Programmes for the eradication, control and monitoring of certain animal diseases and zoonoses

Survey programme for Avian Influenza

Romania

Approved* for 2014 by Commission Decision 2013/722/EU

* in accordance with Council Decision 2009/470/EC
PROGRAMME for ERADICATION:
ANNEX IV

Member States seeking a financial contribution from the Community for national programmes for the control and monitoring of avian influenza in poultry and wild birds shall submit applications containing at least the information set out in this form.

The central data base keeps all submissions. However only the information in the last submission is shown when viewing and used when processing the data.

If encountering difficulties, please contact SANCO-BO@ec.europa.eu

Instructions to complete the form:

1) In order to fill in and submit this form you must have at least the Adobe Reader version 8.1.3

or higher otherwise you will not be able to use the form.

Your version of Acrobat is: 10.104

2) Please provide as much information as possible, in addition to those required by fields marked with an asterisk (*) which are mandatory. Should you need clarifications on some of the information requested, please contact SANCO-BO@ec.europa.eu.

3) To verify your data entry while filling your form, you can use the “verify form” button at the top of each page.

4) When you have finished filling the form, verify that your internet connection is active and then click on the submit notification button below. If the form is properly filled, the notification will be submitted to the server and a Submission number will appear in the corresponding field.

5) IMPORTANT: Once you have received the Submission number, save the form on your computer.

6) If the form is not properly filled, an alert box will appear indicating the number of incorrect fields. Please check your form again and try to re-submit it according to steps 4), 5) and 6). Should you still have any difficulties, please contact SANCO-BO@ec.europa.eu.

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1379594772034-2708
1. Identification of the programme

Member state: ROMANIA

Disease: avian influenza in poultry and wild birds

Request of Community co-financing from beginning of: 2014 to end of 2014

1.1 Contact

Name: Cristian DUICU - General Director of the General Sanitary Veterinary Authority

Phone: +40374150258

Fax: +40374150258

Email: duicu.cristian@ansvsa.ro

2. Description and implementation of the surveillance programme in poultry

2.1.1 Designation of the central authority in charge of supervising and coordinating the departments responsible for implementing the programme

(max. 32000 chars):

At central level, the sanitary veterinary activity is represented by the National Sanitary Veterinary and Food Safety Authority (NSVFSA), lead by a President–Secretary of State and two vice-presidents.

In conformity with the provisions of the Ordinance no. 42/2004 on the organization of veterinary activity: The sanitary veterinary and food safety services are organized and they function as an unique veterinary system and they are structured as it follows:

a) the public sanitary veterinary and food safety services;

b) the free practice sanitary - veterinary activities.

The public sanitary veterinary services are organized following an unitary concept, as a system having pyramidal hierarchy control flow, on the territorial principle, as distinct and independent sector, having
Standard requirements for the submission of surveillance programmes for avian influenza

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the following structure:

a). the National Sanitary Veterinary and Food Safety Authority, denominated the Authority, is the central sanitary-veterinary and food safety authority, which technically and administratively coordinates the entire activity of the sanitary-veterinary and food safety services, it organizes and controls the carrying out of the public sanitary-veterinary and food safety activities;

b). veterinary institutes, reference institutes at national level for the specific competence fields, with legal competence, under the subordination of the Authority; there are three national reference institutes:
- The Institute of Diagnosis and Animal Health (I.D.A.H.) within which, there are national reference laboratories for all animal diseases; its representatives participate annually in „inter-laboratory tests” organized by the community reference laboratories; county sanitary veterinary and food safety laboratories functions under the technical subordination of I.D.A.H.
- Institute for Hygiene and Veterinary Public Health (I.H.V.P.H.);
- Institute for Control of Veterinary Biological Products and Medicines (ICVBM)

c). the county sanitary-veterinary and food safety directions and of Bucharest municipality, county sanitary-veterinary and food safety authorities and of Bucharest municipality, with legal competence, under the subordination of the Authority, which are constituted by the reorganization of the county sanitary-veterinary and food safety directions and of Bucharest municipality, decentralized public services which function under the subordination of the Ministry of Agriculture, Forests and Rural Development;

d) the zonal sanitary-veterinary circumscriptions and sanitary-veterinary and food safety circumscriptions, without legal competence, organized within the structure of the county sanitary veterinary and food safety directions and of Bucharest municipality;

e) the border inspection posts, without legal competence, organized within the structure of the Authority.

The state supports the activity on the protection of animal health, the surveillance, prevention and control of diseases which can be transmitted from animals to humans, by ensuring institutional and legal framework, financial resources, technical and material basis needed to develop the activities within sanitary veterinary field under the best conditions. The Romanian territory is constituted of 42 counties and Bucharest municipality, in each of them being one county sanitary veterinary and food safety direction / of Bucharest municipality (CSVFSD) which represents the competent veterinary authority at the territorial level; there are also 41 county sanitary veterinary and food safety laboratories /of Bucharest municipality.

In Romania, there are developed yearly programmes of active and passive surveillance of animals regarding major diseases and in conformity with European legislative requirements. There are approved programmes that are co-financed by the European Commission and there are national programmes yearly approved by Government Decisions and Orders of the NSVFSA President.

To promote sanitary veterinary policies in agriculture, National Sanitary Veterinary and Food Safety Authority participate quarterly at meetings with representatives of Veterinarian Society in Avian Pathology and Small Animals of Romania and Poultry Breeders Union of Romania.
2.1.2 System in place for the registration of holdings

Each commercial animal holding is sanitary veterinary authorized only if, it has biosecurity conditions and develops programmes for the sanitary veterinary surveillance of animal livestocks. All the commercial holdings are located on the map of Romania using GIS programme. In case of a disease suspicion/confirmation, the affected holding is isolated and it is maintained under control by establishing certain (minimum 3km radius) protection zones and (minimum 10 km radius) surveillance zones. Within the eradication activities, there are also used the natural and artificial barriers.

