



FAO GUIDELINES FOR
SURVEILLANCE OF
PANDEMIC H1N1/2009 AND
OTHER INFLUENZA VIRUSES IN
SWINE POPULATIONS

March 2010



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1. Background

1.1 PANDEMIC H1N1/2009 INFLUENZA

The pandemic H1N1/2009 influenza A virus (pH1N1) is a genetic re-assortment of four different influenza virus strains that includes: (i) a human influenza gene segment, (ii) avian gene segments from North America, (iii) swine influenza gene segments from North America, and (iv) Eurasian avian-like swine gene segments. The initial triple re-assortment, that includes genes of first three virus strains (i, ii and iii) was first seen at least ten years ago and has likely been circulating in pigs since then, causing only very occasional and mild disease in humans in close contact with pigs. The fourth and recent re-assortment between the first triple re-assortment and a Eurasian pig influenza virus had not been detected previously in pigs or humans.

Although the novel virus was initially denominated “swine flu”, evidence of infection of humans from pigs is so far absent, while human-to-human transmission is clearly common and easy.

This is a new infection in humans, pigs and other species. Information is still accumulating on transmission of Influenza A viruses within and between species and its clinical manifestation in domestic species. For this reason, this document should be regarded as a working document. It is intended to formulate and standardise surveillance protocols (why, when and how to sample) in order to gain information and knowledge. It is important to state that there should be no trade implications derived from the detection of the pandemic H1N1 virus in pigs of any kind nor are disease control measures such as culling recommended in response to detection.

1.2 THE HUMAN HEALTH SITUATION

Pandemic H1N1/2009 influenza A virus has been circulating since mid-March 2009 and it has spread worldwide with many millions of cases. On 28 April 2009, WHO raised the pandemic alert from phase 4 to phase 5, indicating that the new virus had spread to several WHO regions around the world, and on 11 June 2009, from phase 5 to phase 6 indicating a global pandemic. Whilst there are mortalities associated with human cases, many are in people with other pre-existing conditions. Of additional concern is the expected increase in number of cases that require hospitalisation potentially overwhelming health services and the impact on business continuity, livelihoods and human welfare.

1.3 “CLASSICAL INFLUENZA VIRUSES” IN PIGS

Classical swine influenza viruses are influenza A viruses that infect pigs. The most common subtypes are H1N1, H1N2 and H3N2. Classical swine flu is common in North and South America, Europe and parts of Asia; it has also been reported in Africa.



Acute swine influenza (SI) is characterized by a short incubation period (1-3 days) after which animals appear anorexic, inactive and have the tendency to huddle and pile. Fever (ranging from 40.5 to 41.7°C) at this stage is present (this is why the animals tend to huddle). If animals are forced to move respiratory distress will become more evident. Open-mouthed abdominal breathing may be observed and movements are accompanied by paroxysms of coughing. Conjunctivitis, nasal discharge and sneezing may be observed. Morbidity rapidly reaches close to 100% of the pigs but mortality is low and usually does not exceed 1%. Generally animals recover 5 to 7 days after onset. Recovery of virus from nasal swabs has been successful in the past (Vannier et al, 1985) up to 29 days after initial infection. After 30-45 days and 60 days post-infection the disease failed to be transmitted to susceptible contact pigs. There is no clear evidence to support or reject the existence of long-term carriers.

However, this acute picture is not always seen with the pandemic H1N1 virus even in naïve pig herds. Clinical signs may be mild and apparent morbidity low. In Norway, a country which had been free of Influenza A viruses in pigs, of 22 affected herds, 14 showed no clinical signs. Additionally, there are many other respiratory infections of pigs that can present a similar clinical picture to SI. This makes developing a case definition difficult.

With all swine influenza viruses, in many larger pig herds infection becomes endemic and the clinical picture changes over time to be much less apparent or even sub-clinical. This is because the virus is maintained in the pig population via the constant presence of susceptible young (usually recently weaned) animals created by the continuous flow of pigs through the production cycle. Whether this will be true for the pandemic H1N1 virus is not yet clear.

Preliminary results of a study of the cross-reactivity of serum samples from US pigs against the Pandemic H1N1/2009 virus by the United States Department of Agriculture¹ (USDA), Agricultural Research Service (ARS), indicate that pre-existing immunity induced by swine influenza viruses circulating in the US may not protect pigs against the Pandemic H1N1/2009 virus presently circulating in people. However, field experience suggests that some cross-protection may occur, possibly via cell mediated immunity.

1.4 PIG TO HUMAN AND HUMAN TO PIG TRANSMISSION

Influenza viruses circulating in humans have the potential to transmit to pigs. There have also been instances of influenza A viruses transmitting to humans from pigs (and other species, particularly turkeys).

Because of this interlinking between humans and pigs, there have been instances when countries have banned imports of pork and pig products from countries where cases have been confirmed. Under WTO/OIE rules that govern trade in animals and animal products,

¹ Vannier P., Gourreau J.M. & Kaiser C. (1985) Infection expérimentale de porcs exempts d'organismes pathogènes spécifiques avec une souche du virus de la grippe porcine (H5N1) et étude de la durée d'excrétion virale. *Canadian Veterinary Journal* 26, 138-143
(Experimental infection of specific pathogen free pigs with a swine influenza virus (hsw1n1) and study of the duration of viral shedding.)



there is no justification for such a ban. The principles of safe trade are as described by the OIE http://www.oie.int/eng/press/en_090507.htm. Furthermore as stated in the joint communiqué between FAO, OIE and WHO, swine influenza viruses do not constitute a food safety problem http://www.oie.int/eng/press/en_090507_bis.htm.

