



Annex IV : Programme for the surveillance of Avian Influenza in poultry and wild birds submitted for obtaining EU cofinancing

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- 7) For simplification purposes you are invited to submit **multi-annual programmes**.
- 8) As mentioned during the Plenary Task Force of 28/2/2014, you are invited to submit your programmes in **English**.

Submission Date

Friday, September 04, 2015 11:17:23

Submission Number

1441354646112-6665

ANNEX 4 : Standard requirements for the submission of surveillance programmes for avian influenza in poultry and wild birds

1. Identification of the programme

Member state: KYPROS

Disease avian influenza in poultry and wild birds

This program is multi annual :

no

Request of Union co-financing
from beginning of:

2016

ANNEX 4 : Standard requirements for the submission of surveillance programmes for avian influenza in poultry and wild birds

1.1 Contact

Name : Georgios Krasias

Phone : +35722805180

Your job type within the CA : Veterinary Officer Animal Health and Welfare Division (AHWD) Veterinarian

Email : gkrasias@vs.moa.gov.cy

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) :

The Veterinary Services of the Ministry of Agriculture, Rural Development and Environment are responsible for the implementation of the control programme of Avian Influenza .
Within the Veterinary Services, the Animal Health and Welfare Division is responsible for the coordination and supervision of the programme. The five District Veterinary Offices with the five Rural Veterinary Stations are responsible for the collection and transport of samples to the Laboratories of the Veterinary Services.

2.1.2 System in place for the registration of holdings

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(max. 32000 chars) :

BREEDING HOLDINGS

The central authority has registered officially all holdings with breeding flocks according to K.Δ.Π. 317/2002, which transfers Directive 90/539/EEC into Cyprus national law.

LAYING FARMS

The central authority has registered officially all holdings with layer flocks according to K.Δ.Π. 761/2003, which transfers Directives 1999/74/EC and 2002/4/EC into Cyprus national law. According to this all laying hen holdings of more than 350 laying hens should be registered with the Veterinary Services. Packing centres are registered according to Cyprus national law N. 276(I)2004.

BROILER FARMS

There is no legal basis for the registration of broiler farms. However the Veterinary Services keep a database with the broiler farms.

TURKEYS

There are no turkey breeding flocks in Cyprus. Day-old chicks are imported from Member States to broiler farms for fattening and slaughter for the period of Christmas.

2.1.3 *Design (risk based surveillance or surveillance based on representative sampling taking into account criteria in Annex I of Commission Decision 2010/367/EC)*

(max. 32000 chars) :

The programme will be implemented from 1st January 2016 until 31 December 2016.

All samples are to be tested in the NRL of Cyprus and in case of positive results, samples will be sent to the CRL for confirmation.

Surveillance is based on representative sampling.

Based on the different types of poultry holdings operating in Cyprus, samples are to be taken from chickens (breeding hens, laying hens, free range laying hens and free range broilers), fattening turkeys, quails and partridges according to Commission Decision 2010/367/EU. Moreover, samples will be collected from backyard poultry in areas defined as high risk.

SAMPLING

Blood sampling for serological examination will be collected from 10 birds per holding or in cases of holdings consisting of more than one shed from 5 birds per shed.

All holdings of each species will be included in the sampling frame for serological examination.

Sampling scheme in breeding flocks

All parent stock is imported to Cyprus as day-old chicks. There are 4 breeding companies, who own in total 9 approved holdings housing breeding flocks, with a total of 26 sheds. Day-old chicks are sold to

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laying hen or broiler farms within Cyprus. It is estimated that 135 blood samples will be collected and tested from breeding flocks (25sheds * 5 birds sampled and 1shed * 10 birds sampled)

Sampling scheme in broilers

There are 3 free range broiler farms, which will be included in the sampling frame for the surveillance of Avian Influenza, with a total of 7 sheds. It is estimated that 45 blood samples will be collected and tested from free range broiler flocks (1st farm: 5 sheds*5 birds sampled per shed and each 2nd farm and 3rd farm:1 shed*10 birds sampled).

Sampling scheme in layers

There are 27 conventional laying hen holdings with 59 sheds. 10 blood samples will be collected from holdings having 1 shed and 5 blood samples per shed in holding with more than 1 shed. It is estimated that 340 blood samples will be collected and tested from conventional laying flocks.

