



Integrated Plan for the Surveillance of Swine Diseases

2nd Edition

Ministry of Agriculture and Livestock
Secretary of Animal Health
Department of Animal Health

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LIST OF ABBREVIATIONS

CC: Complete cycle

DSA: Department of Animal Health

ELISA: Enzyme-linked immunosorbent assay

e-Sisbravet: Specific electronic tool for the management of passive surveillance data in animal health, developed for the registration and follow-up of immediate notifications of suspected diseases and investigations carried out by the Official Veterinary Service.

GRSC: Certified Swine Breeding Farm

MAPA: Ministry of Agriculture, Livestock and Supply

OESA: State Agency for Agricultural Health **OMSA:**

World Organization for Animal Health **PCR:**

Polymerase chain reaction

PSA: African Swine Fever

PSC: Classical Swine Fever

PRRS: Swine Reproductive and Respiratory Syndrome

PNSS: National Swine Health Program

SEI: Electronic Information System **SDA:**

Secretary of Animal Health **SVO:** Official

Veterinary Service

UPL: Piglet Producing Unit

VN: Viral Neutralization

PSC ZL: Classical swine fever virus free zone

PSC ZnL: Classical swine fever virus not free zone



1. INTRODUCTION

Brazil is the world's fourth largest producer of pork, with a herd of more than 40 million animals, supplying the domestic market with about 80% of this production and exporting the remainder, being the world's fourth largest exporter of such protein. The Brazilian swine industry has a very favorable sanitary condition, as it is considered to be free from economically very important diseases that occur in many parts of the world, notably **African Swine Fever (PSA)** and **Swine Reproductive and Respiratory Syndrome (PRRS)** and because it has a large free zone of **Classical Swine Fever (PSC)**. The maintenance of this sanitary condition in Brazil guarantees lower production costs and a competitive advantage in the access to international markets.

However, the increasing international movement of people, international trade in animals and products, intensification of livestock production, and other factors contribute to an increased risk of introduction and spread of diseases, the social, economic, and environmental impact of which can be extremely high. In addition, in the face of growing health risks, trading partners require increasingly robust evidence for the certification of traded animals and products. In addition, the World Organization for Animal Health's (OIE) requirements for disease-free zone certification are based on technical and scientific principles that are constantly evolving.

In this sense, surveillance is the main activity in animal health that allows the early detection of emerging and re-emerging animal diseases, allowing the control and efficient eradication, as well as the certification of disease-free zones, maintaining the access of production systems to national and international trade.

This **Integrated surveillance plan** revises the first version, published in 2021 and executed 2021 and 2022, by the Department of Animal Health (DSA), linked to the Animal Health Secretariat (SDA) of the Ministry of Agriculture, Livestock and Supply (MAPA), for the surveillance of CSP, ASF and PRRS. The Plan was initially outlined for the three diseases, but can be applied and adapted for others, considering changes in the epidemiological situation, trade-related demands, public and private sector interests, and resource availability. The satisfactory results and the lessons learned from the first cycle of the surveillance plan allow us to promote adjustments and improvements for the second cycle.

The Integrated Swine Disease Surveillance Plan was developed by the Official Veterinary Service (SVO) in collaboration with the private sector, representing the commitment to maintain and improve the animal surveillance implemented in Brazil.

2. CURRENT DISEASE CONTEXT IN BRAZIL (PSC, PSA and PRRS)

2.1 Classical Swine Fever (PSC)

The process of international recognition of CSF virus-free zones (FZ) prioritized the most relevant regions for production and export of swine and its products. Currently, about 83% of the Brazilian swine herd is in an FZ of the CSP virus, covering, approximately, 50% of the national territory. The animal health status of the disease in Brazil, recognized by OMSA, is made up as follows:

- **three Free zones:** one comprising the states of Rio Grande do Sul and Santa Catarina; another comprising the states of Acre, Bahia, Distrito Federal, Espírito Santo, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, Rondônia, São Paulo, Sergipe, Tocantins and the municipalities of Guajará, Boca do Acre, south of the municipality of Canutama and southwest of the municipality of Lábrea, belonging to the state of Amazonas; and another formed by the state of Paraná.
- **a Not Free zone (ZnL):** formed by the states of Alagoas, Amapá, Amazonas (except region belonging to the FZ), Ceará, Maranhão, Pará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, and Roraima.