If swine influenza viruses are identified in swine holdings, including the Pandemic H1N1/2009 virus, FAO recommends that the animals be given supportive care and allowed to recover, as the disease is self limiting unless there is movement away from the farm or if susceptible animals are brought in during the acute phase of the disease. **Culling of affected swine is not recommended.**

Animal handlers working with ill swine should protect themselves from potential zoonotic agents and should seek early medical attention if they become ill, feverish, or have respiratory or other symptoms. Pig workers with symptoms of flu should not work with pigs whilst they are likely to be shedding the virus. Medical sources will give guidance on how long this period is or refer to the WHO guidelines (<http://www.who.int/csr/disease/swineflu/guidance/en/index.html>).



2. Guidelines for surveillance of pandemic H1N1/2009 influenza

2.1 GENERAL PRINCIPLES

Animal disease surveillance is defined by the World Organisation for Animal Health (OIE) (2009) as “the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information to those who need to know so that action can be taken.”

Disease surveillance is a tool for action; it should be undertaken to address specific questions and with a pre-determined use for the information generated.

Currently, there are no guidelines or set of defined strategies to address surveillance for this virus. Swine influenza is not notifiable according to most country-level legislation nor internationally. However, all exceptional epidemiological events related to the occurrence of the Pandemic H1N1/2009 virus in swine or other species should be reported (including to the OIE).

In some countries, authorities and pig producers have begun routine surveillance to detect the presence of the Pandemic H1N1/2009 virus and to complete regular surveillance activities undertaken for other respiratory syndromes, such as porcine reproductive and respiratory syndrome (PRRS) to facilitate early detection or identify any change in the prevalence of diseases that do occur. However there are many countries in the world where surveillance systems do not have sufficient capacity in place to provide precise information on the extent and evolution of influenza virus circulation in pigs. Many countries do not consider swine influenza virus infections as a high priority in relation to other animal and public health problems.

The adaptation of disease surveillance schemes to different pig production systems, especially disease surveillance targeting backyard or small pig producers, and in particular in developing countries is encouraged. Surveillance at this level should include the active participation of local communities and farmers to report actively respiratory cases in pigs.

2.2 TESTS AVAILABLE FOR SWINE INFLUENZA VIRUSES

Current serologic tests do not differentiate between H1N1 strains; therefore serology to detect antibodies may be of low value for surveillance given that influenza viruses belonging to the H1N1 sub-type are common among pig populations and that vaccination against swine influenza viruses is carried out on a significant proportion of the industrial pig farms around the world in order to control clinical disease caused by classical swine influenza viruses. These vaccines contain inactivated H1N1 (and in Europe H3N2) virus strains.

For these reasons, positive serology for H1N1 should not be used as the sole indicator of previous infection with the Pandemic H1N1/2009 influenza virus. Serology may be useful in holdings where swine influenza vaccination is not practiced to detect post-infection sero-



conversion i.e. a significant increase of antibody titers against the same antigen in samples taken with a 3-4 weeks interval. However, only detection and sequencing of viral RNA can confirm the presence of the novel H1N1 virus in pigs.

For surveillance, virological assays are currently preferred over serology for the reasons described above. Molecular assays and virus isolation techniques are the most sensitive and specific options for detection of Pandemic H1N1/2009 influenza viruses in pigs; however, as of July 2009 there is no standardized veterinary laboratory protocol available. Ideally, new protocols should allow the differentiation between the classical swine influenza viruses (H1N1, H1N2 and H3N2) and the Pandemic H1N1/2009 virus. While research and development are ongoing, the joint OIE-FAO network of expertise on animal influenza (OFFLU) offers a list of international laboratories for submission of samples, a testing algorithm, and technical recommendations for sample collection and shipment. (www.offlu.net: "Detection of influenza in swine")

Molecular sequencing of the HA gene is currently the most definitive confirmation for the Pandemic H1N1/2009 influenza virus. As several different molecular assays have been modified and developed in recent months for detection of swine influenza, the H1 subtype, and differentiation between classical and Pandemic H1N1/2009 strains, consultation with an influenza reference laboratory (www.offlu.net: "Pandemic H1N1 List of Diagnostic Laboratories") is strongly recommended for the development of region-specific testing protocols; a brief outline of the molecular assays presently available is included in Annex 3. Additionally, national laboratory testing capacities should be taken into consideration when designing the surveillance plan (e.g. which tests can be performed by national/provincial laboratories, and sample throughput per week) to ensure that adequate resources are available to conduct the testing.

2.3 CRITERIA TO ESTABLISH SURVEILLANCE PROGRAMME FOR PANDEMIC H1N1/2009 AND CLASSICAL INFLUENZA VIRUSES IN SWINE

Surveillance for the Pandemic H1N1/2009 influenza virus is usually going to be for one of the following purposes:

- Detect the presence of Pandemic H1N1/2009 virus
- Provide supporting evidence for the absence of the Pandemic H1N1/2009 virus
- Build up a picture of what influenza viruses are circulating in the pig population and detect new introductions and variants

2.4 EPIDEMIOLOGICAL UNIT AND INDIVIDUAL UNITS OF INTEREST

The pig herd is the epidemiological unit. A pig herd is a group of pigs under common management at a given site. It may be one pig kept in a backyard or a unit with many thousands of pigs of all ages from piglets to breeding animals.