In case, the outbreak is located at the border, they should collaborate with the central veterinary authority of that country.

Poultry commercial holdings are registred and sanitary veterinary approved in order to be able to operate. The legal framework for carrying out commercial activity with poultry and poultry products is represented by:
- Council Directive 2009/158/EC on animal health conditions governing intra-Community trade in, and imports from third countries of poultry and hatching eggs;
- The Order of the President of the National Sanitary Veterinary and Food Safety Authority  no. 16/2010 for approving the sanitary and veterinary norm on the sanitary- veterinary registration / authorization  of the units / collection centers / farms of origin and of the means of transport in the field of health and animal welfare, of the establishments involved in the storage and neutralization of animal by-products not intended for human consumption and of processed products.

In order to be sanitary veterinary approved, poultry holdings shall satisfy, in accordance with the legislation in force, the following conditions:
- appropriate facilities and operation;
- application of the “Program of surveillance, prevention and animal disease control, of the diseases transmissible from animals to humans, animal protection and environment protection” approved by Order of the National Sanitary Veterinary and Food Safety Authority President .
- at least one inspection visit per year by the official veterinarian;
- additional checks to verify the compliance of the establishment with the hygiene measures and the operation of the establishments.

2.1.3 Design (risk based or surveillance based on representative sampling)

The objectives of the surveillance programmes for avian influenza in poultry are to inform the
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competent authority of circulating avian influenza virus with a view to controlling the disease in accordance with Directive 2005/94/EC by the annual detection through active surveillance for:

(a) low pathogenic avian influenza (LPAI) of subtypes H5 and H7 in gallinaceous birds (namely chickens, turkeys, guinea fowl, pheasants, partridges and quails) and ratites thereby complementing other existing early detection systems;

(b) LPAI of subtypes H5 and H7 and highly pathogenic avian influenza (HPAI) in domestic waterfowl (namely ducks, geese and mallards for re-stocking supplies of game).

2.1.3.1 Short description of predominant poultry population and types of poultry production

(max. 32000 chars)

In Romania there are: 206 holdings of laying hens, 47 holdings of chicken breeder, 1 holdings of ratites, 7 holdings of pheasants, 11 holdings of quails, 9 holdings of turkies, 2 holdings of palmipedes.
Also, the noncommercial holdings will be sampled situated in target localities, accordingly with the specific requirements for detection of infections with H5/H7 subtypes of Avian Influenza in poultry, so that samples can be considered as representative for the whole territory. The carried out risk analysis revealed the existence of 729 „target” localities. We mention that within the same noncommercial holdings situated in the respective 729 “target”localities, there are reared gallinaceae as well as palmipedes.
The poultry from backyards are grown just for family consumption.

The surveillance program for poultry from backyards is risk-based and the surveillance program for poultry from commercial farms is representative sampling based.
We sent to you (by e-mail SANCO-VET-PROG@ec.europa.eu, in August 12, 2013) the map of Romania with the “target” localities identified by the 42 sanitary veterinary and food safety counties directorates (the territorial competent sanitary veterinary authorities).

A ‘target’ locality is a territorial administrative unit consisting of 80-400 of backyards where are grown open-air birds (gallinaceous birds and palmipedes together), located beside commercial farms, wet areas, pieces of water, swamps, rivers, Danube Delta, Black Sea.

2.1.3.2 Criteria and risk factors for risk based surveillance(1)
Risk factors involved in determining high risk areas are, in principle:
- location of the holding along migratory flight paths of birds, in particular if proceeding from central and eastern Asia, the Caspian Sea and the Black Sea areas;
- distance of the holding from wet areas, ponds, swamps, lakes or rivers where migratory water fowl may gather;
- location of the poultry holdings in areas with a high density of migratory birds, particularly waterfowl;
- poultry or other captive birds kept in an open-air (outdoors) holdings, or in any other premises in which contact between wild birds and poultry or other captive birds cannot be sufficiently prevented;
- location of poultry holding in areas with high densities of poultry holdings;
- intensity of movements of poultry and other captive birds, vehicles and persons within and from holdings and other direct and indirect contacts between holdings.

Based on a risk assessment and the specific situation concerning Romania, the sampling design also considered:
(a) The types of production specific for Romania, and their specific risks: commercial farms positioned in areas that are the biotope of wild birds, backyard flocks where poultry are keeping outdoor, the vicinity with wetlands, the vicinity with water sources, lakes and pools, the presence of more than one species on the holding or other relevant factors;
(b) The number of turkey, duck and geese from commercial or non-commercial holdings to be sampled will be defined to ensure the identification of at least one infected holding if the prevalence of infected holdings is at least 5%, with a 99% confidence interval.
(c) Where holdings producing game, ratites and quails are present, they shall be included in the programme. With regard to quails only adult (or laying) breeders shall be sampled.
(d) The sample will be harvested in the seasonal production, for the commercial farms, and during the entire year, for backyards. However, where appropriate, sampling can be adapted to other identified periods at local level, during which time the presence of other poultry hosts on a holding might pose a greater risk for disease introduction (e.g. in the Danube Delta area, along Danube River and in the neighbourhood of pools and lakes that are biotope for wild birds).
(e) Surveillance will be obligatory extended to backyards flocks, their number being significant for Romania.

(1) Including maps showing target sampling sites identified as being particularly at risk for the introduction of avian influenza virus, taking into account criteria set out in point 4 of Annex I to Commission Decision 2010/367/EC.

