



EUROPEAN COMMISSION  
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

Director General

SANCO/10252/2013

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

## **Survey programme for Avian Influenza**

**Romania**

**Approved\* for 2013 by Commission Decision 2012/761/EU**

\* in accordance with Council Decision 2009/470/EC

# *Standard requirements for the submission of surveillance programmes for avian influenza*

version : 2.2

## *1. Identification of the programme*

Member state : ROMANIA

Disease : avian influenza in poultry and wild birds

Request of Community co-financing from beginning of: 2013 to end of 2013

### *1.1 Contact*

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## *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

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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, technico-material basis necessary for the development of 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 (D.S.V.S.A.) which represents the competent veterinary authority at the territorial level; there are also 41 county sanitary veterinary and food safety laboratories /of Bucharest municipality .

The department responsible for implementing the programme is subordinated to the General Sanitary Veterinary and Food Safety Directorate within National Sanitary Veterinary and Food Safety Authority.

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.

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## **2.1.2      System in place for the registration of holdings**

(max. 32000 chars) :

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 registered 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;
- Commission Decision 2011/214/EU of 1 April 2011 amending Annexes II to IV to 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)**

(max. 32000 chars) :

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: 183 holdings of laying hens, 41 holdings of chicken breeder, 268 holdings of broilers, 1 holdings of ratites, 7 holdings of pheasants, 8 holdings of quails, 10 holdings of turkeys, 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 766 „target” localities. We mention that within the same noncommercial holdings situated in the respective 766 “target”localities, there are reared gallinaceae as well as palmipedes.

The poultry from backyards are grown just for family consumption.

## ***2.1.3.2 Criteria and risk factors for risk based surveillance(1)***

*(max. 32000 chars) :*

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.

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- (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)*

*(max. 32000 chars):*

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) backyards flock galinacee;
- (h) backyards flock palmipedes;
- (i) palmipedes holdings.

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- (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

Category : laying hens

**delete this category**

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	
RO 1	92	43	30	2 580	Haemagglutination-inhibition-test (HI)	X
RO 2	34	17	30	1 020	Haemagglutination-inhibition-test (HI)	X
RO 3	30	17	30	1 020	Haemagglutination-inhibition-test (HI)	X
RO 4	27	14	30	840	Haemagglutination-inhibition-test (HI)	X
<b>Total</b>	183	91	120	5 460		
					<b>Add a new row</b>	

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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.

Category : chicken breeders

**delete this category**

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 1	14	14	30	840	Haemagglutination-inhibition-test (HI)
RO 2	12	12	30	720	Haemagglutination-inhibition-test (HI)
RO 3	14	14	30	840	Haemagglutination-inhibition-test (HI)
RO 4	1	1	30	60	Haemagglutination-inhibition-test (HI)
<b>Total</b>	41	41	120	2 460	

**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) 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 : fattening turkeys

**delete this category**

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 1	9	9	9	30	540 Haemagglutination-inhibition-test (HI)
RO 4	1	1	1	30	60 Haemagglutination-inhibition-test (HI)
<b>Total</b>	10	10	10	600	
<b>Add a new row</b>					

(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.

Category : ratites

**delete this category**

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 3	1	1	1	30	60 Haemagglutination-inhibition-test (HI)

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<b>Total</b>	1	30	60
<b>Add a new row</b>			

(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.

Category : pheasants

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis	<b>delete this category</b>
RO 2	2	2	30	120	Haemagglutination-inhibition-test (HI)	X
RO 3	3	3	30	180	Haemagglutination-inhibition-test (HI)	X
RO 4	2	2	30	120	Haemagglutination-inhibition-test (HI)	X
<b>Total</b>	7	7	90	420		
<b>Add a new row</b>						

(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 : Quails

**delete this category**

NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 2	1	1	1	30	60 Haemagglutination-inhibition-test (HI)
RO 3	6	6	6	20	240 Haemagglutination-inhibition-test (HI)
RO 4	1	1	1	30	60 Haemagglutination-inhibition-test (HI)
<b>Total</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>360</b>	

**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) 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.

Category : backyard flocks gallinacee

**delete this category**

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NUTS (2) (b)	Total number of holdings(c)	Total number of holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 1	132	60	30	3 600	Haemagglutination-inhibition-test (HI) <b>X</b>
RO 2	255	107	25	5 350	Haemagglutination-inhibition-test (HI) <b>X</b>
RO 3	216	90	25	4 500	Haemagglutination-inhibition-test (HI) <b>X</b>
RO 4	163	77	30	4 620	Haemagglutination-inhibition-test (HI) <b>X</b>
<b>Total</b>	<b>766</b>	<b>334</b>	<b>110</b>	<b>18 070</b>	<b>Add a new row</b>

(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.