The PNSS is working on two fronts: to maintain the animal health status in the LFA and to develop the process of eradication of the CSP virus in the LFA, seeking recognition of the whole country as free. The goals are associated, since eradicating the CSP virus in the ZnL contributes to ensuring the health status in the LZ.

2.2 African Swine Fever (PSA)

PSA was introduced in Brazil in 1978, in the municipality of Paracambi in Rio de Janeiro state. The investigations carried out at the time revealed that the swine in the establishment characterized as the index focus became infected by eating leftover food served on board airplanes from Portugal and Spain, countries where the disease was rampant.

The last occurrence of PSA in Brazil was recorded in November 1981 in the municipality of Moreno, State of Pernambuco. The measures applied by the Brazilian SVO allowed the eradication of the disease throughout its territory and the declaration of a PSA-free country in 1984, but at a high cost to the country.

As of 2018, PSA has entered and dispersed widely in the Asian and European continents, reaching the American continent in 2021. MAPA, the State Agricultural Health Organizations (OESA), and the private swine sector have developed and reinforced actions to prevent the entrance of PSA in Brazil and to mitigate the economic and social impacts in case of disease introduction.

Surveillance for PSA is one of the ways to achieve the objectives of the "Action Plan for the Prevention of PSA - Version 2.0 February 2020" (SEI 21000022137/2020-47), which aims to list, organize and guide priority actions, define responsibilities, identify the main obstacles and the resources needed to strengthen the prevention and surveillance of the disease, in order to maintain Brazil's sanitary status as a PSA-free country.

2.3 Swine Reproductive and Respiratory Syndrome (PRRS)

PRRS was first described in the United States in 1987, in Europe in 1990, and soon afterwards in Asia. PRRS causes high mortality in newborn and weaned pigs, low conception rates in breeding herds, increased abortion rates, stillbirths and the birth of weak piglets, resulting in enormous economic losses to producers.

From the experience of countries with highly specialized pig production where the disease has entered, very worrying features of PRRS have been identified, such as the high rate of spread, the lack of effective vaccines, and the inability of strict biosecurity measures to prevent contamination of free-range farms. The PRRS virus has been identified in major swine-producing countries and is endemic in a number of them.

PRRS has never been registered in Brazil, and published scientific papers analyzing several levels of the swine production chain in Brazil have consistently demonstrated the absence of antibodies or RNA for the PRRS virus. In this sense, Brazil adopts strict controls on the import of pigs for breeding and genetic material, in order to mitigate the risk of introducing this disease into the national territory.

Given the importance of the economic and social impact of the introduction of PRRS in Brazil, it is necessary to strengthen the system of prevention, surveillance and response to the possible detection of the disease.

3. DISEASE DESCRIPTION AND CASE DEFINITIONS

3.1. Classical Swine Fever

3.1.1. PSC Suspected Case

1. Pigs (domestic or wild) with clinical signs or lesions consistent with PSC, whether or not associated with increased mortality.

3.1.2. PSC Probable Case

1. a pig with clinical signs or injuries consistent with PSC, as determined by the SVO; or
2. positive result in RT-PCR test on active surveillance sample for PSC.
3. The finding of a probable case of PSC requires the immediate adoption of biosecurity measures and arrangements for laboratory diagnosis to rule out or confirm the disease.

3.1.3. PSC Confirmed Case

1. isolation and identification of the PSC virus in samples from one or more pigs with or without clinical signs of disease; or
2. identification of viral antigen, excluding vaccine strains, or nucleic acid specific for PSC virus in samples from one or more pigs with clinical signs or injuries consistent with PSC; or epidemiologically linked to a confirmed case of PSC; or with evidence of exposure to PSC virus; or
3. detection of antibodies specific for PSC virus, other than those due to vaccination or infection with another Pestivirus, in samples from one or more pigs with clinical signs or injuries consistent with PSC; or epidemiologically linked to a confirmed case of PSC; or with evidence of exposure to the PSC virus.

NOTE 1: the first case/outbreak in a PSC-free zone must be confirmed with isolation and identification of the virus.

NOTE 2: In an already confirmed PSC outbreak, all pigs with clinical signs consistent with PSC will be considered confirmed cases.

3.2. African Swine Fever

3.2.1. PSA Suspected Case

Pigs (domestic or wild) with clinical signs or injuries consistent with PSA, whether or not associated with increased mortality.