However, in larger herds, pigs are often confined in a series of pens or in a series of houses which alters transmission dynamics. Within a herd, the unit of interest for selecting pigs to be sampled is constituted by a group of pigs that are physically confined within the same space with direct and frequent contact between any other animal present in this physical space. The space could be an entire shed (if there is no further separation inside



the shed) or could be individual pens (if sheds are divided into different pens) or, in case of backyard pigs, could simply be the place where animals are kept.

2.5 CASE DEFINITION

The health event that should be linked with a field investigation is the reporting of respiratory syndrome in pigs. A possible case of swine influenza is defined as: *A cluster of clinical cases in pigs showing fever or sneezing or coughing or nasal or ocular discharge in at least one unit of interest, developed within a one-week period and affecting at least 10% of the animals present in the unit.*

It should be noted that there are a number of respiratory infections in pigs that can produce clinical signs that are consistent with the above case definition and that in larger pig herds, the finding of some animals showing respiratory signs is often the norm. Equally, as noted above, infection with pandemic H1N1 does not always cause clear clinical signs even in naïve pigs. This makes it impossible to create a case definition of high sensitivity or specificity. However, using the above case definition will ensure that the animals to be sampled will be those most likely to be affected by and shedding the virus.

Should the event occur in backyard pigs where the number of animals is less than 10 it would be sufficient to observe at least two respiratory cases within a one-week period or in a single pig where the herd size is one.

Farms may be selected for sampling in various ways. The owner may request assistance from his veterinarian to deal with a current outbreak and the veterinarian may then inform the veterinary authorities either immediately or at some later time. Alternatively, farms to be sampled may be sought via some form of active surveillance. In both cases, the clinical situation at the time of the sampling visits may vary and should be determined in accordance with the following guidelines.

This, combined with the purpose of sampling will determine the sampling strategy and number of samples to be taken as shown in the above table.

These are all detection surveys rather than attempts to estimate prevalence or within herd dynamics. The epidemiological unit is the herd, but within the herd, the units of interest vary as described above. For attempted virus detection or to build up a collection of cir-

Clinical situation at time of sampling visit

Current clinical signs	Farms where there are on-going respiratory cases that match the case definition adopted (on-going)
Recent clinical signs	Farms where respiratory cases (matching the case definition adopted) have occurred within the previous 30 days but at the date of the visit there are no cases occurring (occurrence of clinical cases in the previous 30 days)
No recent clinical signs	Farms where respiratory cases have occurred more than one month ago (occurrence of clinical cases more than 1 month ago)



	Detection of presence of H1N1	Support for absence of H1N1	Establishment of circulating viruses and detection of new introductions and variants in pigs
Current clinical signs	20 pigs with clinical signs or recently recovered	No sampling	20 pigs with clinical signs or recently recovered
Recent clinical signs	95/1 sample from recently affected animals	No sampling	No sampling
No recent clinical signs	No sampling	95/1 or 99/1*	No sampling

* Detection of 1% virus shedding at 95% and 99% confidence respectively

culating viruses, the samples should be taken from animals that are either showing clinical signs or have recently done so i.e. those are highest probability of shedding the virus.

Where information is sought to provide supporting evidence for absence of infection, samples should be taken either randomly from the whole herd or randomly from within the age group most likely to be shedding were the virus to be present i.e. recently weaned animals where this age group is present in the herd. In these cases, the sample size may be either 99/1 or 95/1 depending on the reason for requiring this evidence and the level of confidence required. This should be determined locally. In general, for international purposes, a 99/1 sample is preferred but the sample size may be large compared to the resources available.

Sampling for each of the purposes in Section 2.3 of this document is described in more detail below.

2.6 DETECT THE PRESENCE OF PANDEMIC H1N1/2009 VIRUS

This option may often be used when a confirmed human case is reported to have had contact with pigs. It is assumed that human cases are being investigated and that the information on whether or not the identified case had been exposed to pigs would be available. For the purpose of these guidelines for surveillance, having consumed pork products is not be considered as a risk factor for influenza but only contact with live animals. A window period of exposure for the person/s affected should be identified in order to optimize which holdings should be investigated (in early 2009 in Mexico for instance, timely exposure was defined as within the two weeks preceding the onset of human symptoms).

Epidemiological investigations of human cases of the Pandemic H1N1/2009 influenza virus by tracing back potential contact(s) with pig herds to investigate if pigs are infected. Veterinary and Public Health authorities should coordinate efforts at the national and local level to standardize epidemiological investigations at the animal-human interface.



Rather than considering only occupational exposure (which assumes daily contact with pigs) non-occupational exposure to pigs should also be considered such as visiting a farm. The definition of exposure and its possible gradient should be established in close cooperation with the Public Health Authorities.

