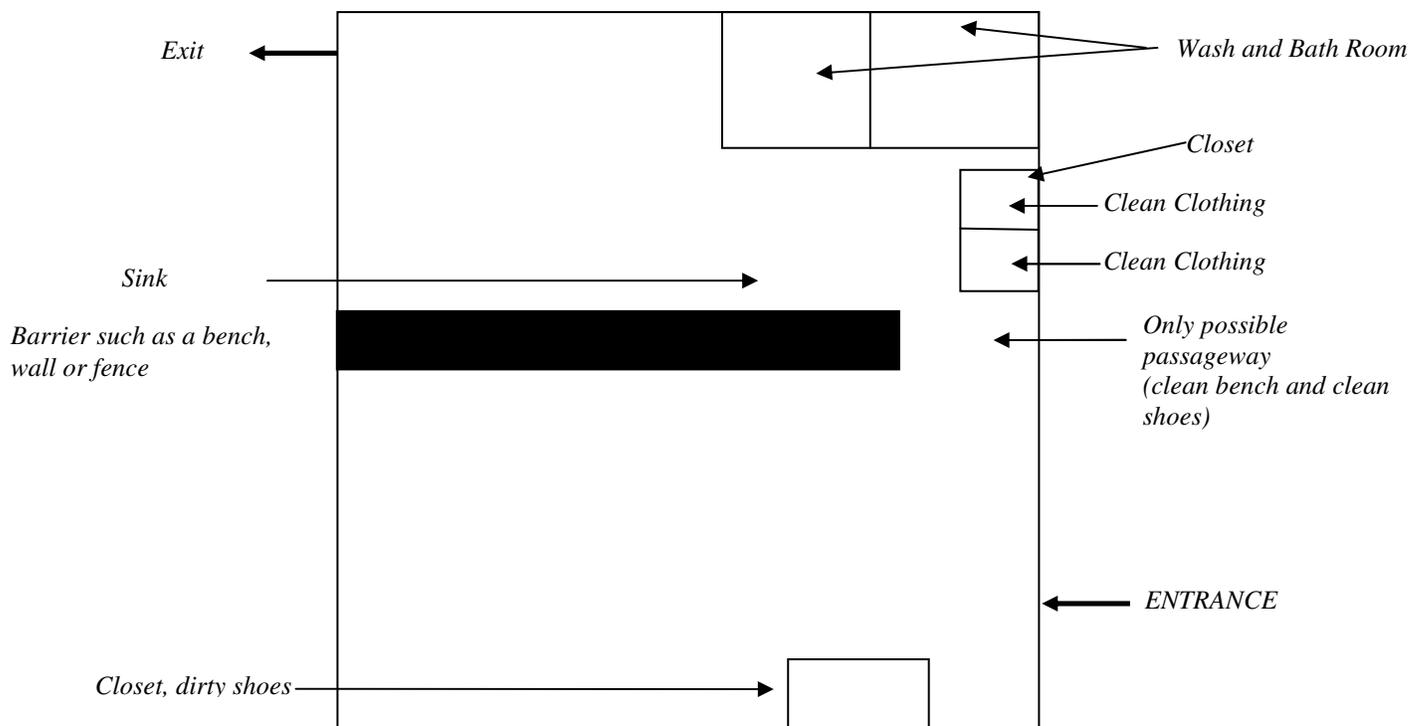
- The nature of contaminants, in particular the operational conditions which facilitate their multiplication and transmission.
- Cleanliness and disinfection.
- The role of staff in the transmission of contaminants.
- The rationale for sanitary protocols and implementation of measures for prevention.
- The potential risks to staff health from zoonotic pathogens and toxins.
- The potential human health risks associated with poultry raising both at home and in the workplace.

## 5.2 STAFF CHANGING ROOM

### 5.2.1 PRINCIPLE OF THE CHANGING ROOM

The changing room is a key barrier against human transmission of pathogens to the hatchery. The staff changing room constitutes the entry point to the clean zone(s) of the hatchery; the changing room must be the only passage from dirty zones (within or outside the hatchery) to the clean zone. The changing room must comply with the one-way flow principle. The changing room should be designed with a physical separation (bench or other appropriate barrier) between clean and dirty sections. A strict protocol for moving from the dirty section to the clean section (e.g. change of shoes, clothing and washing of hands) must be instigated. The changing room should be kept clean at all times. Cleaning should occur at the end of each work day.

