TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION
SEPTEMBER 2010 REPORT

CHAPTER 10.4.

# AVIAN INFLUENZA

Article 10.4.1.

**General provisions**

1. For the purposes of ~~*international trade*~~ the *Terrestrial Code*, avian influenza in its notifiable form (NAI) is defined as an *infection* of *poultry* caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI):

   a) HPNAI viruses have an IVPI in 6-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in 4-to 8-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other HPNAI isolates, the isolate being tested should be considered as HPNAI;

   b) LPNAI are all influenza A viruses of H5 and H7 subtype that are not HPNAI viruses.

2. *Poultry* is defined as 'all domesticated birds, including backyard *poultry*, used for the production of *meat* or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose'.

   Birds that are kept in captivity for any reason other than those reasons referred to in the preceding paragraph, including those that are kept for shows, races, exhibitions, competitions or for breeding or selling these categories of birds as well as pet birds, are not considered to be *poultry*.

3. For the purposes of the *Terrestrial Code*, the *incubation period* for NAI shall be 21 days.

~~3~~4. ~~For the purposes of *international trade*, t~~This chapter deals not only with the occurrence of clinical signs caused by NAI virus, but also with the presence of infection with NAI virus in the absence of clinical signs.

~~4. For the purposes of *international trade*, a Member should not impose immediate bans on the trade in *poultry commodities* in response to a notification, according to Article 1.2.3. of the *Terrestrial Code*, of infection with HPAI and LPAI virus in birds other than *poultry*, including wild birds.~~

5. Antibodies to H5 or H7 subtype of NAI virus, which have been detected in *poultry* and are not a consequence of vaccination, have to be immediately investigated. In the case of isolated serological positive results, NAI infection may be ruled out on the basis of a thorough epidemiological and laboratory investigation that does not demonstrate further evidence of NAI infection.

6. The following defines the occurrence of infection with NAI virus:

   a) HPNAI virus has been isolated and identified as such or viral RNA specific for HPNAI has been detected in *poultry* or a product derived from *poultry*; or

   b) LPNAI virus has been isolated and identified as such or viral RNA specific for LPNAI has been detected in *poultry* or a product derived from *poultry*.

7. For the purposes of the *Terrestrial Code*, 'NAI free establishment' means an *establishment* in which the *poultry* have shown no evidence of NAI infection, based on *surveillance* in accordance with Articles 10.4.27. to 10.4.33.

~~For the purposes of the *Terrestrial Code*, the *incubation period* for NAI shall be 21 days.~~

8. Standards for diagnostic tests, including pathogenicity testing, are described in the *Terrestrial Manual*. Any vaccine used should comply with the standards described in the *Terrestrial Manual*.

9. A Member should not impose immediate bans on the trade in *poultry commodities* in response to a notification, according to Article 1.2.3. of the *Terrestrial Code*, of infection with HPAI and LPAI virus in birds other than *poultry*, including wild birds.

Article 10.4.2.

**Determination of the NAI status of a country, zone or compartment**

The NAI status of a country, a *zone* or a *compartment* can be determined on the basis of the following criteria:

1. NAI is notifiable in the whole country, an on-going NAI awareness programme is in place, and all notified suspect occurrences of NAI are subjected to field and, where applicable, *laboratory* investigations;

2. appropriate *surveillance* is in place to demonstrate the presence of *infection* in the absence of clinical signs in *poultry*, and the risk posed by birds other than *poultry*; this may be achieved through a NAI *surveillance* programme in accordance with Articles 10.4.27. to 10.4.33.;

3. consideration of all epidemiological factors for NAI occurrence and their historical perspective.

Article 10.4.3.

**NAI free country, zone or compartment**

A country, *zone* or *compartment* may be considered free from NAI when it has been shown that neither HPNAI nor LPNAI infection in *poultry* has been present in the country, *zone* or *compartment* for the past 12 months, based on *surveillance* in accordance with Articles 10.4.27. to 10.4.33.

If *infection* has occurred in *poultry* in a previously free country, *zone* or *compartment*, NAI free status can be regained:

1. In the case of HPNAI infections, 3 months after a *stamping-out policy* (including *disinfection* of all affected *establishments*) is applied, providing that *surveillance* in accordance with Articles 10.4.27. to 10.4.33. has been carried out during that three-month period.

2. In the case of LPNAI infections, *poultry* may be kept for *slaughter* for human consumption subject to conditions specified in Article 10.4.19. or a *stamping-out policy* may be applied; in either case, 3 months after the *disinfection* of all affected *establishments*, providing that *surveillance* in accordance with Articles 10.4.27. to 10.4.33. has been carried out during that three-month period.

Article 10.4.4.

**HPNAI free country, zone or compartment**

A country, *zone* or *compartment* may be considered free from HPNAI when:

1. it has been shown that HPNAI infection in *poultry* has not been present in the country, *zone* or *compartment* for the past 12 months, although its LPNAI status may be unknown; or

2. when, based on *surveillance* in accordance with Articles 10.4.27. to 10.4.33., it does not meet the criteria for freedom from NAI but any NAI virus detected has not been identified as HPNAI virus.