There are 12 free range and organic laying holdings, with a total of 29 sheds. It is estimated that 150 blood samples will be collected and tested from free range and organic laying flocks.

Sampling scheme in turkey holdings

There are no breeding farms for turkeys in Cyprus. Based on the number of flocks for 2014 ,it is estimated that 5 holdings will place turkeys for fattening, with a total of 8 sheds. It is estimated that 65 blood samples will be collected and tested from turkeys flocks (5 holdings with 1 shed: 5 sheds*10 birds sampled=50 samples and 1 holding with 3 sheds: 3 sheds*5 birds sampled per shed=15 samples).

Sampling scheme in partridges

There are 3 holdings for partridges with an average of 2 sheds per holding. It is estimated that 30 blood samples will be collected and tested from partridge flocks (6 sheds*5 birds sampled per shed).

Sampling scheme for quails:

There are 3 quail holdings. It is estimated that 30 blood samples will be collected and tested from quail flocks (3 sheds*10 birds sampled per shed).

Sampling scheme in duck, goose and mallard holdings

There are no duck, goose or mallard holdings in Cyprus. In case such holdings are operating in 2016, 20 blood samples will be collected and tested per holding.

Sampling scheme in backyard poultry

Blood samples from backyard poultry will be collected from high risk areas in Cyprus. According to the 2006 census there were 1247 backyard holdings in these high risk areas. It is estimated that 600 blood samples will be collected and tested from 60 backyard holdings (60 sheds * 10 birds sampled per shed).

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

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(max. 32000 chars) :

BREEDING FLOCKS GALLUS GALLUS

In Cyprus there are only 4 breeding companies which have in total 9 breeding holdings and 3 hatcheries. There are in total 25 sheds housing parent stock for broilers and 1 shed housing parent stock for layers.

LAYING FLOCKS GALLUS GALLUS

There are 27 conventional laying hen holdings with 59 sheds and 12 free range and organic laying holdings with 29 sheds.

BROILER FLOCKS GALLUS GALLUS

There are 97 broiler farms operating in Cyprus, out of which only 3 are free range broiler farms.

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

(max. 32000 chars) :

The surveillance programme is based on Representative Sampling, according to Annex I to Commission Decision 2010/367/EU.

Due to the small number of holdings in each poultry category, all poultry holdings will be tested in the framework of the surveillance programme.

- (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) :

Samples are to be taken from chickens (breeding hens, laying hens, free range laying hens and free range broilers), fattening turkeys, quails, partridges and backyard poultry.

- (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

Targets for year

2016

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 |
|--------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|
| CY00 | 5 | 5 | 10 | 50 HI-test (H5) | X |
| CY00 | 1 | 1 | 30 | 30 HI-test (H5) | X |
| CY00 | 2 | 2 | 20 | 40 HI-test (H5) | X |
| CY00 | 1 | 1 | 15 | 15 HI-test (H5) | X |
| CY00 | 5 | 5 | 10 | 50 HI-test (H7) | X |
| CY00 | 1 | 1 | 30 | 30 HI-test (H7) | X |
| CY00 | 2 | 2 | 20 | 40 HI-test (H7) | X |
| CY00 | 1 | 1 | 15 | 15 HI-test (H7) | X |

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| | | | | | |
|----------------------|---|--|--|-----|--|
| Total | | | | 270 | |
| Add a new row | | | | | |
| (a) | <i>Holdings or herds or flocks or establishments as appropriate.</i> | | | | |
| (b) | <i>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</i> | | | | |
| (c) | <i>Total number of holdings of one category of poultry in concerned NUTS 2 region.</i> | | | | |

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 |
|--------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|
| CY00 | 20 | 20 | 10 | 200 | HI-test (H5) |
| CY00 | 3 | 3 | 15 | 45 | HI-test (H5) |
| CY00 | 2 | 2 | 20 | 40 | HI-test (H5) |
| CY00 | 1 | 1 | 25 | 25 | HI-test (H5) |
| CY00 | 1 | 1 | 30 | 30 | HI-test (H5) |
| CY00 | 20 | 20 | 10 | 200 | HI-test (H7) |
| CY00 | 3 | 3 | 15 | 45 | HI-test (H7) |
| CY00 | 2 | 2 | 20 | 40 | HI-test (H7) |
| CY00 | 1 | 1 | 25 | 25 | HI-test (H7) |
| CY00 | 1 | 1 | 30 | 30 | HI-test (H7) |
| Total | | | | 680 | |