This preliminary information may lead to the identification of one (or more) pig farm(s) where investigation is required. In case of occupational exposure at gathering points such as workers in a slaughterhouse or market, the potential number of pig farms requiring investigation may be high. Confirmation that pigs were the source of infection might be supported by virus sequence comparison between pig and human viruses

2.6.1 Target pig population for sampling

The target pig population to be investigated is those pig farms where a contact with human cases was established or where occupational exposure in slaughterhouses/markets occurs. In this case, the source farm or farms of the pigs slaughtered or sold should be established and these farm(s) included in the target population for sampling.

2.6.2 Sampling criteria

The clinical scenario on a farm should be determined as described in section 2.4 and 2.5 above. However, it is important to remember that the case definition for swine influenza is neither sensitive nor specific.

Should a farm be categorized as currently or recently infected, the herd will be eligible for the collection of nasal swabs (for virological analysis). Should the farm be categorized as not currently or recently infected, the herd will be not eligible for immediate sampling.

2.6.3 Sample type and size

If it is determined that the herd should be sampled, nasal swabs should be collected as follows:

- (a) in currently infected farms, samples should be collected from no more than 20 animals. Should the number of clinical cases be more than 20 all nasal swabs will be collected from clinically affected animals; if the number of cases is less than 20 samples will be collected from all the clinically affected animals plus other in contact animals to obtain the necessary 20 samples. The clinical status of the pigs should be indicated on the recording form A.1. Should the overall number of pigs present in the unit of interest be less than 20, samples will be collected from all of them. For each sample collected indication (see form A.3) should be provided whether the sample has been collected from an apparently healthy or clinically affected pig.
- (b) in recently infected farms, the number of nasal swabs to be collected follows the criteria of 1% of prevalence and 95% confidence (see table in Annex 1).
- (c) In farms that have not shown recent clinical signs (and consequently is not eligible for immediate sampling) a monitoring period should be initiated. The starting date (time 0) for the follow-up will be the date of the visit, the end of the follow-up period will be the date of the visit + 14 days. During this period the pig farm should ideally be visited at least twice (i.e. weekly) to verify that there are no signs of respiratory disease deve-



loping. The owner is obliged to report any unusual sign detected in the farm. Should cases of disease be detected consistent with the case definition used, the farm will be sampled according to the procedures already established in this document for currently clinically affected units.

Note: The sample size as a function of the group size can be estimated from the tables in Annex 1

2.7 EVIDENCE TO SUPPORT THE ABSENCE OF PANDEMIC H1N1/2009 VIRUS IN PIGS

Pig herds or compartments wanting to demonstrate freedom from the Pandemic H1N1/2009 influenza virus.

There are no official international requirements for, nor recognition of countries, zones, compartments or individual farms as free from classical swine influenza viruses or from the Pandemic H1N1/2009 virus. Demonstration of freedom from infection lies outwith the scope of this document. However, there may be circumstances where some degree of confidence is required that a herd is not infected with the virus. It should be noted that sample sizes are likely to be large and can only provide supporting evidence for absence unless carried out repeatedly over a significant period which is likely to be costly in terms of manpower and reagents.

2.7.1 Target population

This is the population of pigs for which evidence to support absence is required. This may be a single herd or a larger population such as a compartment.

2.7.2 Sampling criteria

Only units where there have been no recent clinical signs of swine influenza should be sampled.

The capacity of veterinary services for detecting and reporting swine influenza and the support from laboratories to make differential diagnoses with other respiratory syndromes is more critical in this situation and should also be considered.

2.7.3 Sample type and size

Sample size for different group sizes for this question is given in tables in Annex 1. For evidence to support absence of virus circulation, particularly where this is for trade purposes, it is recommended that a sample size be used that is sufficient to detect a level of 1% circulation with 99% confidence. For more complicated situations such as multiple strata or repeated surveys, specialist advice on sample design should be sought.



2.8 General surveillance strategy for influenza viruses in swine populations?

Targeted or active surveillance of pigs could be considered at slaughterhouses, abattoirs, and in some countries animal markets as well as at farms. Surveillance should be based on animals showing clinical respiratory signs.

2.8.1 Target population

Pigs on farms with respiratory disease, particularly when the signs are consistent with the case definition of swine influenza, at markets or in slaughterhouses and abattoirs. If events that match the case definition are reported to the Veterinary Services the location should be visited by an official or authorized veterinarian. It is important that the reporting of such disease events to the veterinary authorities is encouraged.

The veterinarian will proceed to inspect the unit of interest/s where animals are reported to be affected. Pigs on slaughterhouses and markets with respiratory diseases should be investigated and traced back to pig farm of origin and these should also be included in the target population for sampling as described below.

2.8.2 Sampling criteria

Each location should be screened in order to demonstrate the current presence of clinical signs.

2.8.3 Sample type and size

Samples should be collected from no more than 20 animals for each unit of interest. Should the number of clinical cases be more than 20 all nasal swabs will be collected from clinically affected animals; if the number of cases is less than 20 samples will be collected from all the clinically affected animals plus other in contact animals to obtain the necessary 20 samples.

Should the overall number of pigs present in the unit of interest be less than 20, samples will be collected from all of them.

For each sample collected indication (see form A.3) should be provided whether the sample has been collected from an apparently healthy or clinically affected pig.