The *surveillance* may need to be adapted to parts of the country or existing *zones* or *compartments* depending on historical or geographical factors, industry structure, population data, or proximity to recent *outbreaks*.

If *infection* has occurred in *poultry* in a previously free country, *zone* or *compartment*, HPNAI free status can be regained 3 months after a *stamping-out policy* (including *disinfection* of all affected *establishments*) is applied, providing that *surveillance* in accordance with Articles 10.4.27. to 10.4.33. has been carried out during that three-month period.

Article 10.4.5.

**Recommendations for importation from a NAI free country, zone or compartment**

for live poultry (other than day-old poultry)

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the *poultry* showed no clinical sign of NAI on the day of shipment;

2. the *poultry* were kept in a NAI free country, *zone* or *compartment* since they were hatched or for at least the past 21 days;

3. the *poultry* are transported in new or appropriately sanitized *containers*;

4. if the *poultry* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.6.

**Recommendations for the importation of live birds other than poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. on the day of shipment, the birds showed no clinical sign of *infection* with a virus which would be considered NAI in *poultry*;

2. the birds were kept in isolation approved by the *Veterinary Services* since they were hatched or for at least the 21 days prior to shipment and showed no clinical sign of *infection* with a virus which would be considered NAI in *poultry* during the isolation period;

3. a statistically valid sample of the birds, selected in accordance with the provisions of Article 10.4.29., was subjected to a diagnostic test within 14 days prior to shipment to demonstrate freedom from *infection* with a virus which would be considered NAI in *poultry*;

4. the birds are transported in new or appropriately sanitized *containers*;

5. if the birds have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.7.

**Recommendations for importation from a NAI free country, zone or compartment**

for day-old live poultry

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the *poultry* were kept in a NAI free country, *zone* or *compartment* since they were hatched;

2. the *poultry* were derived from parent *flocks* which had been kept in a NAI free country, *zone* or *compartment* for at least 21 days prior to and at the time of the collection of the eggs;

3. the *poultry* are transported in new or appropriately sanitized *containers*;

4. if the *poultry* or the parent *flocks* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.8.

**Recommendations for importation from a HPNAI free country, zone or compartment**

for day-old live poultry

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the *poultry* were kept in a HPNAI free country, *zone* or *compartment* since they were hatched;

2. the *poultry* were derived from parent *flocks* which had been kept in a NAI free *establishment* for at least 21 days prior to and at the time of the collection of the eggs;

3. the *poultry* are transported in new or appropriately sanitized *containers*;

4. if the *poultry* or the parent *flocks* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.9.

**Recommendations for the importation of day-old live birds other than poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. on the day of shipment, the birds showed no clinical signs of *infection* with a virus which would be considered NAI in *poultry*;

2. the birds were hatched and kept in isolation approved by the *Veterinary Services*;

3. the parent *flock* birds were subjected to a diagnostic test at the time of the collection of the eggs to demonstrate freedom from *infection* with NAIV;

4. the birds are transported in new or appropriately sanitized *containers*;

5. if the birds or parent *flocks* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.10.

**Recommendations for importation from a NAI free country, zone or compartment**

for hatching eggs of poultry

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the eggs came from a NAI free country, *zone* or *compartment*;

2. the eggs were derived from parent *flocks* which had been kept in a NAI free country, *zone* or *compartment* for at least 21 days prior to and at the time of the collection of the eggs;

3. the eggs are transported in new or appropriately sanitized packaging materials;

4. if the parent *flocks* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.11.

**Recommendations for importation from a HPNAI free country, zone or compartment**

for hatching eggs of poultry

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the eggs came from a HPNAI free country, *zone* or *compartment*;

2. the eggs were derived from parent *flocks* which had been kept in a NAI free *establishment* for at least 21 days prior to and at the time of the collection of the eggs;

3. the eggs have had their surfaces sanitized (in accordance with Chapter 6.4.);

4. the eggs are transported in new or appropriately sanitized packaging materials;

5. if the parent *flocks* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.12.

**Recommendations for the importation of hatching eggs from birds other than poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the parent *flock* birds were subjected to a diagnostic test 7 days prior to and at the time of the collection of the eggs to demonstrate freedom from infection with NAIV;

2. the eggs have had their surfaces sanitized (in accordance with Chapter 6.4.);

3. the eggs are transported in new or appropriately sanitized packaging materials;

4. if the parent *flocks* have been vaccinated against NAI, it has been done in accordance with the provisions of the *Terrestrial Manual* and the nature of the vaccine used and the date of vaccination have been attached to the *certificate*.

Article 10.4.13.

**Recommendations for importation from a NAI free country, zone or compartment**

for eggs for human consumption

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the eggs were produced and packed in a NAI free country, *zone* or *compartment*;

2. the eggs are transported in new or appropriately sanitized packaging materials.

Article 10.4.14.

**Recommendations for importation from a HPNAI free country, zone or compartment**

for eggs for human consumption

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the eggs were produced and packed in a HPNAI free country, *zone* or *compartment*;

2. the eggs have had their surfaces sanitized (in accordance with Chapter 6.4.);

3. the eggs are transported in new or appropriately sanitized packaging materials.