2.2 Target populations (2)

The number of samples per holdings is minimum 5 for gallinaceous and minimum 20 for palmipedes. In
our opinion, determining the number of samples is conditioned by many factors, from biosecurity conditions, detected risk factors, the recorded morbidity and mortality percentage. The sampling of the following poultry species and production categories shall be included in the surveillance programme:

(a) laying hens;  
(b) chicken breeders;  
(c) fattening turkeys;  
(d) ratites  
(e) pheasants;  
(f) quails;  
(g) gallinaceous birds from backyards;  
(h) palmipedes from backyards;  
(i) palmiped holdings.

(2) including MS specific exceptional circumstances as described in Annex I point 3 of Commission Decision 2010/367/EU)
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2.2.1 Poultry Holdings (a) (except ducks, geese and farmed game birds (waterfowl e.g. mallards) to be sampled

Serological investigation according to Annex I to Commission Decision 2010/367/EU

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<thead>
<tr>
<th>Category:</th>
<th>laying hens</th>
</tr>
</thead>
</table>

<table>
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<th>Total number of holdings (c)</th>
<th>Total number of holdings to be sampled</th>
<th>Number of samples per holding</th>
<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
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<td><strong>120</strong></td>
<td><strong>6 360</strong></td>
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</tr>
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</table>
### Standard requirements for the submission of surveillance programmes for avian influenza

**version : 2.2**

- **(a)** Holdings or herds or flocks or establishments as appropriate.
- **(b)** Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested.
- **(c)** Total number of holdings of one category of poultry in concerned NUTS 2 region.

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**Category : chicken breeders**

<table>
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<th>Total number of holdings</th>
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<th>Number of samples per holding</th>
<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
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<td><strong>2 160</strong></td>
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(b) Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested.  
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#### Category: fattening turkeys

<table>
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<tr>
<th>NUTS (2) (b)</th>
<th>Total number of holdings</th>
<th>Total number of holdings to be sampled</th>
<th>Number of samples per holding</th>
<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
</tr>
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<td>9</td>
<td>30</td>
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**Add a new row**

(a) Holdings or herds or flocks or establishments as appropriate.
(b) Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) cannot be used, region as defined in the programme by the Member States is requested.
(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.

#### Category: ratites

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<th>Total number of holdings to be sampled</th>
<th>Number of samples per holding</th>
<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
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<tr>
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<td>Haemagglutination-inhibition-test (HI)</td>
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<td>1</td>
<td>30</td>
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Standard requirements for the submission of surveillance programmes for avian influenza

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(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.

<table>
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<th>Category : pheasants</th>
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<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
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<td><strong>Total</strong></td>
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<td><strong>7</strong></td>
<td><strong>90</strong></td>
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(a) Holdings or herds or flocks or establishments as appropriate.
(b) Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested
(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.
### Standard requirements for the submission of surveillance programmes for avian influenza

**Category:** Quails

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<th>Total number of holdings (c)</th>
<th>Total number of holdings to be sampled</th>
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<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
</tr>
</thead>
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<tr>
<td>RO 2</td>
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**Category:** Backyard flocks gallinaceae

<table>
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(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.
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<tr>
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<td>20 020</td>
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(a) Holdings or herds or flocks or establishments as appropriate.
(b) Refers to the location of the holding of origin. In case NUTS (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member States is requested.
(c) Total number of holdings of one category of poultry in concerned NUTS 2 region.

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Add a new row

Add a category
### Serological investigation according to Annex I to Commission Decision 2010/367/EU

**Category:**  backyard flocks palmipedes

<table>
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<tr>
<th>NUTS (2) (b)</th>
<th>Total number of duck and goose holdings</th>
<th>Total number of duck and goose holdings to be sampled</th>
<th>Number of samples per holding</th>
<th>Total number of tests</th>
<th>Method of laboratory analysis</th>
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<tr>
<td>RO 1</td>
<td>108</td>
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<td>30</td>
<td>3 000</td>
<td>Haemagglutination-inhibition-test (HI)</td>
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**Add a new row**
Standard requirements for the submission of surveillance programmes for avian influenza

(a) Holdings or herds or flocks or establishments as appropriate.
(b) Refers to the location of the holding of origin. In case NUTS (2) code can not be used, region as defined in the programme by the Member State is requested

Category: palmipedes holdings

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</table>

(a) Holdings or herds or flocks or establishments as appropriate.
(b) Refers to the location of the holding of origin. In case NUTS (2) code can not be used, region as defined in the programme by the Member State is requested

Add a category
## 2.3 Sampling procedures, sampling periods and frequency of testing

(max. 32000 chars):

Sampling of birds from backyards is performed by the free practice veterinarian, authorized by the sanitary veterinary and food safety county directions. By bio-security reasons, these free practice veterinarians who have backyards access, do not have access in commercial farms.
Sampling of birds from commercial farms is accomplished by the veterinarian that provides specialized technical assistance in farm (having own veterinarian’s office legally established) and he is empowered by the sanitary veterinary and foods safety authority of the county.

In the Annex IX A of The Final and Intermediate Technical and Financial Reports is mentioned, in each county, the total number of the existing commercial holdings and the total number of commercial holdings sampled (tables 1.1 – 1.7).
Also, in the Annex IX A, in each county is mentioned the total number of the identified target localities and the total number of the target localities sampled (table 1.8). In the table 1.8/IXA “holdings” means in fact, “target localities from each county”.

3. The surveillance program for poultry from backyards is risk-based and the surveillance program for poultry from commercial farms is representative sampling based.