## 5.2.2 CHANGING ROOM DESIGN



## 5.3 WORK WEAR

Upon entry into the hatchery, staff must change into appropriate work clothing. Work clothing should protect the personnel from dirt and other contaminants; work clothing should not be worn outside the hatchery and should be cleaned frequently (this will depend on the level of activity). Showering using anti-microbial products is recommended where feasible.

Work clothing should include:

- A cap covering hair, especially for staff working in the hatching zone.
- A face mask.
- A coat or a blouse.
- Shoes.

The coats should be made of an easy-to-clean fabric such as cotton-polyester. Coats and shoes must be washed regularly. Hair caps and masks should be disposable and not reused. Maintenance personnel must also adhere to work clothing requirements. Visitor clothing kits, providing the same level of protection, should be available for visitors. Entry into the hatchery should be forbidden for those failing to comply with clothing regulations. Irregular external employees such as de-beakers, sexing specialists, and maintenance technicians, must also meet these standards before entry. People represent a major

contamination risk to any hatchery. This is why minimizing the risk of human transmission of contaminants through the implementation of strict sanitary measures is paramount.

## 5.4 FEET AND HAND WASHING

The use of a pediluvium at the entrance to critical areas is a decision to be made by the company; it is recommended. To be effective, footbaths must be:

- Strategically positioned.
- Unavoidable and readily usable—ensure correct placement and sufficient length and depth.
- Well-maintained and regularly disinfected; the solution should be replaced regularly dependent on through-traffic and location in the hatchery.

Staff must wash their hands:

- At the entrance to the hatchery. If possible hand-washing should occur after removing shoes and before handling clean clothing for the hatchery.
- Between workstation changes.
- After use of toilets.

It is also recommended that for critical, high-risk operations (e.g. sexing), personnel wash their hands between the handling of different batches.

Hands-free taps are compulsory outside toilets. Sinks should be positioned throughout the hatchery at points considered critical and especially at cross-points between clean and dirty zones.

Each sink must be equipped with:

- A bactericidal liquid soap dispenser.
- Disposable hand towels.
- A disposal receptacle for used hand towels.
- A nail brush, particularly in locker rooms and the “down” zone.

# 6. HEALTH PROGRAM

## 6.1 REGISTRATION

All breeding flock farms and hatcheries should have written and displayed protocols for vaccinations, surveillance (sampling and testing) and biosecurity.

Key events in poultry breeding flock farms and hatcheries must be recorded on a daily basis. This information should include:

- Entry and exit of poultry (date, number, origin/destination).
- Health issues: disease incidence and mortality (date, number of animals concerned, diagnosis, treatments administered).
- Vaccinations delivered (date, vaccine and batch number, concerned poultry).
- Monitoring activities (date, number, type and batch of samples collected, results).
- Cleanup, disinfection, maintenance activities (date, product used, operation carried out).
- Other remarkable events.

## 6.2 INFLUENZA PROTOCOL

### 6.2.1 ORIGIN OF POULTRY

HEs and DOCs introduced into the hatchery or poultry farms must come from countries, zones or compartments declared free of AI. The supplying farms must evidence the use of appropriate disease control/surveillance measures such as regular sampling.

### 6.2.2 MONITORING PROTOCOL

Blood samples must be collected every six months and tested by polymerase chain reaction (PCR) in a certified laboratory. A minimum 15 birds must be sampled per sample batch.

## 6.3 NEWCASTLE DISEASE PROTOCOL

Breeding flocks and chicks will be vaccinated against newcastle disease according to the manufacturer's recommendations and the national legislation. As mentioned above, the recording of vaccination dates, vaccine lot numbers and method of administration must be recorded.

## **6.4 SALMONELLA AND MYCOPLASMA PROTOCOL**

### **6.4.1 BREEDING FLOCK (GROWTH PERIOD)**

#### **6.4.1.1 SALMONELLA GALLINARUM PULLORUM MONITORING**

At one day of age, 20 chicks are to be collected for serological testing by rapid plate agglutination (RPA).

At 10 days of age, bacteriological analyses are to be performed on 20 chicks (among those eliminated during sorting or recently dead chicks which have been stored frozen); analyses are performed on the organs of two sets of 10 chicks, excluding the cæcums.

Two weeks before the start of lay, for birds remaining in the same poultry building, or two weeks before transfer to the egg-laying building, 60 blood samples are to be collected for serological testing by RPA. In cases where pullets have been vaccinated, serological tests are to be performed on 60 sentinel chicks, non-vaccinated and easily identifiable. Alternatively, bacteriological analyses of organs are to be performed on a minimum 10 dead or sorted poultry. The chicks are to be tested in groups of five.