Article 10.4.15.

**Recommendations for importation of egg products of poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the *commodity* is derived from eggs which meet the requirements of Articles 10.4.13. or 10.4.14.; or

2. the *commodity* has been processed to ensure the destruction of NAI virus in accordance with Article 10.4.25.;

AND

3. the necessary precautions were taken to avoid contact of the *commodity* with any source of NAI virus.

Article 10.4.16.

**Recommendations for importation from a NAI free country, zone or compartment**

for poultry semen

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the donor *poultry*:

1. showed no clinical sign of NAI on the day of semen collection;

2. were kept in a NAI free country, *zone* or *compartment* for at least the 21 days prior to and at the time of semen collection.

Article 10.4.17.

**Recommendations for the importation from a HPNAI free country, zone or compartment**

for poultry semen

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the donor *poultry*:

1. showed no clinical sign of HPNAI on the day of semen collection;

2. were kept in a HPNAI free country, *zone* or *compartment* for at least the 21 days prior to and at the time of semen collection.

Article 10.4.18.

**Recommendations for the importation of semen of birds other than poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the donor birds:

1. were kept in isolation approved by the *Veterinary Services* for at least the 21 days prior to semen collection;

2. showed no clinical sign of *infection* with a virus which would be considered NAI in *poultry* during the isolation period;

3. were tested within 14 days prior to semen collection and shown to be free of NAI infection.

Article 10.4.19.

**Recommendations for importation from either a NAI or HPNAI free country, zone or compartment**

for fresh meat of poultry

*Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that the entire consignment of *fresh meat* comes from *poultry*:

1. which have been kept in a country, *zone* or *compartment* free from HPNAI since they were hatched or for at least the past 21 days;

2. which have been slaughtered in an approved *abattoir* in a country, *zone* or *compartment* free from HPNAI and have been subjected to ante-mortem and post-mortem inspections in accordance with Chapter 6.2. and have been found free of any signs suggestive of NAI.

Article 10.4.20.

**Recommendations for the importation of meat products of poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. the *commodity* is derived from *fresh meat* which meet the requirements of Article 10.4.19.; or

2. the *commodity* has been processed to ensure the destruction of NAI virus in accordance with Article 10.4.26.;

AND

3. the necessary precautions were taken to avoid contact of the *commodity* with any source of NAI virus.

Article 10.4.21.

**Recommendations for the importation of products of poultry origin, other than feather meal and poultry meal, intended for use in animal feeding, or for agricultural or industrial use**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. these *commodities* were processed in a NAI free country, *zone* or *compartment* from *poultry* which were kept in a NAI free country, *zone* or *compartment* from the time they were hatched until the time of *slaughter* or for at least the 21 days preceding *slaughter*; or

2. these *commodities* have been processed to ensure the destruction of NAI virus (under study);

AND

3. the necessary precautions were taken to avoid contact of the *commodity* with any source of NAI virus.

Article 10.4.22.

**Recommendations for the importation of feathers and down of poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. these *commodities* originated from *poultry* as described in Article 10.4.19. and were processed in a NAI free country, *zone* or *compartment*; or

2. these *commodities* have been processed to ensure the destruction of NAI virus (under study);

AND

3. the necessary precautions were taken to avoid contact of the *commodity* with any source of NAI virus.

Article 10.4.23.

**Recommendations for the importation of feathers and down of birds other than poultry**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. these *commodities* have been processed to ensure the destruction of NAI virus (under study); and

2. the necessary precautions were taken to avoid contact of the *commodity* with any source of NAI virus.

Article 10.4.24.

**Recommendations for the importation of feather meal and poultry meal**

Regardless of the NAI status of the country of origin, *Veterinary Authorities* should require the presentation of an *international veterinary certificate* attesting that:

1. these *commodities* were processed in a NAI free country, *zone* or *compartment* from *poultry* which were kept in a NAI free country, *zone* or *compartment* from the time they were hatched until the time of *slaughter* or for at least the 21 days preceding *slaughter*; or

2. these *commodities* have been processed either:

   a) with moist heat at a minimum temperature of 118ºC for minimum of 40 minutes; or

   b) with a continuous hydrolysing process under at least 3.79 bar of pressure with steam at a minimum temperature of 122ºC for a minimum of 15 minutes; or

   c) with an alternative rendering process that ensures that the internal temperature throughout the product reaches at least 74ºC;

AND

3. the necessary precautions were taken to avoid contact of the *commodity* with any source of NAI virus.

Article 10.4.25.

**Procedures for the inactivation of the AI virus in eggs and egg products**

The following times for industry standard temperatures are suitable for the inactivation of AI virus present in eggs and egg products:

| | **Core temperature (°C)** | **Time** |
|---|---|---|
| Whole egg | 60 | 188 seconds |
| Whole egg blends | 60 | 188 seconds |
| Whole egg blends | 61.1 | 94 seconds |
| Liquid egg white | 55.6 | 870 seconds |
| Liquid egg white | 56.7 | 232 seconds |
| 10% salted yolk | 62.2 | 138 seconds |
| Dried egg white | 67 | 20 hours |
| Dried egg white | 54.4 | 513 hours |

The listed temperatures are indicative of a range that achieves a 7-log kill. Where scientifically documented, variances from these times and temperatures may also be suitable when they achieve the inactivation of the virus.