In Romania, about 60% of poultry are reared in commercial farms which carry out biosecurity conditions and 40% of poultry are reared in noncommercial farms which don’t carry out biosecurity conditions and the poultry are reared in open-air.
Sampling from poultry and wild birds is performed according to the procedure established by National Reference Laboratory for AI/ND and delivered to all 42 county sanitary veterinary directorates/Bucharest.

The sampling period is different according to the holding type/profile, such as:
- in noncommercial holdings where the biosecurity level is low (including the method of storage of feed) the sampling is performed as a rule in spring and in autumn when the phenomenon of migration is highly; but sampling and testing are performed whenever we identify high level, in these type of holdings we use the Risk-based surveillance (RBS) method;
- in commercial holdings, we perform the surveillance representative sampling method depending on the type of holding, the category of poultry and biosecurity level.

(a) Sampling procedures shall be carried out in accordance with the Diagnostic Manual.

(b) Cloacal and tracheal/oropharyngeal swabs and/or tissues from wild birds found dead or moribund shall be sampled for molecular detection (PCR) and/or virus isolation.

(c) Specific care must be taken for the storage and transport of samples in accordance with paragraphs 5 and 6 of Chapter IV of the Diagnostic Manual. All avian influenza virus isolates of cases in wild birds shall be submitted to the EURL, unless a derogation has been granted as provided for in paragraph 4(d) of Chapter V of the Diagnostic Manual. Viruses of the H5/H7 subtype shall be submitted to the EURL without delay and shall be subjected to the standard characterisation tests (nucleotide sequencing/IVPI) according to the Diagnostic Manual.
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(d) Sampling shall not extend beyond 31 December of the year of implementation of the surveillance programme.

2.4. Laboratory testing: description of the laboratory tests used and follow up investigations

Description of the used serological tests: (max 32000 chars)

The main laboratory is the National Reference Laboratory for Avian Influenza and Newcastle disease, within IDAH Institute.

NRL for avian influenza and Newcastle disease is structured as follows:

a). Viral Serology Laboratory in which active surveillance is done for avian influenza and Newcastle disease by serological tests:
   - Haemagglutination inhibition test for detection of antibodies against avian influenza virus subtypes H5 and H7 for samples taken from Ilfov County and Bucharest
   - Haemagglutination inhibition test for detection of antibodies post vaccine against Newcastle disease virus for taken from Ilfov County and Bucharest

b). Viral Biology Laboratory in which monitoring is performed for avian influenza and Newcastle disease by virological tests:
   - Isolation and identification of avian influenza virus on embryonated SPF eggs by inoculating
   - Identify the subtype of neuraminidase by neuraminidase inhibition test
   - Isolation and identification of Newcastle disease virus by inoculation into embryonated SPF eggs
   - Pathogenicity of Newcastle disease virus by intracerebral pathogenicity index.

c). Molecular Biology Laboratory:

There are 10 accredited county labs of molecular biology, and there are situated in different parts of Romania. There are: Arad, Braşov, Braila, Calarasi, Constanţa, Iaşi, Suceava, Satu-Mare, Tulcea and Vrancea.

Experts from the NRL for avian influenza and Newcastle disease participated in a training course in Community Reference Laboratories and International Reference Laboratories. Support staff has been trained by specialists in training programs organized by the NRL.

NRL for avian influenza and Newcastle disease participate every year at the ring tests organized by LIR and LCR and other laboratories.

Avian Influenza:
   - hemagglutination and hemagglutination inhibition test for detection of antibody for avian influenza
   - diagnosis of avian influenza by virus isolation into embryonated SPF chicken eggs
   - neuraminidase inhibition test (macromethod) in avian influenza

The diagnostic tests include: screening tests (real time RT-PCR for detection of matrix protein), identification tests (identification of H5, H7 and N1) and
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viral characterization (sequencing analyses and pathotyping).

In the NRL of Molecular Biology, there are SOPs for both diseases: Avian Influenza and Newcastle disease. The protocols were recommended by the CRL of AI and ND, Weybridge, United Kingdom. The protocols described in the Avian Influenza manual are used according to our lab conditions and equipments.

In the NRL for avian influenza and Newcastle disease the methods of diagnostics are in according with OIE Manual and are accredited and validated. The reagents and kits are recommended by the CRL Weybridge. The extraction kits and the One step RT-PCR kits are validated by the producers and with these kits was performed the entire validation of the genome identification of Avian Influenza virus. All the methodology for diagnostic of avian influenza is validated and RENAR accredited.

Handling of pathological materials for diagnosis are performed in a high containment unit. The main features are: laboratories with negative pressure, ventilation system with two HEPA filters for exhaust air, decontamination of waste water, sterilization of biological waste by a double door autoclave, restricted access for trained people and mandatory shower for exit. Biorisk manual and standard operating procedures contains details about routine activity inside laboratories.

In attach you can find the laboratories coordinate surveillance for avian influenza and Newcastle disease.

Laboratory tests shall be carried out in accordance with the Diagnostic Manual.

Testing of samples shall be carried out at National Laboratories for avian influenza (NL) in Romania and at county sanitary veterinary and food safety laboratories and Bucharest sanitary veterinary and food safety laboratory under the control of the NRL.

- All results will be sent to the Community Reference Laboratory for Avian Influenza (CRL) for collation. A good flow of information must be ensured. The CRL shall provide technical support and keep an enlarged stock of diagnostic reagents. Antigens for use in the surveillance shall be supplied to NLs by the CRL to ensure uniformity.

- All avian influenza virus isolates of cases in wild birds shall be submitted to the CRL in accordance with Community legislation, unless a derogation according to paragraph 4 of Chapter V under Differential diagnosis in the avian influenza Diagnostic Manual laid down in Decision 2006/437/EC is granted. Viruses of H5/H7 subtype shall be submitted without delay and shall be subjected to the standard characterisation tests (nucleotide sequencing/IVPI) according to the said diagnostic manual.
3. Description and implementation of the surveillance programme in wild birds

3.1.1 Designation of the central authority in charge of supervising and coordinating the departments responsible for implementing the programme and relevant collaborating partners (e.g. epidemiologists, ornithologists, nature bird observation and hunter organisations).