#### **6.4.1.2 SALMONELLA TYPHIMURIUM AND ENTERITIDIS SCREENING**

Environmental samples for bacteriological analysis are to be taken.

On day one, 10 samples are to be collected for analysis from the bottom of boxes used for the transportation of DOCs. A further 60 manure samples are to be collected and analyzed at four weeks, and again two weeks prior to laying.

#### **6.4.1.3 MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SYNOVIAE SCREENING**

For both blood and tracheal swabs, samples must be taken on the same day, analyzed individually and analyzed using the same techniques.

At one day of age, 20 chicks are to be collected (the same individuals as sampled for the detection of salmonella gallinarum pullorum) and analyzed using the ELISA or the RPA technique.

At age 8 to 12 weeks, and then two weeks prior to laying for birds remaining on the farm premises, and two weeks prior to transfer for birds moved to a layer-house, 60 blood samples are to be collected and analyzed by RPA or ELISA. Alternatively, 60 tracheal swabs are to be collected and analyzed using culture or PCR techniques. For mycoplasma synoviae, only 30 samples are necessary. If the flock is receiving antibiotic treatment against mycoplasma, analyses must be serological.

## **6.4.2 BREEDING FLOCK (EGG-LAYING PERIOD)**

### **6.4.2.1 *SALMONELLA GALLINARUM PULLORUM* MONITORING**

Thirty percent into the laying period, 60 blood samples are to be collected for serological analysis by RPA. (In other words, if the laying period lasts 12 months, the sampling should occur around 4 months after the laying starts.) The results of these analyses must be known before the first departure from the premises (for both chicks and HEs).

During the laying period, analyses are performed every eight weeks; these analyses should alternate between serological testing on breeding flocks and bacteriological analysis on HEs. Serological analyses by RPA are performed on 60 blood samples collected from hens. Bacteriological analyses are to be conducted on samples at the hatchery taken from 20 chicks from unhatched eggs or sorted chicks (analyses are to be performed in series of 10 on organs).

For hatcheries that systematically ship HEs prior to hatching, bacteriology on chicks is to be replaced by serology on parental stock.

### **6.4.2.2 *SALMONELLA ENTERITIDIS* AND *TYPHIMURIUM* SCREENING**

Environmental samples are to be taken for bacteriological analysis. Litter or manure samples are to be collected and analyzed every four weeks.

### **6.4.2.3 *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* SCREENING**

At the inception of the laying period, 60 blood samples are to be collected for serological analysis by rapid agglutination (ARL) or ELISA. Every eight weeks during the laying period, either 60 blood samples are to be collected for analysis by ARL or ELISA or 60 tracheal swabs are to be collected and analyzed using culture or PCR techniques. The 60 samples (either all blood samples or all swabs) must be collected on the same day, and analyzed individually using the same technique. For mycoplasma synoviae, only 30 samples are required.

## 7. SUMMARY TABLE: PROTOCOLS FOR THE MONITORING OF POULTRY BREEDING FLOCKS

<b>Disease</b>	<b>Poultry-farming period</b>	<b>Egg-laying period</b>
Highly pathogenic avian influenza	PCR by accredited laboratory. At least 15 animals taken from each breeding flock batch every 6 months.	
Newcastle disease	Vaccination of all birds according to manufacturer's recommendations. Record dates of vaccination, vaccinated birds, vaccine lot #.	
<i>S. gallinarum pullorum</i>	Serology (RPA) on 20 one day-old chicks. Bacteriology on 20 sorted or dead chicks at 10 days old. Serology (RPA) on 60 birds 2 weeks prior to laying or transfer.	Alternating analysis every 8 weeks: Serology (RPA) on 60 birds. Bacteriology on sorted chicks or unhatched eggs.
<i>S. enteritidis</i> and <i>typhimurium</i>	Bacteriology on 10 samples from bottom of box. Bacteriology on samples of manure 4 weeks and then 2 weeks prior to laying.	Bacteriology on manure or swabs on litter every 4 weeks.
<i>M. gallisepticum</i> and <i>synoviae</i>	Serology (ELISA or RPA) on 20 DOCs. Serology (ELISA or RPA) on 60 samples at 4-8 weeks and 2 weeks prior to laying (or PCR on tracheal swabs).	Serology every 8 weeks (60 samples) or PCR on tracheal swabs.