3.2.2 PSA Probable Case

1. a pig with clinical signs or injuries consistent with PSA, as determined by the SVO; or
2. positive result in PCR test on active surveillance sample for PSA.

The finding of a probable case of PSA requires the immediate adoption of biosecurity measures and arrangements for laboratory diagnosis to rule out or confirm the disease.

3.2.3. PSA Confirmed Case

1. isolation and identification of the PSA virus in samples from one or more pigs with or without clinical signs of disease; or
2. identification of viral antigen or nucleic acid specific for PSA virus in samples from one or more pigs with clinical signs or injuries consistent with PSA; or epidemiologically linked to a confirmed case of PSA; or with evidence of exposure to PSA virus.

NOTE 1: the first case/focus of PSA in Brazil must be confirmed with isolation and identification of the virus followed by genetic sequencing.

NOTE 2: In an already confirmed PSA outbreak, all pigs with clinical signs consistent with PSA will be considered confirmed cases.

3.3. Swine Reproductive and Respiratory Syndrome- PRRS

3.3.1. PRRS Suspected Case

1. Pigs (domestic or wild) with clinical signs or injuries consistent with PRRS, whether or not associated with increased mortality.

3.3.2. PRRS Probable Case

1. a pig with clinical signs or injuries consistent with PRRS, as determined by the SVO; or
2. positive result in PCR test on active surveillance sample for PRRS.

The finding of a probable case of PRRS requires the immediate adoption of biosecurity measures and arrangements for laboratory diagnosis to rule out or confirm the disease.

3.3.3. PRRS Confirmed Case

1. isolation and identification of the PRRS virus in samples from one or more pigs with or without clinical signs of disease; OR
2. identification of viral antigen or nucleic acid specific for PRRS virus in samples from one or more pigs with clinical signs or injuries consistent with PRRS; or epidemiologically linked to a confirmed case of PRRS.

NOTE 1: the first case/focus of PRRS in Brazil must be confirmed with isolation and followed by genetic sequencing.

NOTE 2: In an already confirmed PRRS outbreak, all pigs with clinical signs consistent with PRRS will be considered confirmed cases.

For more information and details regarding the diseases and their case definitions, see: PSA Datasheet and PRRS Datasheet (https://sistemasweb.agricultura.gov.br/pages/fichas_tecnicas/ficha_tecnica.html).

4. PURPOSES AND REASONS

The situation of PSC in the country's ZnL, the occurrence of PRRS in the main pig-producing countries, the increasing spread of PSA in Asia, Europe and other parts of the world, and its reintroduction in the Americas, increase the concern about the possible introduction and spread of these diseases in Brazil or in the Free Zone, in the case of PSC.

The rapid detection of a possible introduction of these diseases is essential for the success of emergency response actions, the control and eradication of the outbreak, with the goal of rapid recovery of the sanitary condition. This early detection may be hampered by the similarity of the clinical picture to other diseases present in production systems. It is necessary to update the knowledge of producers, handlers, and private sector technicians about diseases, as well as strengthen interaction with the SVO to ensure fast and accurate disease detection.

The active surveillance components of PSC, PSA and PRRS, in the current PSC-free zone, are of great importance to demonstrate the absence of these diseases, with the aim of obtaining certification for the trade of swine and their products from Brazil to the most diverse markets. Conducted on a continuous basis and with the appropriate level of sensitivity, it also enables the identification of the emergence or change of risk factors and the adoption of management measures that promote mitigation, as well as the efficient allocation of resources to strategic areas and sectors.

The standardized and auditable data from the surveillance system should be able to support risk analysis processes and evaluations of the integrated surveillance plan itself, in order to support the definition of health policies and short, medium and long-term strategies of the PNSS.

Therefore, after reviewing the procedures in place, the DSA proposes an Integrated Surveillance Plan for PSC, PSA and PRRS aimed at strengthening the surveillance and response to emergencies for these diseases, as well as optimizing the use of the resources committed, with the main objective of protecting the pig industry and the national economy from the occurrence of the aforementioned diseases and their economic and social impact, in addition to ensuring certification for market access.

5. GOALS

This document describes an Integrated Surveillance Plan that builds on currently available diagnostic methodologies and aims to achieve the following objectives:

Goal 1: Strengthen capacity for early case detection of PSC, PSA, and PRRS. Early detection of suspected cases of PSC, PSA and PRRS, followed by prompt and accurate handling of reports, is the basis for passive surveillance and emergency preparedness and response.