Article 10.4.26.

**Procedures for the inactivation of the AI virus in meat**

The following times for industry standard temperatures are suitable for the inactivation of AI virus present in *meat*.

| | Core temperature (°C) | Time |
|---|---|---|
| Poultry meat | 60.0 | 507 seconds |
| | 65.0 | 42 seconds |
| | 70.0 | 3.5 seconds |
| | 73.9 | 0.51 seconds |

The listed temperatures are indicative of a range that achieves a 7-log kill. Where scientifically documented, variances from these times and temperatures may also be suitable when they achieve the inactivation of the virus.

Article 10.4.27.

**Surveillance: introduction**

Articles 10.4.27. to 10.4.33. define the principles and provide a guide on the *surveillance* for NAI complementary to Chapter 1.4., applicable to Members seeking to determine their NAI status. This may be for the entire country, *zone* or *compartment*. Guidance for Members seeking free status following an *outbreak* and for the maintenance of NAI status is also provided.

The presence of avian influenza viruses in wild birds creates a particular problem. In essence, no Member can declare itself free from avian influenza (AI) in wild birds. However, the definition of NAI in this chapter refers to the *infection* in *poultry* only, and Articles 10.4.27. to 10.4.33. were developed under this definition.

The impact and epidemiology of NAI differ widely in different regions of the world and therefore it is impossible to provide specific recommendations for all situations. *Surveillance* strategies employed for demonstrating freedom from NAI at an acceptable level of confidence will need to be adapted to the local situation. Variables such as the frequency of contacts of *poultry* with wild birds, different biosecurity levels and production systems and the commingling of different susceptible species including domestic waterfowl require specific *surveillance* strategies to address each specific situation. It is incumbent upon the Member to provide scientific data that explains the epidemiology of NAI in the region concerned and also demonstrates how all the risk factors are managed. There is therefore considerable latitude available to Members to provide a well-reasoned argument to prove that absence of NAI virus (NAIV) infection is assured at an acceptable level of confidence.

*Surveillance* for NAI should be in the form of a continuing programme designed to establish that the country, *zone* or *compartment*, for which application is made, is free from NAIV infection.

Article 10.4.28.

**Surveillance: general conditions and methods**

1. A *surveillance* system in accordance with Chapter 1.4. should be under the responsibility of the *Veterinary Authority*. In particular:

   a) a formal and ongoing system for detecting and investigating *outbreaks* of *disease* or NAI infection should be in place;

   b) a procedure should be in place for the rapid collection and transport of samples from suspect cases of NAI to a *laboratory* for NAI diagnosis as described in the *Terrestrial Manual*;

   c) a system for recording, managing and analysing diagnostic and *surveillance* data should be in place.

2. The NAI *surveillance* programme should:

   a) include an early warning system throughout the production, marketing and processing chain for reporting suspicious cases. Farmers and workers, who have day-to-day contact with *poultry*, as well as diagnosticians, should report promptly any suspicion of NAI to the *Veterinary Authority*. They should be supported directly or indirectly (e.g. through private *veterinarians* or *veterinary para-professionals*) by government information programmes and the *Veterinary Authority*. All suspected cases of NAI should be investigated immediately. As suspicion cannot always be resolved by epidemiological and clinical investigation alone, samples should be taken and submitted to a *laboratory* for appropriate tests. This requires that sampling kits and other equipment are available for those responsible for *surveillance*. Personnel responsible for *surveillance* should be able to call for assistance from a team with expertise in NAI diagnosis and control. In cases where potential public health implications are suspected, notification to the appropriate public health authorities is essential;

   b) implement, when relevant, regular and frequent clinical inspection, serological and virological testing of high-risk groups of *animals*, such as those adjacent to a NAI infected country, *zone* or *compartment*, places where birds and *poultry* of different origins are mixed, such as live bird markets, *poultry* in close proximity to waterfowl or other potential sources of NAIV.

An effective *surveillance* system will periodically identify suspicious cases that require follow-up and investigation to confirm or exclude that the cause of the condition is NAIV. The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably. Applications for freedom from NAIV infection should, in consequence, provide details of the occurrence of suspicious cases and how they were investigated and dealt with. This should include the results of *laboratory* testing and the control measures to which the *animals* concerned were subjected during the investigation (quarantine, movement stand-still orders, etc.).

Article 10.4.29.

**Surveillance strategies**

1. Introduction

   The target population for *surveillance* aimed at identification of *disease* and *infection* should cover all the susceptible *poultry* species within the country, *zone* or *compartment*. Active and passive *surveillance* for NAI should be ongoing. The frequency of active *surveillance* should be at least every 6 months. *Surveillance* should be composed of random and targeted approaches using molecular, virological, serological and clinical methods.

The strategy employed may be based on randomised sampling requiring *surveillance* consistent with demonstrating the absence of NAIV infection at an acceptable level of confidence. Random *surveillance* is conducted using serological tests described in the *Terrestrial Manual*. Positive serological results should be followed up with molecular or virological methods.