3. Risk management of influenza viruses in swine populations and at the animal/human interface

In order to reduce the risk of transmission of Pandemic H1N1/2009 influenza within the animal population or between animals and humans FAO recommends the following:

- *Case definition* of swine influenza such as the one defined in point 2.5 should be identified before starting the surveillance programme.
- *Outbreak investigation* protocols and laboratory sampling procedures should be developed and disseminated to all veterinary professionals and animal health workers.
- *Regular surveillance* for porcine respiratory diseases should be intensified, cases of porcine respiratory syndrome should be investigated by the national veterinary authorities and differential diagnosis carried out by the national laboratory. If Pandemic H1N1/2009 influenza virus is suspected, samples should be confirmed using molecular sequencing techniques with support from international reference laboratories if needed (www.offlu.net: "Pandemic H1N1 List of Diagnostic Laboratories"). OIE and FAO as international organisations dealing with animal health issues should be informed if the Pandemic H1N1/2009 influenza virus is confirmed in pigs.
- *Movement restrictions* should be implemented by farms or holdings with swine after a confirmatory diagnosis of Pandemic H1N1/2009 influenza virus. Restriction measures should be in force until at least one week to ten days after the last animal has recovered. The overall duration of the disease and virus excretion is difficult to estimate, because it depends on the number of susceptible animals present in any given farm, the layout of the farm and work practices. In industrial pig farming systems, restriction measures may rapidly cause overcrowding. In such circumstances sending clinically healthy animals for regular slaughter, under veterinary inspection, may represent an alternative and avoid animal welfare issues (i.e., overcrowding). Animals suffering from swine influenza can be separated from healthy herd-mates and allowed to recover; there is no need to cull affected animals. In case of suspected outbreak movement restriction should be in place until a laboratory diagnosis is available.
- *Biosecurity and personal protection*. Animal handlers and veterinarians should wear protective gear and ensure that proper cleaning and disinfection is conducted on equipment and material between units to minimize the risk of spreading pathogens between pigs at different locations and being infected by zoonotic agents, including influenza. Workers in one house should not be allowed to visit or work in other houses nor have pig sites of their own.



- Persons who work directly with swine should be urged not to go to work if they have any signs of respiratory disease, fever or any influenza-like illness.
- Biosecurity should be increased in pig herds to prevent transmission on fomites and mechanical vectors such as vehicles.
- *Vaccination for swine influenza.* In high risk areas, a swine influenza vaccine could be used in swine as long as it is considered effective against the circulating strain and is permitted by the relevant authorities.

FAO encourages country authorities to remain vigilant, gathering epidemiologic data, reporting unusual influenza-like illnesses in swine, assisting in facilitating the forwarding of appropriate specimens to international influenza reference laboratories (OFFLU Network) and responding to urgent animal health and zoonotic disease problems.

Through the FAO/OIE/WHO Global Early Warning System (GLEWS), timely data and information on disease outbreaks is shared between the three international organizations, which communicate and coordinate with other partners, including reference laboratories, Centers for Disease Control and Prevention (CDC) and the European Center for Disease Prevention and Control (ECDC), key national and academic institutions, and lending or development grant organisations.

Information sharing between countries at international level and communication of the results of surveillance activities for classical swine influenza, the emergent Pandemic H1N1/2009 influenza virus, and other novel viruses would improve the overall understanding of influenza dynamics and the different types of pig production systems where they circulate.

What is needed is to improve early detection and reporting and the knowledge of the epidemiological situation of animal influenza viruses in order to monitor any potential shifting to viruses of zoonotic potential or those of increased virulence.

FAO member countries can request assistance for sample or specimen dispatch to through the EMPRES shipping facilities (EMPRES-Shipping-Service@fao.org) for confirmation or agent characterisation.



Annex 1

Type of samples and sample size

(For more detailed information on collection of samples, please see http://www.offlu.net/OFFLU%20Site/OFFLU_SwInf.pdf)

Nasal swabs (for virological examination) are the sample of choice. Sampling should give priority to animals that show signs of disease (fever, sneezing and nasal/ocular discharges). Animals to be sampled will need to be restrained and deep intranasal swabs collected while avoiding contamination of the swab with organic material on the snout. It is preferable to use sterile commercial swabs that come with their own tube and viral transport medium. When using separate swabs and tubes, use sterile swabs and avoid wooden shafts. Synthetic tips are preferred (rayon or dacron). After sampling, each swab should be placed into a sterile glass or plastic container with a screw cap; the shaft of the swab might need to be cut fit into the tube. The containers should contain viral transport medium or equivalent (phosphate buffered saline [PBS], supplemented with antibiotics and bovine serum albumin [5 mg/ml]); foetal bovine serum should not be included. Samples should not be pooled.

All samples must be labelled with a unique identification code; the same code will be used when recording animal information on the sampling protocol. Samples are to be sealed and maintained in a refrigerated environment (icepacks or other).

Collection of blood samples (for antibody determination) is likely to be of low value as H1N1 influenza is a common disease in pigs and they are also often vaccinated against this infection.