In addition, early and consistent reporting and investigation of suspected cases provides a solid database of disease absence that contributes to the assessment of the situation at the beginning of an outbreak and to the demonstration of the absence of the investigated diseases.

This integrated surveillance plan expands the scope of surveillance of suspected cases, seeking to detect not only swine hemorrhagic syndrome, targeting PSC and PSA, but also pictures compatible with PRRS.

Another important aspect is that these investigations, together with the other surveillance components, maintain the actions and capacity of the animal health information systems, sample collection and dispatch, laboratories and emergency management, essential conditions for an adequate response in case of a confirmed case, when the demand is suddenly and massively increased.

Goal 2: Demonstrate the absence of infection with PSC, PSA, and PRRS in domestic and wild swine populations.

The data generated by the surveillance system for PSC, PSA and PRRS in domestic and wild swine must be able to certify the status of free zones providing ongoing support for health status confirmations with the OMSA and trading partners. This support can be obtained from the data resulting from the implementation of the activities foreseen in this Plan, without the need for additional studies and sampling.

Continuous active surveillance (clinical and laboratory) for target diseases in higher risk sectors of the production chain also helps to increase the chances of early detection of cases or serologic reactions consistent with the occurrence of viral transmission.

At first, considering the absence of cases in South America and the characteristics of PSA, the serological surveillance component for demonstrating disease-free status will not be carried out. If there is a relevant change in the epidemiologic situation of PSA in the region, serologic surveillance samples for PSC and PRRS shall also be tested for PSA.

6. EXPECTED RESULTS

The development of the actions foreseen in this Plan is related to meeting the objectives described in item 5.

All data collected must be recorded in the specific systems for passive (e-SISBRAVET) and active surveillance defined by MAPA.

The results will be presented as:

- Records and databases of the respective information systems used for surveillance management;
- Annual surveillance system report; and
- Performance analyses of system components and evaluation of surveillance indicators.

The resulting information will be used by system managers for decision making and specific actions such as:

- Immediate care and investigation within 12 hours of suspected Hemorrhagic Syndromes reported to the official animal health service, to rule out or confirm the target diseases;
- Performance evaluation in the conduct of passive surveillance to guide procedures during investigations;
- Activate the rapid response system in the event of a confirmed outbreak;
- Adequacy of detection and response capabilities based on the results of surveillance indicators or when there is an indication of a potential increase in risk to the susceptible population;
- To subsidize certification guarantees and trade negotiations that require proof of disease-free status; and
- Revising strategies and procedures when target diseases are detected.

The Integrated Surveillance Plan should be evaluated in its parameters and structure every 3 years. Updates may be made following changes in the risk assessments or when the DSA deems necessary.

Significant changes in risk factors that increase the likelihood of disease introduction into the country's PSC-free zone should lead to a review of this Plan, even if outside the established frequency.

7. STAKEHOLDERS

All those involved in the breeding, handling, transport, production, surveillance, inspection, diagnosis, training, research and care of domestic pigs and the control of wild swine, among other activities, are considered stakeholders in the Integrated Swine Disease Surveillance Plan and are responsible for carrying out parts of the surveillance tasks.

In Brazil, the SVO is comprised of government authorities, with MAPA representing the central and superior instance at the federal level, and the OESAs representing the intermediate and local instances at the state level. The SVO is one of the main players in the surveillance system for PSC, PSA and PRRS, as it is responsible for their coordination, implementation and evaluation, in addition to the inspection of products of animal origin, analyses in the Federal Laboratories of Animal Health and international agricultural surveillance.

Producers and members of the agribusinesses also participate in an important way, as well as service providers and input suppliers, professionals and institutions involved with wild pigs. Table 1 shows the responsibilities of the segments involved or interested in the Plan.

Table 1. Accountabilities of segments either INVOLVED or interested in the Integrated SURVEILLANCE Plan for PSC, PSA and PRRS.