Targeted *surveillance* (e.g. based on the increased likelihood of *infection* in particular localities or species) may be an appropriate strategy. Virological and serological methods should be used concurrently to define the NAI status of high risk populations.

A Member should justify the *surveillance* strategy chosen as adequate to detect the presence of NAIV infection in accordance with Chapter 1.4. and the prevailing epidemiological situation, including *cases* of HPAI detected in any birds. It may, for example, be appropriate to target clinical *surveillance* at particular species likely to exhibit clear clinical signs (e.g. chickens). Similarly, virological and serological testing could be targeted to species that may not show clinical signs (e.g. ducks).

If a Member wishes to declare freedom from NAIV infection in a specific *zone* or *compartment*, the design of the survey and the basis for the sampling process would need to be aimed at the population within the *zone* or *compartment*.

For random surveys, the design of the sampling strategy will need to incorporate epidemiologically appropriate design prevalence. The sample size selected for testing will need to be large enough to detect *infection* if it were to occur at a predetermined minimum rate. The sample size and expected *disease* prevalence determine the level of confidence in the results of the survey. The Member should justify the choice of design prevalence and confidence level based on the objectives of *surveillance* and the epidemiological situation, in accordance with Chapter 1.4. Selection of the design prevalence in particular clearly needs to be based on the prevailing or historical epidemiological situation.

Irrespective of the survey approach selected, the sensitivity and specificity of the diagnostic tests employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated for the vaccination/*infection* history and the different species in the target population.

Irrespective of the testing system employed, *surveillance* system design should anticipate the occurrence of false positive reactions. If the characteristics of the testing system are known, the rate at which these false positives are likely to occur can be calculated in advance. There needs to be an effective procedure for following up positives to ultimately determine with a high level of confidence, whether they are indicative of *infection* or not. This should involve both supplementary tests and follow-up investigation to collect diagnostic material from the original sampling unit as well as *flocks* which may be epidemiologically linked to it.

The principles involved in *surveillance* for *disease*/*infection* are technically well defined. The design of *surveillance* programmes to prove the absence of NAIV infection/circulation needs to be carefully followed to avoid producing results that are either insufficiently reliable, or excessively costly and logistically complicated. The design of any *surveillance* programme, therefore, requires inputs from professionals competent and experienced in this field.

2. Clinical surveillance

Clinical *surveillance* aims at the detection of clinical signs of NAI at the *flock* level. Whereas significant emphasis is placed on the diagnostic value of mass serological screening, *surveillance* based on clinical inspection should not be underrated. Monitoring of production parameters, such as increased mortality, reduced feed and water consumption, presence of clinical signs of a respiratory *disease* or a drop in egg production, is important for the early detection of NAIV infection. In some cases, the only indication of LPNAIV infection may be a drop in feed consumption or egg production.

Clinical *surveillance* and *laboratory* testing should always be applied in series to clarify the status of NAI suspects detected by either of these complementary diagnostic approaches. *Laboratory* testing may confirm clinical suspicion, while clinical *surveillance* may contribute to confirmation of positive serology. Any

sampling unit within which suspicious *animals* are detected should have restrictions imposed upon it until NAI infection is ruled out.

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

3. Virological surveillance

Virological *surveillance* using tests described in the *Terrestrial Manual* should be conducted:

a) to monitor at risk populations;

b) to confirm clinically suspect cases;

c) to follow up positive serological results;

d) to test 'normal' daily mortality, to ensure early detection of *infection* in the face of vaccination or in *establishments* epidemiologically linked to an *outbreak*.

4. Serological surveillance

Serological *surveillance* aims at the detection of antibodies against NAIV. Positive NAIV antibody test results can have four possible causes:

a) natural *infection* with NAIV;

b) vaccination against NAI;

c) maternal antibodies derived from a vaccinated or infected parent *flock* are usually found in the yolk and can persist in progeny for up to 4 weeks;

d) false positive results due to the lack of specificity of the test.

It may be possible to use serum collected for other survey purposes for NAI *surveillance*. However, the principles of survey design described in these recommendations and the requirement for a statistically valid survey for the presence of NAIV should not be compromised.

The discovery of clusters of seropositive *flocks* may reflect any of a series of events, including but not limited to the demographics of the population sampled, vaccinal exposure or *infection*. As clustering may signal *infection*, the investigation of all instances should be incorporated in the survey design. Clustering of positive *flocks* is always epidemiologically significant and therefore should be investigated.

If vaccination cannot be excluded as the cause of positive serological reactions, diagnostic methods to differentiate antibodies due to *infection* or vaccination should be employed.

The results of random or targeted serological surveys are important in providing reliable evidence that no NAIV infection is present in a country, *zone* or *compartment*. It is therefore essential that the survey be thoroughly documented.

5. Virological and serological surveillance in vaccinated populations

The *surveillance* strategy is dependent on the type of vaccine used. The protection against AI is haemagglutinin subtype specific. Therefore, two broad vaccination strategies exist: 1) inactivated whole AI viruses, and 2) haemagglutinin expression-based vaccines.