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| |
|--|
| Add a new row |
| <p>(a) <i>Holdings or herds or flocks or establishments as appropriate.</i> (b) <i>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</i> (c) <i>Total number of holdings of one category of poultry in concerned NUTS 2 region.</i></p> |

Category : **free range 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 | |
|----------------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|----------|
| CY00 | 8 | 8 | 10 | 80 | HI-test (H5) | X |
| CY00 | 3 | 3 | 15 | 45 | HI-test (H5) | X |
| CY00 | 1 | 1 | 25 | 25 | HI-test (H5) | X |
| CY00 | 8 | 8 | 10 | 80 | HI-test (H7) | X |
| CY00 | 3 | 3 | 15 | 45 | HI-test (H7) | X |
| CY00 | 1 | 1 | 25 | 25 | HI-test (H7) | X |
| Total | | | | 300 | | |
| 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 : broilers (only when at risk)

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 |
|----------------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|
| CY00 | 1 | 1 | 25 | 25 | HI-test (H5) |
| CY00 | 2 | 2 | 10 | 20 | HI-test (H5) |
| CY00 | 1 | 1 | 25 | 25 | HI-test (H7) |
| CY00 | 2 | 2 | 10 | 20 | HI-test (H7) |
| Total | | | | 90 | |
| 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 : 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 |
|--------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|
| CY00 | 5 | 5 | 10 | 50 | HI-test (H5) |
| CY00 | 1 | 1 | 15 | 15 | HI-test (H5) |
| CY00 | 5 | 5 | 10 | 50 | HI-test (H7) |

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| | | | | | |
|----------------------|---|---|----|-----------------|----------|
| CY00 | 1 | 1 | 15 | 15 HI-test (H7) | X |
| Total | | | | 130 | |
| 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.

delete this category

Category : farmed game birds (gallinaceous)

| 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 |
|----------------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|
| CY00 | 6 | 6 | 10 | 60 HI-test (H5) | X |
| CY00 | 6 | 6 | 10 | 60 HI-test (H7) | X |
| Total | | | | 120 | |
| 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.

delete this category

Category : backyard flocks

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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 |
|----------------------|-----------------------------|--|-------------------------------|-----------------------|-------------------------------|
| CY00 | 1 247 | 60 | 10 | 600 | HI-test (H5) |
| CY00 | 1 247 | 60 | 10 | 600 | HI-test (H7) |
| Total | | | | 1 200 | |
| 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.

Add a category

| Totals | Total number of tests |
|---------------------------|-----------------------|
| Total poultry 2016 | 2 790 |

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

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Targets for year **2016**

delete this category

Category : N/A

| 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 |
|----------------------|---|---|-------------------------------|-----------------------|-------------------------------|
| CY00 | 0 | 0 | 0 | 0 | Virus isolation test |
| Total | | | | 0 | |
| 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

| | | | | |
|---|--|-----------------------|--|--|
| | | | | |
| NUTS (2) (b) | | Total number of tests | | |
| Total ducks and geese and farmed game birds 2016 | | 0 | | |
| | | | | |

TOTALS for Poultry (2.2.1) + Ducks and Geese (2.2.2) and farmed game birds for year :

2016

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| Poultry + Ducks/Geese /farmed game birds | Total number of tests |
|--|-----------------------|
| Grand Total | 2 790 |
| Grand Total ELISA | 0 |
| Grand Total agar | 0 |
| Grand Total HI tests (H5) | 1 395 |
| Grand Total HI tests (H7) | 1 395 |
| Grand Total Virus Isolation test | 0 |
| Grand Total PCR test | 0 |
| Grand Total Other test | 0 |
| Grand Total Samplings | 0 |

2.3 Sampling procedures, sampling periods and frequency of testing taking into account criteria set out in Annex I of Commission Decision 2010/367/EC

(max. 32000 chars) :

Sampling from all categories of poultry holdings (breeding, conventional laying hen, free range laying hen, free range broiler, fattening turkey, partridge, quail and backyard) will be distributed throughout the year.