STAKEHOLDERS	Description	Accountabilities	Type of participation
Official Veterinary Service	Veterinarians and support staff directly in contact with SVO through institutional affiliation	Standardization, database management, maintenance and analysis, information dissemination, investigation of suspected cases, inspection of pigs, sample collection, training, education, communication and funding	Clinical Surveillance and diagnosis
Producers/Farmers	Owners of pigs	Notification of suspected cases; adoption of good production practices, biosecurity measures, and records of activities; funding.	Clinical Surveillance
Embrapa and other research institutions	Veterinarians, zootechnicians, agronomists and assistants	Surveys, notification of suspected cases; information dissemination, training	Clinical Surveillance and diagnosis
Industry	Agribusiness segment for animal origin products and suppliers of livestock inputs	Notification of any suspected cases; dissemination of information; provision of indirect surveillance information, funding, biosecurity	Clinical Surveillance
Accredited Laboratories	Public or private MAPA accredited labs for conducting screening tests	Notification of any suspected cases; dissemination of information; performance of screening serological tests	Diagnostic surveillance
Private Laboratories	Private laboratories that perform diagnostic tests of diseases in the production system	Notification of any suspected cases; dissemination of information, submitting samples received from compatible clinical cases to the LFDA	Diagnostic surveillance
Qualified Veterinarians	Private veterinarians with delegated competence from the SVO to perform specific action	Suspected cases notification; sample collection; generate information of interest (productivity reports); biosecurity; information dissemination	Clinical Surveillance
Service Providers	Occasional or permanent services: consultancies, clinical care, agricultural resales, vaccinators, collection of dead pigs	Notification of any suspected cases; dissemination of information, biosecurity	Clinical Surveillance

STAKEHOLDERS	Description	Accountabilities	Type of participation
Veterinarians and environmental services professionals	veterinarians, biologists, zoo-technicians and other professionals who work in the area of environmental conservation and preservation areas management	Notification of any suspected cases; dissemination of information, biosecurity	Clinical Surveillance and Sample collection
Population management agents	individuals or legal entities authorized by the environmental agency to carry out the population management of wild pigs	Notification of any suspected cases; dissemination of information, biosecurity	Clinical Surveillance and Sample collection
CNA	Confederation of Agriculture and Livestock of Brazil	Dissemination of information; funding; institutional support	Clinical Surveillance
ABCS	Brazilian Association of Swine Breeders and state associations of breeders	Dissemination of information; funding; institutional support	Clinical Surveillance
ABPA	Brazilian Association of Animal Protein	Dissemination of information; funding; institutional support	Clinical Surveillance
ABEGS	Brazilian Association of Swine Genetic Companies	Dissemination of information; funding; institutional support	Clinical Surveillance
Animal Health Funds	Associations created for the purpose of raising funds for indemnities in support of Animal Health actions.	Dissemination of information; funding;	Clinical Surveillance
Rural extension	Veterinarians and assistants	Notification of any suspected cases; dissemination of information, biosecurity	Clinical Surveillance
Swine Carriers	Professionals who transport pigs between farms and to slaughter	Notification of any suspected cases; dissemination of information, biosecurity	Clinical Surveillance
International agricultural surveillance	Veterinarians and assistants related to international-transit surveillance Of animals and goods	Notification of any suspected cases; dissemination of information, biosecurity	Clinical Surveillance



8. DESCRIPTION OF TARGET POPULATION

This Integrated Plan for surveillance of swine diseases should initially be applied throughout the geographical area of the PSC-free zone in Brazil (RS, SC, PR, AC, BA, DF, ES, GO, MG, MS, MT, RJ, RO, SE, SP, TO and the municipalities of Guajará, Boca do Acre, south of the municipality of Canutama and southwest of the municipality of Lábrea, belonging to the state of AM).

Figure 1. Geographic area of the CSP FREEZONES of Brazil, 2021 representing the coverage area of the Integrated SURVEILLANCE PLAN.



This Plan is based on the characterization of the Brazilian swine population, separated into three distinct parts so named: **Tech Swine Farming**, **Non-tech Swine Farming**, and **Wild Swine Population**. This division is detailed below:

TECH SWINE FARMING: represents the set of breeds carried out by tech producers, i.e., who incorporate the technological advances in genetics, nutrition, health, biosecurity and who follow the zootechnical indexes of their production. This group includes genetics companies, large and medium-sized agribusinesses, integrated, cooperative, and independent swine producers that access the main processing and distribution channels in the production chain.