Sample size for different group sizes, probabilities of detection and expected levels of shedding

Detection of 5% shedding, 95% confidence										
Group size	≥2000	1000	500	400	300	200	100	50	25	
Sample size	59	56	53	52	50	46	38	28	18	
Detection of 1% shedding, 95% confidence										
Group size	≥10,000	5000	3000	2000	1000	500	400	300	200	100
Sample size	299	283	272	261	231	188	172	150	120	75
Detection of 1% shedding, 99% confidence										
Group size	≥10,000	5000	3000	2000	1000	500	400	300	200	100
Sample size	459	429	399	374	315	240	214	182	140	83



Annex 2

Production system form

FORM A.1

Contact details of the staff person visiting the pig farm

Name _____

Address _____

Telephone (if available) _____

E-mail address (if available) _____

Preliminary data

(1) Province _____

(2) District _____

(3) Town/village _____

(4) Owner of farm _____

(5) Latitude/Longitude of the farm (if available) Lat _____ Long _____

(6) Type of pig production system:

large/industrialized medium/commercial small scale/backyard

free ranging closed housing

(7) Total number of pigs present in the farm:

_____ piglets _____ weaners _____ growers/finishers _____ sows _____ boars

(8) The farm is investigated because of:

notification of respiratory cases in relation to human case/s

(9) If the investigation is undertaken in relation with human cases please indicate if the pig farm is categorized as:

(clinical disease ongoing) (clinical disease in past 30 days)

(history of clinical disease >30 days)

(10) Indicate if a vaccination program is carried out in the farm:

no yes, every 6 months yes, every year yes, irregularly

(13) Please indicate when the last vaccination against swine influenza (if any) was performed:

_____/_____/_____

(14) Commercial name of the vaccine _____ Manufacturer _____

Virus strains included _____

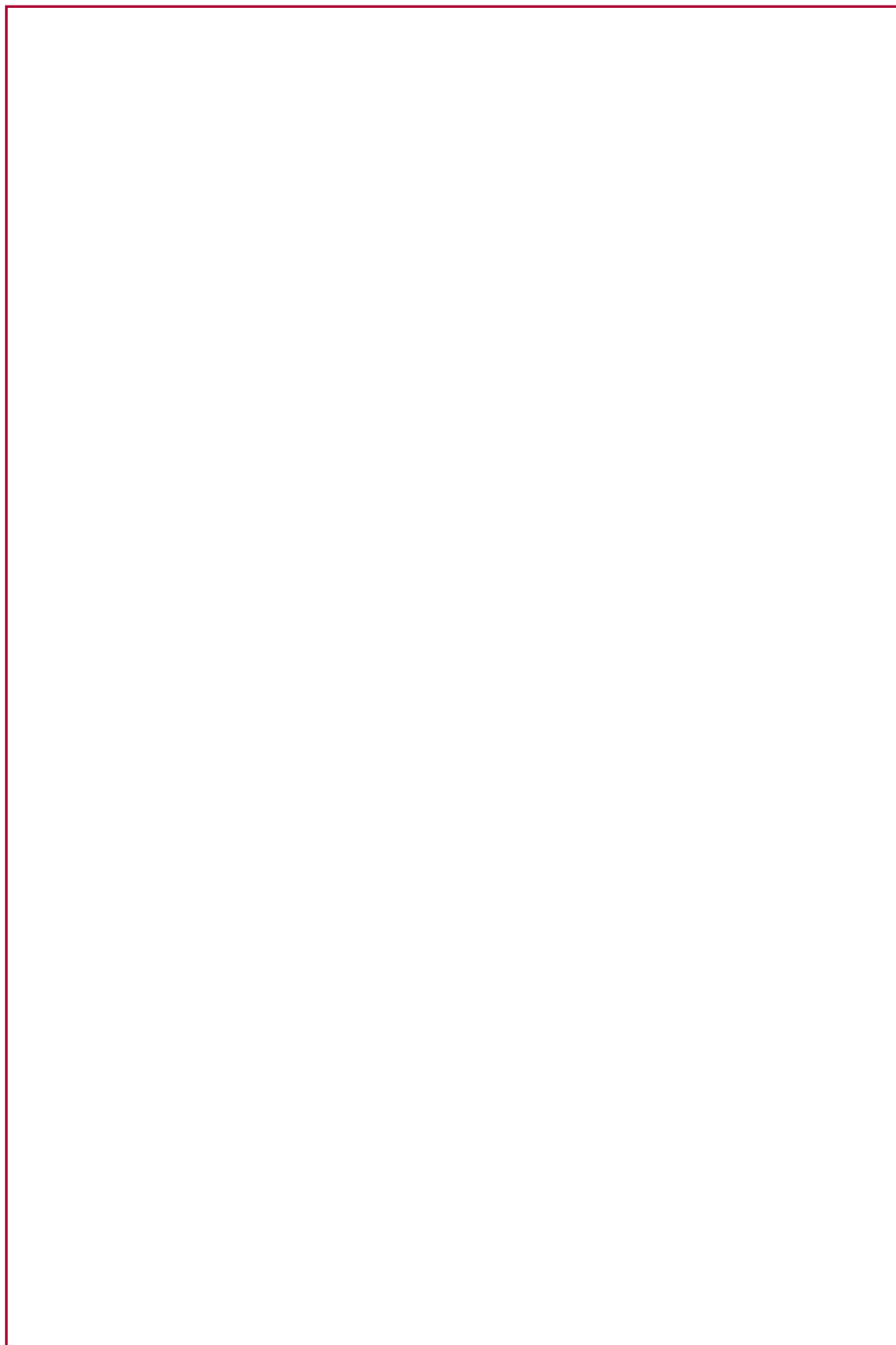
(15) Date of the visit ____/____/_____

Signature



FORM A.2

Farm layout of the farming system and description of the production system



Annex 3

Swine detection

Summary of molecular assays, range of detection - specifically with regards to the Pandemic H1N1 2009 virus, and target gene for identification of Type A influenzas