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2.4. Laboratory testing : description of the laboratory tests used and follow up investigations taking into account criteria set out in the *Diagnostic Manual for avian influenza (Common Decision 2006/437/EC)*

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

According to Chapter VIII of the Annex to Commission Decision 2006/437/EC. HI serological testing is used for the present of antibodies H5 and H7 subtypes

The reagents required for these tests are isotonic PBS (0.01 M), pH 7.0–7.2, red blood cells (RBCs) taken from a minimum of three SPF chickens and pooled into an equal volume of Alsever's solution and H5/ H7 antigens. Cells are washed three times in PBS before use as a 1% (packed cell v/v) suspension. Positive and negative control antigens and antisera are run with each test.

Procedure:

- i) Dispense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate.
- ii) Place 0.025 ml of serum into the first well of the plate.
- iii) Make twofold dilutions of 0.025 ml volumes of the serum across the plate.
- iv) Add 4 HAU of antigen H5/H7 in 0.025 ml to each well and leave for a minimum of 30 minutes at room temperature (i.e. about 20°C) or 60 minutes at 4°C.
- v) Add 0.025 ml of 1% (v/v) chicken RBCs to each well and mix gently, allow the RBCs to settle for about 40 minutes at room temperature, i.e. about 20°C, or for 60 minutes at 4°C if ambient temperatures are high, by which time control RBCs should have formed a distinct button.
- vi) The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) are considered to show inhibition.
- vii) The validity of results are assessed against a negative control serum, which should not give a titre $>1/4$ (>22 or $>\log_2 2$ when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre.

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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 Veterinary Services of the Ministry of Agriculture, Rural Development and Environment are responsible for the implementation of the control programme of Avian Influenza .
Within the Veterinary Services, the Animal Health and Welfare Division is responsible for the coordination and supervision of the programme. The five District Veterinary Offices with the five Rural Veterinary Stations are responsible for the collection and transport of samples to the Laboratories of the Veterinary Services.

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

max. 32000 chars) :

Samples for PCR will be collected from wild birds in high risk areas.

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Collection of dead or moribund wild birds for sampling in the frame of the A.I. surveillance programme is performed in collaboration with the Game fund, a governmental service. It must be noted that the presence in Cyprus of birds listed in part 2 of Annex II of Commission Decision 2010/367/EC depends mainly on the weather conditions in the island during the winter and that according to the Game Fund's data the Bean Goose (*Anser fabalis*) does not pass from Cyprus.

Passive surveillance

Birds found dead or moribund in high risk areas will be collected and examined for AI virus.

3.1.3 Estimation of the local and/or migratory wildlife population

(max. 32000 chars) :

Monitoring of local and migratory wildlife population is performed by the Game Fund. According to its reports there was an increase in the number of migratory birds, including waterfowl, until the 1st semester of 2013, due to the higher level of rainfall. However, during the 2nd semester of 2013 and the beginning of 2014, drought conditions were observed in the region. In general the number of migratory wild birds depends on the weather conditions (rainfall and temperature) each year .

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

(max. 32000 chars) :

The surveillance programme for Avian Influenza in wild birds aims at the early detection of HPAI viruses of the subtype H5N1 in wild birds in order to prevent the introduction of the infection in poultry.

Samples for PCR will be collected from wild birds in high risk areas.

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Game fund is a governmental service that cooperates with veterinary services and provides dead or moribund wild birds that can be sampled for the A.I. surveillance program. The samples will be either a) oropharyngeal and/or cloacal swabs from moribund, or b) faeces, cloacal swabs, tracheal/ oropharyngeal swabs or tissues (brain, heart, lung, kidney and intestines) from dead birds.

It must be noted that the presence in Cyprus of birds listed in part 2 of Annex II of Commission Decision 2010/367/EU depends mainly on the weather conditions in the island during the winter. It can not be ensured that samples from all the species of these wild birds will be collected. It must also be noted that according to the Game Fund's data the Bean Goose (*Anser fabalis*) does not pass from Cyprus.

Passive surveillance

Birds found dead or moribund in high risk areas will be collected and examined for AI virus. Based on the experience of previous years, it is expected to examine around 180 samples from approximately 180 birds.