In this group you will find establishments of the following categories:

- Certified Swine Breeding Establishment (GRSC): an establishment that fully meets the requirements established for certification of compliance with differentiated biosecurity standards and is certified as free of the specified diseases;
- Piglet Production Unit ("UPL"): this involves the covering, gestation, maternity, and nursery phases and, sometimes, an exclusive use insemination center;
- Nursery and wean to finish: establishment that receives weaned piglets from the UPL to grow them only in the nursery phase or until they are ready to be sent to slaughter;
- Termination: an establishment that receives pigs for the purpose of fattening them for subsequent dispatch to slaughter;
- Complete Cycle (CC): predominant establishment among independent swine farms. This model encompasses all phases of production, i.e., the same establishment contemplates from the arrival of piglets destined for reproduction until the end of termination.

NON-TECH SWINE FARMING: the group of non-technical producers who do not incorporate technological advances (especially in genetics, nutrition, health and biosecurity) and for whom the production of pigs is intended for their own consumption (subsistence) or for local or micro-regional trade (commercial), with limited access to some processing and distribution channels of the production chain.

This population is comprised of:

- Non-tech subsistence swine farming: establishments where the swine production is for own consumption;
- Non-tech Local Trade Swine Farming: establishments that, as a rule, are small-sized and destined for local trade, with limited access to some processing and distribution channels in the production chain.

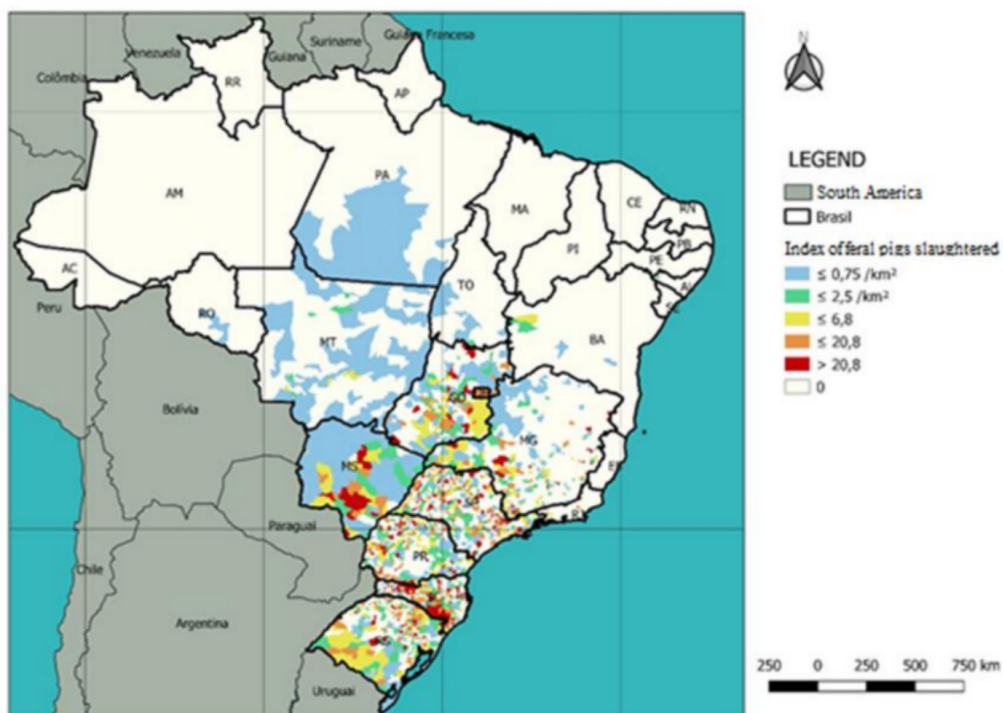
The characteristics for identifying this type of breeding are described in Annex I.

WILD SWINE POPULATION: animals of the species *Sus scrofa*, which includes the domestic pig (*Sus scrofa domesticus*), their different forms, breeds, and strains, the European wild boar (*Sus scrofa scrofa*) and all the different degrees of interbreeding between these subspecies in the wild.

Note: The species *Tayassu tajacu* and *Tayassu pecari*, popularly known as cateto and queixada, respectively, belong to the family *Tayassuidae*, which, despite being popularly known as porco-do-mato, do not belong to the family *Suidae* and, therefore, are not the target of this Plan.

Although there are no quantitative data on the population of wild swine in Brazil, their distribution throughout the national territory can be accessed by the Fauna Management Information System - SIMAF, managed by IBAMA, and by maps of perceived presence of wild swine in Brazil, made by MAPA. Figure 2 illustrates this distribution in the country.

Figure 2. Index of WILD swine slaughtered in Brazil by municipality, SIMAF, 2020.



9. DATA SOURCES AND USE

Table 2. Main PNSS data sources.