Assay	Target gene	Type A Swine origin	Type A Human origin	Type A Avian origin	Pandemic 2009 H1N1	Comment	Validation reference
CDC InfA	M	✓ / -	✓	✓	✓	Testing at AFSSA failed to detect very recent Eurasian lineage, other laboratories report detection of avian- and swine-like H1s	CDC, CFIA, VLA, AFSSA
2002 Spackman SEPRL	M	✓	✓	✓	✓ / -	Decreased sensitivity in detecting pH1N1	Spackman et al 2002
2009-modified Spackman SEPRL	M	✓	✓	✓	✓	50:50 mix 100% pH1N1 match plus original reverse primer, may have decreased sensitivity for avian-like influenza	SEPRL, NVSL, VLA
2009-modified Spackman CFIA	M	✓	✓	✓ / -	✓	Some decrease in sensitivity for avian-like influenza as compared to others. Degeneracies in reverse primer require HPLC purification	CFIA, AFSSA, NVSL
2002-modified Spackman AAHL	M	✓	✓	✓	✓ / -	Degeneracies in forward and reverse primer - recommend HPLC purification for primers	AAHL - standard matrix test in use
2009-modified AAHL	M	✓	✓	✓ / -	✓	100% pH1N1 match in reverse primer, may have decreased sensitivity for avian-like influenza	AAHL
2009-modified Spackman IZSVe	M	✓	✓	✓ / -	✓	100% pH1N1 match in reverse primer, may have decreased sensitivity for avian-like influenza	IZSVe
2009-modified Spackman CVI	M	✓	no data	no data	✓	100% pH1N1 match in reverse primer, may have decreased sensitivity for avian-like influenza	CVI
FLI- IVA-NP2RT-qPCR	NP	✓	✓	✓	✓	Detects pH1N1 without modification - in use for >2 years; sensitivity similar to Spackman M PCR; used in at least three VLA/Epizone ring trials with correct results; assay is combined with an internal control based on a heterologous in-vitro RNA transcript	FLI, AFSSA
Combo M gene VLA	M	✓	✓	✓	✓		AFSSA
LSI M-gene	M	✓	✓	✓	✓	Internal control included (B-actin)	AFSSA
Adiavet SIV Realtime	M	✓	✓	✓	✓	Internal control included (GADPH)	AFSSA
Applied Biosystems NP	N/P	✓ / -	✓ / -	?	✓ / -	not recommended by AAHL	AAHL
Applied Biosystems M	M	✓	✓	?	✓		AAHL



Summary of molecular assays, range of detection - specifically with regards to the Pandemic H1N1 2009 virus and target gene for identification of H1/N1/H3 in pigs

Assay	Target gene	Pandemic H1N1 2009	H1 Eurasian swine	H1 N.A. swine	H1 human	Comment	Validation reference
CDC SwInfA	NP	✓	✓	✓	-	Decreased sensitivity as compared to modified Spackman M-gene	CDC, CFIA, VLA
CDC SwH1	H1	✓	-	✓ / -	-	Assay to differentiate swine-origin H1 from other swine influenzas - however, testing at AFSSA detected classical swine H1N1	CDC, CFIA, VLA, AFSSA
2009 SEPRL N1 Novel	N1	✓	-	-	-	Assay differentiates the Pandemic N1 2009 from the North American and Eurasian classical N1s	SEPRL, NVSL, AAHL, AFSSA
2009 SEPRL-modified N1 [210-330] AAHL	N1	✓	-	pending	-	Mismatches in SEPRL N1 addressed in modification to improve specificity	AAHL
2009 NADC pH1N1	M	✓	?	-	-		NADC
VLA H1-118	H1	✓	-	-	-		VLA
HKU SWF - CONVENTIONAL RT-PCR	H1	✓	✓ / -	?	-	One of the positive controls recommended in this assay is a swine H1 virus isolated in Hong Kong. This is designed to minimize the shipping and handling of A/California/04/2009-like H1N1 viruses to laboratories which do not have the recommended biosafety facility. If the test is run without controls, products should be confirmed by sequencing and comparison with sequences in deposited databases. The absence of the correct PCR products (i.e. a negative result) does not rule out the presence of influenza virus. Results should be interpreted together with the available clinical and epidemiological information.	IZSvE
HKU-WHO real-time	H1	✓	-	✓ / -	-		AFSSA
Pasteur Institute, Paris	H1	✓	-	✓ / -	-		AFSSA
FLI- RT-qPCR-IVA-H1N1-1	H1	✓	- / ✓	-	-	Testing at AFSSA detected very recent Eurasian lineage	FLI publication pending, AFSSA
FLI- RT-qPCR-IVA-H1N1-2	H1	✓	-	-	-		FLI publication pending, AFSSA
LSI TaqVet A/ H1N1(2009) H1 detection	H1	✓	-	-	-		AFSSA
LSI TaqVet A/ H1N1(2009) N1 detection	N1	✓	-	-	-		AFSSA
Adiavet A/ H1N1(2009) H1 detection	H1	✓	-	-	-		AFSSA
CVI nH1N1-2009	H1	✓	no data	no data	no data	reported to distinguish pH1N1 2009 from European H1	CVI
CVI-SIV H3	H3	-	no data	no data	no data	reported to distinguish European H3 vs H1	CVI