In the case of vaccinated populations, the *surveillance* strategy should be based on virological and/or serological methods and clinical *surveillance*. It may be appropriate to use sentinel birds for this purpose. These birds should be unvaccinated, AI virus antibody free birds and clearly and permanently identified. Sentinel birds should be used only if no appropriate *laboratory* procedures are available. The interpretation of serological results in the presence of vaccination is described in Article 10.4.33.

Article 10.4.30.

**Documentation of NAI or HPNAI free status**

1. Members declaring freedom from NAI or HPNAI for the country, zone or compartment: additional surveillance procedures

In addition to the general conditions described in above mentioned articles, a Member declaring freedom from NAI or HPNAI for the entire country, or a *zone* or a *compartment* should provide evidence for the existence of an effective *surveillance* programme. The strategy and design of the *surveillance* programme will depend on the prevailing epidemiological circumstances and should be planned and implemented according to general conditions and methods described in this Chapter, to demonstrate absence of NAIV or HPNAIV infection, during the preceding 12 months in susceptible *poultry* populations (vaccinated and non-vaccinated). This requires the support of a *laboratory* able to undertake identification of NAIV or HPNAIV infection through virus detection and antibody tests described in the *Terrestrial Manual*. This *surveillance* may be targeted to *poultry* population at specific risks linked to the types of production, possible direct or indirect contact with wild birds, multi-age *flocks*, local trade patterns including live bird markets, use of possibly contaminated surface water, and the presence of more than one species on the holding and poor biosecurity measures in place.

2. Additional requirements for countries, zones or compartments that practise vaccination

Vaccination to prevent the transmission of HPNAI virus may be part of a *disease* control programme. The level of *flock* immunity required to prevent transmission will depend on the *flock* size, composition (e.g. species) and density of the susceptible *poultry* population. It is therefore impossible to be prescriptive. The vaccine should also comply with the provisions stipulated for NAI vaccines in the *Terrestrial Manual*. Based on the epidemiology of NAI in the country, *zone* or *compartment*, it may be that a decision is reached to vaccinate only certain species or other *poultry* subpopulations.

In all vaccinated *flocks* there is a need to perform virological and serological tests to ensure the absence of virus circulation. The use of sentinel *poultry* may provide further confidence of the absence of virus circulation. The tests have to be repeated at least every 6 months or at shorter intervals according to the risk in the country, *zone* or *compartment*.

Evidence to show the effectiveness of the vaccination programme should also be provided.

Article 10.4.31.

**Countries, zones or compartments declaring that they have regained freedom from NAI or HPNAI following an outbreak: additional surveillance procedures**

In addition to the general conditions described in the above-mentioned articles, a Member declaring that it has regained country, *zone* or *compartment* freedom from NAI or HPNAI virus infection should show evidence of an active *surveillance* programme depending on the epidemiological circumstances of the *outbreak* to demonstrate the absence of the *infection*. This will require *surveillance* incorporating virus detection and antibody tests described in the *Terrestrial Manual*. The use of sentinel birds may facilitate the interpretation of *surveillance* results.

A Member declaring freedom of country, *zone* or *compartment* after an *outbreak* of NAI or HPNAI (with or without vaccination) should report the results of an active *surveillance* programme in which the NAI or HPNAI susceptible *poultry* population undergoes regular clinical examination and active *surveillance* planned and implemented according to the general conditions and methods described in these recommendations. The *surveillance* should at least give the confidence that can be given by a randomized representative sample of the populations at risk.

Article 10.4.32.

**NAI free establishments within HPNAI free compartments: additional surveillance procedures**

The declaration of NAI free *establishments* requires the demonstration of absence of NAIV infection. Birds in these *establishments* should be randomly tested using virus detection or isolation tests, and serological methods, following the general conditions of these recommendations. The frequency of testing should be based on the risk of *infection* and at a maximum interval of 21 days.

Article 10.4.33.

**The use and interpretation of serological and virus detection tests**

*Poultry* infected with NAI virus produce antibodies to haemagglutinin (HA), neuraminidase (NA), nonstructural proteins (NSPs), nucleoprotein/matrix (NP/M) and the polymerase complex proteins. Detection of antibodies against the polymerase complex proteins will not be covered in this chapter. Tests for NP/M antibodies include direct and blocking ELISA, and agar gel immunodiffusion (AGID) tests. Tests for antibodies against NA include the neuraminidase inhibition (NI), indirect fluorescent antibody and direct and blocking ELISA tests. For the HA, antibodies are detected in haemagglutination inhibition (HI), ELISA and neutralization (SN) tests. The HI test is reliable in avian species but not in mammals. The SN test can be used to detect subtype specific antibodies to the haemagglutinin and is the preferred test for mammals and some avian species. The AGID test is reliable for detection of NP/M antibodies in chickens and turkeys, but not in other avian species. As an alternative, blocking ELISA tests have been developed to detect NP/M antibodies in all avian species.

The HI and NI tests can be used to subtype AI viruses into 16 haemagglutinin and 9 neuraminidase subtypes. Such information is helpful for epidemiological investigations and in categorization of AI viruses.