**Add a category**

<b>Total Poultry</b>	1 016	492	610

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## 2.2.2 DUCKS, GEESE AND FARMED GAME BIRDS (WATERFOWL e.g. MALLARD) HOLDINGS (a) to be sampled.

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

Category : backyard flocks palmipedes

**delete this category**

NUTS (2) (b)	Total number of duck and geese holdings	Total number of duck and geese holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 1	132	60	30	3 600	Haemagglutination-inhibition-test (HI) <b>X</b>
RO 2	255	107	25	5 350	Haemagglutination-inhibition-test (HI) <b>X</b>
RO 3	216	90	25	4 500	Haemagglutination-inhibition-test (HI) <b>X</b>
RO 4	163	77	30	4 620	Haemagglutination-inhibition-test (HI) <b>X</b>
<b>Total</b>	<b>766</b>	<b>334</b>	<b>110</b>	<b>18 070</b>	

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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 (2) code can not be used, region as defined in the programme by the Member State is requested

Category : palmipedes holdings

NUTS (2) (b)	Total number of duck and geese holdings	Total number of duck and geese holdings to be sampled	Number of samples per holding	Total number of tests	Method of laboratory analysis
RO 2	1	1	1	25	50 Haemagglutination-inhibition-test (HI)
RO 3	1	1	1	25	50 Haemagglutination-inhibition-test (HI)
<b>Total</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>50</b>	<b>100</b>

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 (2) code can not be used, region as defined in the programme by the Member State is requested

Add a category

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<b>Total Ducks and geese</b>	768	336	160	18 170

<b>Grand Total Poultry + Ducks/Gueese</b>	1 784	828	770	45 600

## *2.3 Sampling procedures, sampling periods and frequency of testing*

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*(max. 32000 chars):*

- (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.

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

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

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.

Virological tests:

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- a. RT – PCR  
The method is based on the amplification and identification of a genetic fragment of matrix protein, the common fragment for all viruses subtype A of A.I.
- b. Virus isolation by the inoculation of embryonated specific pathogen free (SPF) eggs (only for positive samples at PCR).

## *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):*

The central authority in charge with the supervising and coordinating the departments implementing the programme is the National Sanitary Veterinary and Food Safety Authority. The programme for the surveillance of Avian Influenza in poultry and wild birds is drawn up at the Sanitary Veterinary General Directorate, based on the data regarding the risk analysis sent by the county and Bucharest municipality sanitary veterinary and food safety directorates

- ### *3.1.2 Description and delimitation of the geographical and administrative areas in which the programme is to be applied*

*(max. 32000 chars):*

The programme will be applied on the entire territory of Romania, taking account of the high risk areas.

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## *3.1.3 Estimation of the local and/or migratory wildlife population*

*(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):*

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.
- (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*

NUTS (2) code/region (a)	Wild birds to be sampled (b)	Total number of birds to be sampled	Estimated total number of samples to be taken for active surveillance (c)	Estimated total number of samples to be taken for passive surveillance
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RO 1		230		230		460	110 <b>X</b>
RO 2		530		530		1 060	210 <b>X</b>
RO 3		210		210		420	100 <b>X</b>
RO 4		230		230		460	110 <b>X</b>
<b>Total</b>		1 200		1 200		2 400	530
<b>Add a new row</b>							

(a) Refers to the place of collection of birds/samples. In case NUTS 2 (Nomenclature of Territorial Units for Statistics) can not be used, region as defined in the programme by the Member State is requested. Please fill-in these values directly in the field.

(b) General description of the wild birds are intended to be sampled in the framework of the active and passive surveillance.

(c) Voluntary, to be included for information purposes, not eligible for cofinancing.

## 3.3 Sampling procedures and sampling periods

max 32000 chars :

- (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/IVP) 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.

## *3.4 Laboratory testing: description of the laboratory tests used*

*max 32000 chars:*

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.

Virological tests:

a. RT – PCR

The method is based on the amplification and identification of a genetic fragment of matrix protein, the common fragment for all viruses subtype A of A.I. b. Virus isolation by the inoculation of embryonated specific pathogen free (SPF) eggs (only for positive samples at PCR).

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

*max 32000 chars:*

The first highly pathogenic Avian Influenza case H5N1 was diagnosed in Romania on 7-th of October 2005 (first time was declared in 1941) at poultry in

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backyards, in a very exposed at risk area, in Danube Delta. During the winter of 2005-2006 (October – April), the disease extended at 53 outbreaks in nine counties from the South-East area of the country.