Annex 4

Swine reagents

Summary of PCR platforms and mastermix kits validated for Type A influenza assays from Table 1

Assay	Target gene	Master-mix (refer to table below for extraction kit recommendations)	PCR Platform*	Comment
CDC InfA	M	SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen)	ABI 7500, Stratagene MX3005P, BioRad iQ series	TaqMan@[AB] and Invitrogen have Influenza A (H1N1) Assay Sets design based on CDC sequences shortened for use with MGB probe
2002 Spackman SEPRL	M	Ag-Path (Ambion), SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen), Qiagen OneStep	Smart Cyclor 2 , ABI 7500, Roche LightCycler	
2009-modified Spackman SEPRL	M	Qiagen OneStep (for SmartCycler), Ag-Path (for ABI)	Smart Cyclor 2 , ABI 7500	
2009-modified Spackman CFIA	M	Qiagen OneStep (for SmartCycler), Ag-Path (for ABI)	Smart Cyclor 2 , ABI 7500	
2002-modified Spackman AAHL	M	Ag-Path (Ambion)	ABI 7500	
2009-modified AAHL	M	Ag-Path (Ambion)	ABI 7500	
2009-modified Spackman IZSVe	M	QuantiTect Multiplex RT-PCR kit	RotorGene 6000 Corbett	
2009-modified Spackman CVI	M			
FLI- IVA-NP2RT-qPCR	NP	Ag-Path (Ambion), SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen), QuantiTect Probe RT-PCR Kit (Qiagen)	Stratagene 3005P, LightCycler Roche	
LSI M-gene	M	Custom, ready made with kit	ABI 7500, Stratagene MX3005P, BioRad Chromo4	Full kit - mastermix and internal control included (B-actin)
Adiavet SIV Realtime	M	Custom, ready made with kit	ABI 7500 FAST, BioRad Chromo4, Qiagen Rotorgene, Roche LightCycler 2 and 480 - contact Adiagene for calibration	Internal control included (GADPH)
Applied Biosystems NP	NP	Ag-Path (Ambion), SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen)		
Applied Biosystems M	M	Ag-Path (Ambion), SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen)		

* NOTE: because protocols are platform dependent, must optimize assay to new platform if not validated below



Summary of PCR platforms and mastermix kits validated for H1/N1/H3 influenza assays from Table 2

Assay	Target gene	Master-mix (refer to table below for extraction kit recommendations)	PCR Platform*	Comment
CDC SwinfA	NP	SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen)	ABI 7500, Stratagene MX3005P, BioRad iQ series	TaqMan@[AB] and Invitrogen have Influenza A (H1N1) Assay Sets design based on CDC sequences shortened for use with MGB probe
CDC SwH1	H1	SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen)	ABI 7500, Stratagene MX3005P, BioRad iQ series	TaqMan@[AB] and Invitrogen have Influenza A (H1N1) Assay Sets design based on CDC sequences shortened for use with MGB probe
2009 SEPRL N1 Classic/Novel	N1	Qiagen OneStep (for SmartCycler), Ag-Path (for ABI)	Smart Cycler 2 , ABI 7500 FAST	
2009 SEPRL-modified N1 [210-330] AAHL	N1	Ag-Path (Ambion)	ABI 7500 FAST	
2009 NADC pH1N1	M	Ag-Path (Ambion)	ABI 7500 FAST	
VLA H1-118	H1	QuantiTect Probe RT-PCR Kit (Qiagen)	ABI 7500 FAST	
HKU SWF - CONVENTIONAL RT-PCR	H1	QIAGEN OneStep RT-PCR kit	ABI 9700 conventional PCR	
FLI- RT-qPCR-IVA-H1N1-1	H1	Ag-Path (Ambion), SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen), QuantiTect Probe RT-PCR Kit (Qiagen)	Stratagene 3005P, LightCycler Roche	
FLI- RT-qPCR-IVA-H1N1-2	H1	Ag-Path (Ambion), SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen), QuantiTect Probe RT-PCR Kit (Qiagen)	Stratagene 3005P, LightCycler Roche	
LSI TaqVet A/ H1N1(2009) H1 detection	H1	Custom, ready made with kit	ABI 7500, Stratagene MX3005P, BioRad Chromo4	Internal control included (B-actin)
LSI TaqVet A/ H1N1(2009) N1 detection	N1	Custom, ready made with kit	ABI 7500, Stratagene MX3005P, BioRad Chromo4	Internal control included (B-actin)
Adiavet A/ H1N1(2009) H1 detection	H1	Custom, ready made with kit	ABI 7500 FAST, BioRad Chromo4, Qiagen Rotorgene, Roche LightCycler 2 and 480 - contact Adiagene for calibration	Internal control included (GADPH)
CVI nH1N1-2009	H1			
CVI-SIV H3	H3			

* NOTE: because protocols are platform dependent, must optimize assay to new platform if not validated below



Annex 5

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