*Poultry* can be vaccinated with a variety of AI vaccines including inactivated whole AI virus vaccines, and haemagglutinin expression-based vaccines. Antibodies to the haemagglutinin confer subtype specific protection. Various strategies can be used to differentiate vaccinated from infected birds including serosurveillance in unvaccinated sentinel birds or specific serological tests in the vaccinated birds.

AI virus infection of unvaccinated birds including sentinels is detected by antibodies to the NP/M, subtype specific HA or NA proteins, or NSP. *Poultry* vaccinated with inactivated whole AI vaccines containing an influenza virus of the same H sub-type but with a different neuraminidase may be tested for field exposure by applying serological tests directed to the detection of antibodies to the NA of the field virus. For example, birds vaccinated with H7N3 in the face of a H7N1 epidemic may be differentiated from infected birds (DIVA) by detection of subtype specific NA antibodies of the N1 protein of the field virus. Alternatively, in the absence of DIVA, inactivated vaccines may induce low titres of antibodies to NSP and the titre in infected birds would be markedly higher. Encouraging results have been obtained experimentally with this system, but it has not yet been validated in the field. In *poultry* vaccinated with haemagglutinin expression-based vaccines, antibodies are detected to the specific HA, but not any of the other AI viral proteins. *Infection* is evident by antibodies to the NP/M or NSP, or the specific NA protein of the field virus. Vaccines used should comply with the standards of the *Terrestrial Manual*.

All *flocks* with seropositive results should be investigated. Epidemiological and supplementary *laboratory* investigation results should document the status of NAI infection/circulation for each positive *flock*.

A confirmatory test should have a higher specificity than the screening test and sensitivity at least equivalent than that of the screening test.

Information should be provided on the performance characteristics and validation of tests used.

1. The follow-up procedure in case of positive test results if vaccination is used

In case of vaccinated populations, one has to exclude the likelihood that positive test results are indicative of virus circulation. To this end, the following procedure should be followed in the investigation of positive serological test results derived from *surveillance* conducted on NAI-vaccinated *poultry*. The investigation should examine all evidence that might confirm or refute the hypothesis that the positive results to the serological tests employed in the initial survey were not due to virus circulation. All the epidemiological information should be substantiated, and the results should be collated in the final report.

Knowledge of the type of vaccine used is crucial in developing a serological based strategy to differentiate infected from vaccinated *animals*.

a) Inactivated whole AI virus vaccines can use either homologous or heterologous neuraminidase subtypes between the vaccine and field strains. If *poultry* in the population have antibodies to NP/M and were vaccinated with inactivated whole AI virus vaccine, the following strategies should be applied:

i) sentinel birds should remain NP/M antibody negative. If positive for NP/M antibodies, indicating AI virus infection, specific HI tests should be performed to identify H5 or H7 AI virus infection;

ii) if vaccinated with inactivated whole AI virus vaccine containing homologous NA to field virus, the presence of antibodies to NSP could be indicative of *infection*. Sampling should be initiated to exclude the presence of NAIV by either virus isolation or detection of virus specific genomic material or proteins;

iii) if vaccinated with inactivated whole AI virus vaccine containing heterologous NA to field virus, presence of antibodies to the field virus NA or NSP would be indicative of *infection*. Sampling should be initiated to exclude the presence of NAIV by either virus isolation or detection of virus specific genomic material or proteins.

b) Haemagglutinin expression-based vaccines contain the HA protein or gene homologous to the HA of the field virus. Sentinel birds as described above can be used to detect AI infection. In vaccinated or sentinel birds, the presence of antibodies against NP/M, NSP or field virus NA is indicative of *infection*. Sampling should be initiated to exclude the presence of NAIV by either virus isolation or detection of virus specific genomic material or proteins.

2. The follow-up procedure in case of positive test results indicative of infection for determination of infection due to HPNAI or LPNAI virus

The detection of antibodies indicative of a NAI virus infection as indicated in point a)i) above will result in the initiation of epidemiological and virological investigations to determine if the infections are due to HPNAI or LPNAI viruses.

Virological testing should be initiated in all antibody-positive and at risk populations. The samples should be evaluated for the presence of AI virus, by virus isolation and identification, and/or detection of influenza A specific proteins or nucleic acids (Figure 2). Virus isolation is the gold standard for detecting *infection* by AI virus and the method is described in the *Terrestrial Manual*. All AI virus isolates should be tested to determine HA and NA subtypes, and *in vivo* tested in chickens and/or sequencing of HA proteolytic

cleavage site of H5 and H7 subtypes for determination of classification as HPNAI, LPNAI or LPAI (not notifiable) viruses. As an alternative, nucleic acid detection tests have been developed and validated; these tests have the sensitivity of virus isolation, but with the advantage of providing results within a few hours. Samples with detection of H5 and H7 HA subtypes by nucleic acid detection methods should either be submitted for virus isolation, identification, and *in vivo* testing in chickens, or sequencing of nucleic acids for determination of proteolytic cleavage site as HPNAI or LPNAI viruses. The antigen detection systems,

because of low sensitivity, are best suited for screening clinical field cases for *infection* by Type A influenza virus looking for NP/M proteins. NP/M positive samples should be submitted for virus isolation, identification and pathogenicity determination.