- (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 conformity with Part 2 of Annex II to Decision 2010/367/EC

Targets for year

2016

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| NUTS (2) code/region (a) | Total number of birds to be sampled | Estimated total number of samples to be taken for passive surveillance | Type of test | Number of tests |
|--------------------------|-------------------------------------|--|--------------|-----------------|
| CY00 | 180 | 180 | PCR test | 180 |
| Total | | | | X |
| Add a new row | | | | |

(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.

| | Total number of tests |
|------------------------------------|-----------------------|
| Total number of tests | 180 |
| Total Virus isolation tests | 0 |
| Total PCR tests | 180 |
| Total Other tests | 0 |

3.3 Sampling procedures and sampling periods

max 32000 chars :

Sampling from wild birds found dead or moribund will be performed throughout the year.

All samples will be submitted to the Laboratories of the Veterinary Services.

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3.4 Laboratory testing : description of the laboratory tests used taking into account criteria set out in the Diagnostic Manual for avian influenza (Commission Decision 2006/437/EC)

max 32000 chars :

According to Chapter V and Chapter VI of the Annex to Commission Decision 2006/437/EC.
RealTime-RT-PCR

1) Extract RNA using MagNA Pure Compact Nucleic Acid Isolation Kit and MagNA pure compact instrument.

2) Detection of influenza A matrix gene by real time Taqman® RT-PCR

The reagents required for these tests are:

a) Primers and probe

Sep1 AGA TGA GTC TTC TAA CCG AGG TCG

Sep2 TGC AAA AAC ATC TTC AAG TCT CTG

SePRO FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA

b) Real time PCR master mix which utilises the Qiagen Onestep RT-PCR kit (Cat No. 210212).

Volumes for the above master mix are indicated below:

DEPC treated water 13.75µl

(x5) Qiagen 1 step RT PCR buffer 5µl

Rox ref dye(pre diluted 1:500) in DEPC water 0.375µl

Qiagen dNTP mix 1µl

Sep1(50µM) 0,2µl

Sep2(50µM) 0,2µl

SePRO(30µM) 0.25µl

25mM magnesium chloride 1.25µl

RNAasin (40U/µl) 0.1µl

Qiagen 1 stepRT PCR enzyme mix 1µl

Aliquot 23µl of master mix per well of the real time plate/strip(s) and add 2µl of the sample RNA. Once RNA is added, plate/strip(s) are loaded onto a

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Lightcycler 96 real time Machine. The reactions are incubated with the following thermocycling profile:

RT step: 50°C for 30 mins,

95°C for 15 mins

PCR step (x 40 cycles): 95°C for 10 secs

60°C for 20 secs

The collection of fluorescence data is done during the 60°C annealing step using the ROX and FAM filters.

Criteria for assessing whether a sample is positive or negative are as follows:

Ct value <30 - samples are M gene positive

Ct value 30-35 with sigmoidal/logarithmic appearance – samples are weak positive.

Ct value >35 samples are negative if the amplification plot has a linear shape. These plots may represent spurious probe degradation or nonspecific fluorescence.

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

max 32000 chars :

No avian influenza virus was detected in samples collected from poultry farms in the framework of the surveillance programmes carried out by the Veterinary Services during the last years.

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

(max. 32000 chars) :

No HPAI virus was detected in wild birds sampled in the framework of the surveillance programmes. However H1N1 was detected in wild birds in August 2006.

6. *Measures in place as regards the notification of the disease*

(max. 32000 chars) :

According to Animal Health Laws of 2001 until 2014, any case or suspicion of Avian influenza should be notified to the Veterinary Services. Confirmed cases will be notified to the EU through ADNS and OIE according to the relevant legislation provisions.

7. *Costs*

7.1 *Detailed analysis of the costs*

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7.1.1 Poultry including ducks, geese and farmed game birds

(max. 32000 chars) :

The cost of the surveillance programme for poultry consists of the cost for the sampling of the birds and the cost of the tests performed on samples collected.

7.1.2 Wild birds

(max. 32000 chars) :

The cost of the surveillance programme for poultry consists of the cost for the sampling of the birds and the cost of the tests performed on samples collected.