(max. 32000 chars):

a. The program will be performed in close co-operation with epidemiologists and ornithologists;
b. The competent authority for nature conservation (Danube Delta Biosphere, Ministry of Environment and Forests, Ministry of Agriculture and Rural Development, Hunting Directorate, Romanian Ornithological Society) shall be ensured for designing the surveillance, assisting in species identification and optimising sampling. The design of the surveillance shall be adapted to the national situation as regards selection of species to be sampled according to species predominance and bird population sizes. Sampling must consider the seasonality of migration patterns, which may vary in different Member States. It shall take into account the behaviour of bird species as regards migratory flyways, main habitats, gregariousness and degree of mixing during migration and the results obtained from previous surveillance during 2009-2013. For H5N1 HPAI, all those factors shall be considered in relation to the probability of wild bird exposure to infected poultry and wild birds in outbreak areas and the probability of contact of wild birds with domestic poultry, especially the “higher risk” species. Liaisons with bird conservation/watching institutions and ringing stations shall be encouraged. Sampling, where appropriate, shall be carried out under the supervision of staff from these groups/stations, by hunters and other ornithological skilled persons.
3.1.2 Description and delimitation of the geographical and administrative areas in which the programme is to be applied

max. 32000 chars):

1. Passive surveillance of wild birds shall be targeted on:
   a) areas where increased incidence of morbidity and mortality in wild birds occurs;
   b) areas close to the Black Sea, the Danube river, lakes, rivers and waterways which constitute biotopes for wild birds;
   c) areas in close proximity with poultry holdings where live the wild birds;
   d) birds belonging to identified “higher risk” species and which may come into contact with both poultry and wild birds.

2. Active surveillance on living and clinically healthy and/or clinically diseased, injured or hunted birds shall be targeted on:
   a) migratory birds belonging to the order of Anseriformes (water fowl) and Charidriiformes (shorebirds and gulls);
   b) at identified areas for concentration and mixing of high number of migratory birds involving different species and in particular when these areas are in proximity to domestic poultry farms;
   c) a selection of higher risk species.

3. Moreover, the investigations of live and dead wild birds shall be focussed on the birds:
   a) in the areas where cases of HPAI H5N1 were identified at wild birds and poultry;
   b) in the areas epidemiologically related to these cases;
   c) that come in direct contact with the poultry holdings.

3.1.3 Estimation of the local and/or migratory wildlife population
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(max. 32000 chars):

It cannot estimate the flock of migratory birds, but it can confirm that, in Romania, in the Danube Delta there are three migration tracks, creating a favorable biotope for wild birds, especially the water birds. The temperate climate in Romania is favourable to the migration of wild birds in a large number. Within the local flock of bird species, there are usually found out species typical of temperate climate, in particular: sparrow, pigeon, ring dove, red-throated diver, loon, duck, red crow, black crow, hooded crow, moor hen, wild duck etc.

3.2 Design, criteria, risk factors and target population(3)

(max. 32000 chars):

Sampling procedures shall be carried out in accordance with the Diagnostic Manual.

(b) Cloacal and tracheal/oropharyngeal swabs and/or tissues from wild birds found dead or moribund shall be sampled for molecular detection (PCR) and/or virus isolation.

(c) Specific care must be taken for the storage and transport of samples in accordance with paragraphs 5 and 6 of Chapter IV of the Diagnostic Manual. All avian influenza virus isolates of cases in wild birds shall be submitted to the EU RL, unless a derogation has been granted as provided for in paragraph 4(d) of Chapter V of the Diagnostic Manual. Viruses of the H5/H7 subtype shall be submitted to the EU RL without delay and shall be subjected to the standard characterisation tests (nucleotide sequencing/IVPI) according to the Diagnostic Manual.

(d) Sampling shall not extend beyond 31 December of the year of implementation of the surveillance programme. The objective of the surveillance programme for avian influenza in wild birds is the timely detection of HPAI of the subtype H5N1 in wild birds in order to protect poultry in poultry holdings and safeguard veterinary public health.

(a) A risk-based surveillance (RBS) shall be implemented as a "passive" surveillance system by laboratory investigation of moribund wild birds or birds found dead and it shall be specifically directed towards water bird species.

(b) Wild birds, in particular migratory water birds, that have been shown to be at a higher risk of becoming infected with, and transmitting the HPAI H5N1...
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virus, the "target species" (TS), shall be specifically targeted.

(c) Areas close to the sea, lakes and waterways where birds were found dead; and in particular when these areas are in close proximity to poultry holdings, especially in areas where there is a high density of poultry holdings, shall be targeted.

(d) Close cooperation with epidemiologists and ornithologists and the competent authority for nature conservation shall be ensured in the preparation of the surveillance programme, assisting in species identification and optimising sampling adapted to the national situation.

Virological surveillance for avian influenza in wild birds aim to identify the risk of introduction of AI viruses (LPAI and HPAI) to domestic poultry by:

- ensuring early detection of HPAI H5N1 by investigating increased incidence of morbidity and mortality in wild birds, in particular in selected “higher risk” species.

- in the event that HPAI H5N1 is detected in wild birds, then surveillance of live and dead wild birds shall be enhanced to determine whether wild birds of other species can act as asymptomatic carriers or “bridge species”.