*Laboratory* results should be examined in the context of the epidemiological situation. Corollary information needed to complement the serological survey and assess the possibility of viral circulation includes but is not limited to:

a) characterization of the existing production systems;

b) results of clinical *surveillance* of the suspects and their cohorts;

c) quantification of vaccinations performed on the affected sites;

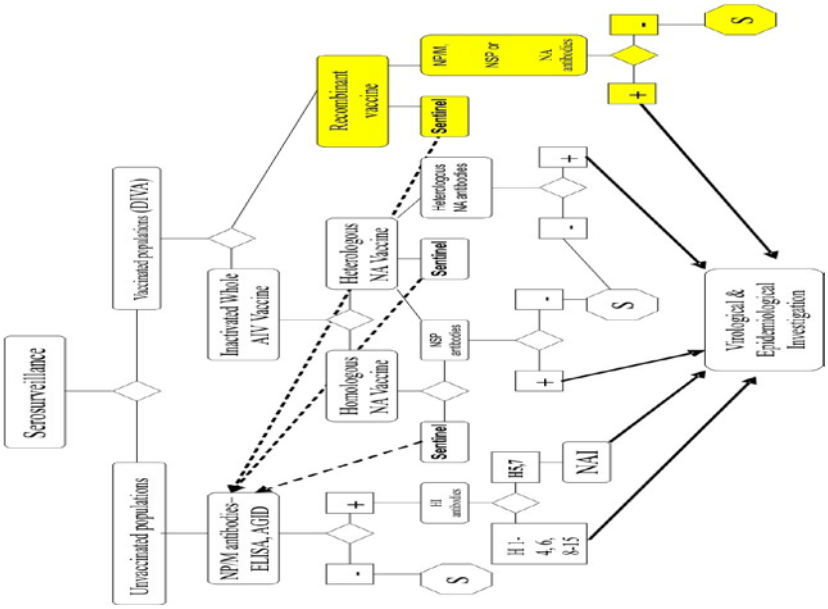
d) sanitary protocol and history of the affected *establishments*;

e) control of *animal identification* and movements;

f) other parameters of regional significance in historic NAIV transmission.

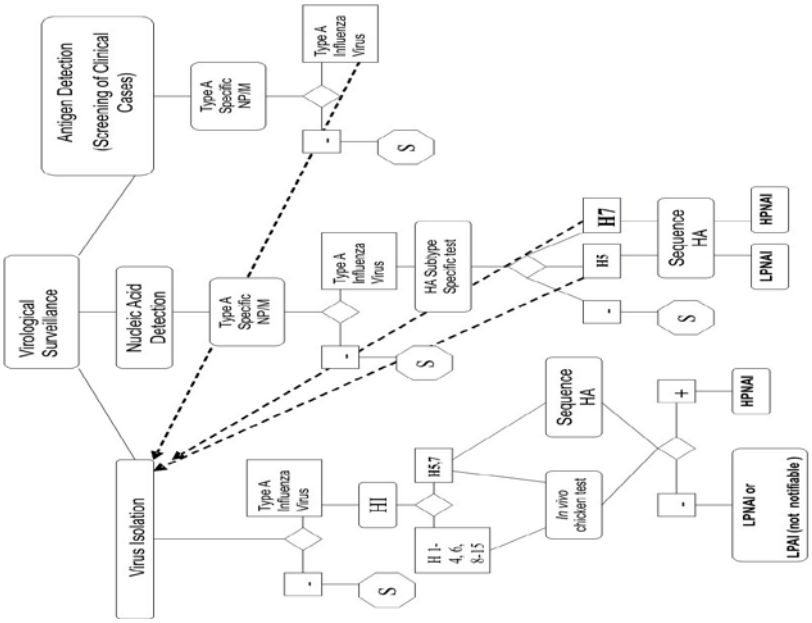
The entire investigative process should be documented as standard operating procedure within the epidemiological *surveillance* programme.

Fig. 1. **Schematic representation of laboratory tests for determining evidence of NAI infection through or following serological surveys**




| Key: | |
|---|---|
| AGID | Agar gel immunodiffusion |
| DIVA | Differentiating infected from vaccinated animals |
| ELISA | Enzyme-linked immunosorbant assay |
| HA | Haemagglutinin |
| HI | Haemagglutination inhibition |
| NA | Neuraminidase |
| NP/M | Nucleoprotein and matrix protein |
| NSP | Nonstructural protein |
| S | No evidence of NAIV |

Fig. 2. **Schematic representation of laboratory tests for determining evidence of NAI infection using virological methods**




The above diagrams indicate the tests which are recommended for use in the investigation of *poultry flocks*.

| Key: | |
|---|---|
| AGID | Agar gel immunodiffusion |
| DIVA | Differentiating infected from vaccinated animals |
| ELISA | Enzyme-linked immunosorbant assay |
| HA | Haemagglutinin |
| HI | Haemagglutination inhibition |
| NA | Neuraminidase |
| NP/M | Nucleoprotein and matrix protein |
| NSP | Nonstructural protein |
| S | No evidence of NAIV |