After a short period of peacetime, highly pathogenic A.I. H5N1 evolved during May - July 2006, in 5 commercial holdings of Brasov County: SC DRAKOM SILVA SRL, SC PATI-PROD SRL - farm no.3, SC PATI-PROD SRL – turkey farm, SC AVIPROD 2002 Ploiesti, SC PATI-PROD SRL farm no. 5, and 122 backyards from 18 counties located in the Centre and East of the country, and in Bucharest City. 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 first outbreak was confirmed on March 15, in the hens of the backyards located in Letea community. 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 10August, 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 Laboratory submitted to the National Reference Laboratory for avian influenza 20 samples for confirmation/validation 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.

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## *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*

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## *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  
a. COMMERCIAL HOLDINGS

Laying hens:

2730 samples x 0,5 euro = 1365 euro

5460 tests x 12 euro = 65 520 euro

Chicken breeders:

1230 samples x 0,5 euro = 615 euro

2460 tests x 12 euro = 29 520 euro

Turkey:

300 samples x 0,5 euro = 150 euro

600 tests x 12 euro = 7 200 euro

Pheasants, quails and ratites:

420 samples x 0,5 euro = 210 euro

840 tests x 12 euro = 10 080 euro

Gees and ducks:

50 samples x 0,5 euro = 25 euro

100 tests x 12 euro = 1200 euro

TOTAL COMMERCIAL HOLDINGS:  
4730 samples x 0,5 euro = 2365 euro

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9460 tests x 12 euro/test = 113 520 euro

## b. NONCOMMERCIAL HOLDINGS (backyards)

Gallinaceae (poultry):

9035 samples x 0,5 euro = 4517,5 euro

18 070 tests x 12 euro = 216 840 euro

Geese and ducks:

9035 samples x 0,5 euro = 4517,5 euro

18070 tests x 12 euro = 216 840 euro

TOTAL NON COMMERCIAL HOLDINGS:

18070 samples x 0,5 euro = 9035 euro

36140 tests x 12 euro/test = 433 680 euro

TOTAL TESTS SEROLOGICAL SURVEILLANCE = 45 600 from which:

TOTAL TESTS- POULTRY IN COMMERCIAL HOLDINGS = 9 460

TOTAL TESTS – POULTRY IN NONCOMMERCIAL HOLDINGS = 36 140

## 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 = 563 400 euro from which:

SAMPLING POULTRY 22 800 X 0,5 euro = 11 400 euro

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## SEROLOGICAL SURVEILLANCE:

HAEMAGGLUTINATION AND HAEMAGGLUTINATION INHIBITION TESTS = 547 200 euro

## VIROLOGICAL SURVEILLANCE:

RT-PCR test and virus isolation= 4800 euro

## 7.1.2 *Wild birds*

(max. 32000 chars):

### VIROLOGICAL SURVEILLANCE IN WILD BIRDS

SAMPLING WILD BIRDS 1200 x 5 euro = 6000 euro

RT-PCR test = 1200 x 20 euro = 24 000 euro

Virus isolation = 30 x 40 Euro = 1200 euro

TOTAL COSTS SURVEILLANCE WILD BIRDS = 31 200 euro

TOTAL COSTS SURVEILLANCE POULTRY AND WILD BIRDS/2013 = 594 600 euro

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## 7.2 Summary of the costs

### 7.2.1 Poultry surveillance

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

Laboratory testing	Methods of laboratory analysis	Number of tests	Unitary test cost (per method) in €	Total cost (€)
ELISA test		0	0	0
agar gel immune diffusion test		0	0	0
Haemagglutination-inhibition-test (HI) for H5 (specify number of tests for H5)		22 800	12	273,600
Haemagglutination-inhibition-test (HI) for H7 (specify number of tests for H7)		22 800	12	273,600
Virus isolation test		80	40	3200
PCR test		80	20	1600
Other please specify here		0	0	0
<b>Add a new row</b>				

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Sampling			Other measures		
	Number of samples	Unitary cost in €		Number of samples	Unitary cost in €
Samples	22 800	0.5		0	0
<b>Other please specify here</b>					
<b>Total poultry</b>					
<b>Testing + Sampling + Other measures</b>	68 560				563 400,00 €
			<b>Add a new row</b>		
			X		

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## 7.2.2 Wild bird surveillance

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

Laboratory testing		
Methods of laboratory analysis	Number of tests	Unitary test cost (per method) in €
Virus isolation test	30	40
PCR test	1 200	20
Other please specify here	0	0
<b>Add a new row</b>		
Sampling		
Samples	Number of samples	Unitary cost in €
Samples	1 200	5
<b>Other measures</b>		

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	Number	Unitary cost in €	Total cost (€)
Other please specify here	0	0	0
			<b>Add a new row</b>
<b>Testing + Sampling + Other measures</b>			
<b>Total wild birds</b>	2430		31 200,00 €
<b>Grand Total</b>	<b>70990</b>		<b>594 600,00 €</b>
<b>Poultry + Wild birds</b>			

## 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,pna.
- 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.

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