- continuing a “baseline” surveillance of different species of free living migratory birds as part of continuous monitoring of LPAI viruses. Anseriformes (water fowl) and Charadriiformes (shorebirds and gulls) shall be the main sampling targets to assess if they carry LPAI viruses of H5 and H7 subtypes (which would in any case also detect HPAI H5N1 and other HPAI, if present). “Higher risk species” must be targeted in particular.

- Sampling shall not extend beyond 31 December of the year of implementation of the programme.

- Testing of samples shall be carried out at National Laboratories for avian influenza (NL) in Member States and at county sanitary veterinary and food safety laboratories and Bucharest sanitary veterinary and food safety laboratory under the control of the NRL.

- All results will be sent to the Community Reference Laboratory for Avian Influenza (CRL) for collation. A good flow of information must be ensured. The CRL shall provide technical support and keep an enlarged stock of diagnostic reagents. Antigens for use in the surveillance shall be supplied to NLs by the CRL to ensure uniformity.

- All avian influenza virus isolates of cases in wild birds shall be submitted to the CRL in accordance with Community legislation, unless a derogation according to paragraph 4 of Chapter V under Differential diagnosis in the avian influenza Diagnostic Manual laid down in Decision 2006/437/EC is granted. Viruses of H5/H7 subtype shall be submitted without delay and shall be subjected to the standard characterisation tests (nucleotide sequencing/IVPI) according to the said diagnostic manual.
(e) If the epidemiological situation for the HPAI H5N1 virus so requires, surveillance activities shall be enhanced by awareness raising and active searching and monitoring for dead or moribund wild birds, in particular for those belonging to TS.

(f) that come in direct contact with the poultry holdings.

(3) Areas at risk (wetlands in particular where links with high density poultry populations), previous positive findings as referred to in point 2 of Part 1 of Annex II to Commission Decision 2010/367/EC should be taken into account and if possible complemented by a map.

### 3.2.1 WILD BIRDS focussed on target species

**Investigations according to the surveillance programme set out in Part 2 of Annex II to Decision 2010/367/EC**

<table>
<thead>
<tr>
<th>NUTS (2) code/region</th>
<th>Wild birds to be sampled (b)</th>
<th>Total number of birds to be sampled</th>
<th>Estimated total number of samples to be taken for active surveillance (c)</th>
<th>Estimated total number of samples to be taken for passive surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO 1</td>
<td>275</td>
<td>275</td>
<td>203</td>
<td>52</td>
</tr>
<tr>
<td>RO 2</td>
<td>419</td>
<td>419</td>
<td>152</td>
<td>381</td>
</tr>
<tr>
<td>RO 3</td>
<td>157</td>
<td>157</td>
<td>176</td>
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<tr>
<td>RO 4</td>
<td>70</td>
<td>70</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>921</strong></td>
<td><strong>921</strong></td>
<td><strong>570</strong></td>
<td><strong>487</strong></td>
</tr>
</tbody>
</table>
3.3 Sampling procedures and sampling periods

(a) Sampling procedures shall be carried out in accordance with the Diagnostic Manual.

(b) Cloacal and tracheal/oropharyngeal swabs and/or tissues from wild birds found dead or moribund shall be sampled for molecular detection (PCR) and/or virus isolation.

(c) Specific care must be taken for the storage and transport of samples in accordance with paragraphs 5 and 6 of Chapter IV of the Diagnostic Manual. All avian influenza virus isolates of cases in wild birds shall be submitted to the EURL, unless a derogation has been granted as provided for in paragraph 4(d) of Chapter V of the Diagnostic Manual. Viruses of the H5/H7 subtype shall be submitted to the EURL without delay and shall be subjected to the standard characterisation tests (nucleotide sequencing/IVPI) according to the Diagnostic Manual.

(d) Sampling shall not extend beyond 31 December of the year of implementation of the surveillance programme.

3.4 Laboratory testing: description of the laboratory tests used

(a) Molecular Biology Laboratory: The method is based on the amplification and identification of a genetic fragment of matrix proteine, the common
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There are 10 accredited county labs of molecular biology, and there are situated in different parts of Romania. There are: Arad, Braşov, Braila, Calarasi, Constanța, Iași, Suceava, Satu-Mare, Tulcea and Vrancea.

b). Virus isolation by the inoculation of embryonated specific pathogen free (SPF) eggs (only for positive samples at PCR).

Experts from the NRL for avian influenza and Newcastle disease participated in a training course in Community Reference Laboratories and International Reference Laboratories. Support staff has been trained by specialists in training programs organized by the NRL. Laboratory tests shall be carried out in accordance with the Diagnostic Manual. Testing of samples shall be carried out at National Laboratories for avian influenza (NL) in Romania and at county sanitary veterinary and food safety laboratories and Bucharest sanitary veterinary and food safety laboratory under the control of the NRL.

- All results will be sent to the Community Reference Laboratory for Avian Influenza (CRL) for collation. A good flow of information must be ensured. The CRL shall provide technical support and keep an enlarged stock of diagnostic reagents. Antigens for use in the surveillance shall be supplied to NLs by the CRL to ensure uniformity.
- All avian influenza virus isolates of cases in wild birds shall be submitted to the CRL in accordance with Community legislation, unless a derogation according to paragraph 4 of Chapter V under Differential diagnosis in the avian influenza Diagnostic Manual laid down in Decision 2006/437/EC is granted. Viruses of H5/H7 subtype shall be submitted without delay and shall be subjected to the standard characterisation tests (nucleotide sequencing/IVPI) according to the said diagnostic manual.

The serological surveillance is not used in Romania for the surveillance of AI in wild birds.