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7.2.2 Wild bird surveillance

Detail analysis of the cost of the programme - wild birds

Targets for year **2016**

| Laboratory testing | | | | |
|--------------------------------|------------------|---|----------------|--|
| Methods of laboratory analysis | Number of tests | Unitary test cost (per method) in € (*) | Total cost (€) | |
| Virus isolation test | 0 | 37.88 | 0 | |
| PCR test | 180 | 19.74 | 3553.2 | |
| Other cost | 0 | 0 | 0 | |
| | | | | |
| Delivery of wild animals | | | | |
| Delivery of wild animals | No of wild birds | Eligible cost in € (*) | Total cost (€) | |
| | 0 | 0 | 0 | |
| | | | | |
| Other measures | | | | |
| Other measures | Number | Unitary cost in € | Total cost (€) | |
| | | | | |

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| | | | | |
|--|---|---|------------|---|
| | 0 | 0 | 0 | X |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| Total wild birds | | | | |
| Testing + Delivery + Other measures | | | | |
| | | | 3 553,20 € | |

(*) as per cofinancing decision for 2014 programmes

TOTALS for Poultry, duck, geese, farmed game birds (7.2.1) + WILD BIRDS (7.2.2) for year :

2016

| | |
|--|------------|
| | Total Cost |
| Grand Total Poultry, Ducks/Geese/Farmed game birds + WILD BIRDS | 34368,75 |

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C. Financial information

1. Identification of the implementing entities - financial circuits/flows

Identify and describe the entities which will be in charge of implementing the eligible measures planned in this programme which costs will constitute the reimbursement/payment claim to the EU. Describe the financial flows/circuits followed.

Each of the following paragraphs (from a to e) shall be filled out if EU cofinancing is requested for the related measure.

a) Implementing entities - **sampling**: who perform the official sampling? Who pays? (e.g. authorised private vets perform the sampling and are paid by the regional veterinary services (state budget); sampling equipment is provided by the private laboratory testing the samples which includes the price in the invoice which is paid by the local state veterinary services (state budget))

(max. 32000 chars):

The samplings for the surveillance programme for poultry is performed by Veterinary Services' personnel.

Concerning the surveillance programme for wild birds ,Game fund is a governmental service that cooperates with the veterinary services and provides dead or moribund wild birds that can be sampled for the A.I. surveillance program.

b) Implementing entities - **testing**: who performs the testing of the official samples? Who pays? (e.g. regional public laboratories perform the testing of official samples and costs related to this testing are entirely paid by the state budget)

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(max. 32000 chars) :

The Laboratories of the Veterinary Services will perform all the testing of official samples.

c) Implementing entities - **compensation**: who performs the compensation? Who pays? (e.g. compensation is paid by the central level of the state veterinary services, or compensation is paid by an insurance fund fed by compulsory farmers contribution)

(max. 32000 chars) :

N/A

d) Implementing entities - **vaccination** : who provides the vaccine and who performs the vaccination? Who pays the vaccine? Who pays the vaccinator? (e.g. farmers buy their vaccine to the private vets, send the paid invoices to the local state veterinary services which reimburse the farmers of the full amount and the vaccinator is paid by the regional state veterinary services)

(max. 32000 chars) :

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e) Implementing entities - **other essential measures**: who implement this measure? Who provide the equipment/service? Who pays?

(max. 32000 chars) :

N/A

2 Co-financing rate (see provisions of applicable Work Programme)

The maximum co-financing rate is in general fixed at 50%. However based on provisions of Article 5.2 and 5.3 of the Regulation (EU) No 652/2014, we request that the co-financing rate for the reimbursement of the eligible costs would be increased:

Up to 75% for the measures detailed below

Up to 100% for the measures detailed below

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3. Source of funding of eligible measures

All eligible measures for which cofinancing is requested and reimbursement will be claimed are financed by public funds.

yes

no

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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 : **jpg, jpeg, tiff, tif, xls, xlsx, doc, docx, ppt, pptx, bmp, pna, pdf.**
- 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) Only use letters from a-z and numbers from 1-10 in the attachment names, otherwise the submission of the data will not work.

List of all attachments

| | Attachment name | File will be saved as (only a-z and 0-9 and -_) | File size |
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| | 6665_4175.ocx | 6665_4175.ocx | 19 kb |
| | 6665_4176.jpg | 6665_4176.jpg | 377 kb |
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