4. Description of the epidemiological situation of the disease in poultry during the last five years

max 32000 chars :

During November-December 2007, in Murighiol a town of Tulcea county, was confirmed an outbreak of highly pathogenic A.I. H5N1 in gallinaceae and palmipedes from a non-commercial holding. Measures have been instituted by the European Commission under the Commission Decision 2007/770/CEE. On 07.02.2009, Community Reference Laboratory confirmed a case of H5N3 low pathogenic AI, in 19 sentinel poultry, placed by Tulcea Sanitary Veterinary and Food Safety Directorate in wild environment of the Danube Delta - Condura channel.

In the first quarter of 2010, in Tulcea County, there were confirmed two outbreaks of Highly pathogenic AI H5N1 in the poultry reared in backyards. The
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First outbreak was confirmed on March 15, in the hens of the backyards located in Letea communality. The case was notified to the European Commission and the OIE Restrictive measures were imposed by the European Commission in accordance with Commission Decision 2010/158/UE The second outbreak was confirmed on March 27, in the hens raised in backyards in Plauru - village located in Area B of the first outbreak. Restrictive measures were imposed by the European Commission in accordance with Commission Decision 2010/218/UE

The outbreak occurred in 20 isolated geese which were the property of Babes - Bolyai University and were brought as sentinel birds to Cot Candura location in August 2010 for avian influenza research. This location is isolated, it is surrounded by lakes Fortuna, Balcanestii Mari, Papadia, Rotelciuc and water channel Sontea Noua and Veche.

Tracheal swabs, cloacal swabs and serological samples were taken on 10 August, 24 September, 29 October and 5 November 2010. On the samples collected on 29 October and 5 November, the RT-PCR test results were positive in tracheal swabs and cloacal swabs; the serological results were negative. On 3 November 2010, Tulcea Sanitary Veterinary and Food Safety Directorate submitted a disease suspicion notification report. According to the procedure, Tulcea Sanitary Veterinary and Food Safety Directorate submitted to the National Reference Laboratory for avian influenza 20 samples for confirmation/invalidation of the avian influenza suspicion. A positive RT-PCR result for H5 subtype was obtained completed with the result of the virological examination on 12 November 2010: the haemagglutination inhibition and positive neuraminidase inhibition reactions for H5 and N3 subtypes. The date of confirmation is 12 November 2010, as during this period (3 to 12 November 2010) the virus isolation and its characterization as being low pathogenic were carried out.

5. **Description of the epidemiological situation of the disease in wild birds during the last five years**

(max. 32000 chars):

In the period 2005 -2006 H.P.A.I. H5N1 virus was isolated in wild life from 24 wild birds: 14 swans, 4 wild geese, 1 water hen, 1 wild pigeon, 2 coots, 1 heron and 1 owl.

In the last 5 years was not isolated any A.I. virus in wild life.
6. Measures in place as regards the notification of the disease

(max. 32000 chars):
There are notified compulsorily all cases of animals’ sickness by farmers or sanitary veterinary staff assuring the surveillance of animals’livestocks. The primary notification is carried out to the territorially competent veterinary authority which, in its turn shall notify the central veterinary authority of Romania by a rapid alert system.
In case it is found out that a disease suspicion was not notified in time, there are applied contraventions according to the specific legislation and there are not granted compensations to animals’ owners.

7. Costs

7.1 Detailed analysis of the costs

7.1.1 Poultry

(max. 32000 chars):
Costs

1. DETAILED ANALYSIS OF THE COSTS:
A.1). SEROLOGICAL SURVEILLANCE IN POULTRY
Haemagglutination and haemagglutination inhibition tests in poultry
## Standard requirements for the submission of surveillance programmes for avian influenza

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<table>
<thead>
<tr>
<th>Category</th>
<th>Sample Size</th>
<th>Test Cost</th>
<th>Total Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMMERCIAL HOLDINGS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laying hens</td>
<td>3180</td>
<td>0.5 euro</td>
<td>1590 euro</td>
</tr>
<tr>
<td>Chicken breeders</td>
<td>1080</td>
<td>0.5 euro</td>
<td>540 euro</td>
</tr>
<tr>
<td>Turkey</td>
<td>270</td>
<td>0.5 euro</td>
<td>135 euro</td>
</tr>
<tr>
<td>Pheasants, quails, and ratites</td>
<td>450</td>
<td>0.5 euro</td>
<td>225 euro</td>
</tr>
<tr>
<td>Geese and ducks</td>
<td>50</td>
<td>0.5 euro</td>
<td>25 euro</td>
</tr>
<tr>
<td><strong>TOTAL COMMERCIAL HOLDINGS</strong></td>
<td>5030</td>
<td>0.5 euro</td>
<td>2515 euro</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample Size</th>
<th>Test Cost</th>
<th>Total Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NONCOMMERCIAL HOLDINGS</strong> (backyards)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallinaceae (poultry)</td>
<td>10010</td>
<td>0.5 euro</td>
<td>5005 euro</td>
</tr>
<tr>
<td>Geese and ducks</td>
<td>9050</td>
<td>0.5 euro</td>
<td>4525 euro</td>
</tr>
<tr>
<td><strong>TOTAL NON COMMERCIAL HOLDINGS</strong></td>
<td>19060</td>
<td>0.5 euro</td>
<td>9530 euro</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample Size</th>
<th>Test Cost</th>
<th>Total Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallinaceae (poultry)</td>
<td>10020</td>
<td>12 euro</td>
<td>240240 euro</td>
</tr>
<tr>
<td>Geese and ducks</td>
<td>18100</td>
<td>12 euro</td>
<td>217200 euro</td>
</tr>
<tr>
<td><strong>TOTAL NON COMMERCIAL HOLDINGS</strong></td>
<td>38120</td>
<td>12 euro/test</td>
<td>457440 euro</td>
</tr>
</tbody>
</table>
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TOTAL TESTS SEROLOGICAL SURVEILLANCE = 48 180 from which:
TOTAL TESTS - POULTRY IN COMMERCIAL HOLDINGS = 10 060
TOTAL TESTS – POULTRY IN NONCOMMERCIAL HOLDINGS = 38 120

A 2). VIRUSOLOGICAL SURVEILLANCE IN POULTRY
a. COMMERCIAL HOLDINGS
RT-PCR test 30 x 20 euro = 600 euro
Virus isolation tests 30 x 40 euro = 1200 euro
b. NONCOMMERCIAL HOLDINGS (backyards)
RT-PCR test 50 x 20 euro = 1000 euro
Virus isolation 50 x 40 euro = 2000 euro

TOTAL TESTS VIROLOGICAL SURVEILLANCE = 160 from which:
TOTAL TESTS - POULTRY IN COMMERCIAL HOLDINGS = 60
TOTAL TESTS – POULTRY IN NONCOMMERCIAL HOLDINGS = 100

TOTAL COSTS SEROLOGICAL AND VIROLOGICAL SURVEILLANCE POULTRY = 595 005 euro from which:
SAMPLING POULTRY 24090 X 0.5 euro = 12 045 euro
SEROLOGICAL SURVEILLANCE:
HAEMAGGLUTINATION AND HAEMAGGLUTINATION INHIBITION TESTS = 578 160 euro
VIROLOGICAL SURVEILLANCE:
RT-PCR test and virus isolation = 4800 euro

7.1.2 Wild birds

(max. 32000 chars):

VIROLOGICAL SURVEILLANCE IN WILD BIRDS
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<table>
<thead>
<tr>
<th>Procedure</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLING WILD BIRDS</td>
<td>2 435 euro</td>
</tr>
<tr>
<td>RT-PCR test</td>
<td>18 420 euro</td>
</tr>
<tr>
<td>Virus isolation</td>
<td>800 euro</td>
</tr>
</tbody>
</table>

**TOTAL COSTS SURVEILLANCE WILD BIRDS** = 21 655 euro

**TOTAL COSTS SURVEILLANCE POULTRY AND WILD BIRDS/2013** = 616 660 euro
### 7.2 Summary of the costs

#### 7.2.1 Poultry surveillance

**Detailed analysis of the cost of the programme - poultry**

<table>
<thead>
<tr>
<th>Methods of laboratory analysis</th>
<th>Number of tests</th>
<th>Unitary test cost (per method) in €</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA test</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>agar gel immune diffusion test</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haemagglutination-inhibition-test (H5) for H5 (specify number of tests for H5)</td>
<td>24 090</td>
<td>12</td>
<td>289,080</td>
</tr>
<tr>
<td>Haemagglutination-inhibition-test (H7) for H7 (specify number of tests for H7)</td>
<td>24 090</td>
<td>12</td>
<td>289,080</td>
</tr>
<tr>
<td>Virus isolation test</td>
<td>80</td>
<td>40</td>
<td>3200</td>
</tr>
<tr>
<td>PCR test</td>
<td>80</td>
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<tr>
<td>Other please specify here</td>
<td>0</td>
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</tr>
</tbody>
</table>

Add a new row
### Standard requirements for the submission of surveillance programmes for avian influenza

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<table>
<thead>
<tr>
<th>Sampling</th>
<th>Number of samples</th>
<th>Unitary cost in €</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>24 090</td>
<td>0.5</td>
<td>12045</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other measures</th>
<th>Number of samples</th>
<th>Unitary cost in €</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other please specify here</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Total poultry Testing + Sampling + Other measures**

| Total poultry                 | 74 430            |                   | 595 005,00 €  |
### Standard requirements for the submission of surveillance programmes for avian influenza

**version : 2.2**

#### 7.2.2 Wild bird surveillance

**Detail analysis of the cost of the programme - wild birds**

<table>
<thead>
<tr>
<th>Laboratory testing</th>
<th>Number of tests</th>
<th>Unitary test cost (per method) in €</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus isolation test</td>
<td>20</td>
<td>40</td>
<td>800</td>
</tr>
<tr>
<td>PCR test</td>
<td>921</td>
<td>20</td>
<td>18420</td>
</tr>
<tr>
<td>Other please specify here</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Add a new row**

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Number of samples</th>
<th>Unitary cost in €</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>487</td>
<td>5</td>
<td>2435</td>
</tr>
</tbody>
</table>

**Other measures**
Standard requirements for the submission of surveillance programmes for avian influenza

version : 2.2

<table>
<thead>
<tr>
<th>Other please specify here</th>
<th>Number</th>
<th>Unitary cost in €</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

[Add a new row]

<table>
<thead>
<tr>
<th>Total wild birds Testing + Sampling + Other measures</th>
<th>1428</th>
<th></th>
<th>21 655,00 €</th>
</tr>
</thead>
</table>

| Grand Total Poultry + Wild birds | 73858 |                   | 616 660,00 € |

Attachments

IMPORTANT :
1) The more files you attach, the longer it takes to upload them.
2) This attachment files should have one of the format listed here: .zip, .jpg, .jpeg, .tiff, .tif, .xls, .doc, .bmp, .png.
3) The total file size of the attached files should not exceed 2 500Kb (+- 2.5 Mb). You will receive a message while attaching when you try to load too much.
4) IT CAN TAKE SEVERAL MINUTES TO UPLOAD ALL THE ATTACHED FILES. Don't interrupt the uploading by closing the pdf and wait until you have received a Submission Number!
5) Zip files cannot be opened (by clicking on the Open button). All other file formats can be opened.
Standard requirements for the submission of surveillance programmes for avian influenza

version : 2.2