Type of data	Data source	Record and access location	Description
Notification records and suspected cases investigation of compulsory notifiable diseases in swine	MAPA and OESA	e-Sisbravet	Inserted under the responsibility of OESA and managed by OESA and MAPA. The PNSS uses this data to follow up on the occurrences of probable cases of PSC, PSA and PRRS and evaluate surveillance from the notification of suspected cases.
Registering establishments and herds with their geolocation	OESA	OESA computerized system	Recorded in databases by the OESA, updated by the farmers, and used in the surveillance design.
Records of animal movement by issuing the government payment forms for Animal Transit (GTA)	OESA	OESA computerized system	Performed by producers, qualified veterinarians, and OESA and used by the PNSS to structure animal health management actions, such as animal transit surveillance, identification of establishments with a higher concentration of movement and inter-relationships between areas in the country.
Registration of slaughterhouses and information on swine slaughtered and official inspection	MAPA and OESA	SVO, PGA-SIGSIF and OESA computerized system structure spreadsheets	Data consolidated by both MAPA (establishments under federal inspection) and OESA (state and municipal slaughterhouses). They are used by the PNSS to analyze surveillance in slaughterhouses.
Recording surveillance data from the inspection of establishments and management of PNSS activities	MAPA and OESA	DSA computerized system	Consolidated by OESA and forwarded to MAPA, as a basis for carrying out analyses to evaluate the surveillance system for notifiable diseases in pigs in the country.
Recording of human, financial and structural resources data from the OESA, the emergency funds and MAPA	MAPA and OESA	Annual report spreadsheets	Updated and consolidated annually by OESA and SFA, serve as a complement to the PNSS analysis.
International surveillance data records	MAPA/ VIGIAGRO	SIGVIG and spreadsheets	Obtained from VIGIAGRO at points of entry of people, animals and various goods
Registration of official and accredited laboratories	LFDA and Accredited Laboratories	DSA computerized system	Used by the PNSS for analyses related to surveillance from notifications and serological surveillance.
Data records from active serological surveillance	MAPA	DSA computerized system	Entered by OESA into the system And managed by DSA
Information about wild swine	IBAMA	SIMAF	Registered by IBAMA and population management agents
Data from studies and surveys	Research Institutions	Scientific papers and results from studies	produced by research institutions And assessed by DSA
Data on occurrences of epidemiologically relevant events and presence or absence of diseases	OMSA and International agencies	OMSA-WAHIS System And others	Data used by PNSS, when necessary, for specific analyses

10. COMPONENTS OF THE SURVEILLANCE SYSTEM

Each component of the surveillance system comprises an activity used to investigate one or more hazards in the target population. The set of surveillance components or activities that are capable of producing data on a particular disease state or on the state of a particular population constitutes a surveillance system.

This plan is based on the guidelines proposed by the OMSA and the Food and Agriculture Organization of the United Nations (FAO) in several of their documents, in particular the OMSA "Land Animal Health Code and the Manual of Diagnostic Tests and Vaccines" and the FAO "Manual for Risk-Based Disease Surveillance".

Taking into account regional diversities, the integrated surveillance plan for PSC, PSA and PRRS sought to establish a more effective and cost-efficient program, especially regarding the existence of differentiated risks of disease occurrence in different regions and in the various types of production and regions of the country.

The Integrated Surveillance Plan consists of five components listed below:

- 1. risk-based serological surveillance**
- 2. inspections in breeding establishments**
- 3. investigations of suspected cases**
- 4. slaughterhouse inspection**
- 5. serological surveillance in wild swine**

Given the availability of scientific evidence and the high degree of predictability and control of the plan's actions by the SVO, it was possible to determine the expected level of sensitivity and the probability of absence of the target diseases in relation to the "risk-based serological surveillance" component. For the other components, there is a lack of elements to enable a prior and complete quantitative analysis of their contributions. Nevertheless, it is important to emphasize that the satisfactory execution of all components, as recommended in this Plan, is essential for the surveillance system to achieve the expected sensitivity and the most complete coverage possible of the swine population.

According to the Land Code, "surveillance strategies used to determine PSA status should be adapted to the situation (...) the approach used should take into account the presence of wild swine, the presence of ticks of the genus *Ornithodoros* and the presence of PSA in neighboring countries or areas".

As PSA has not been present in South America for more than 30 years, the risk-based serosurveillance and wild swine serosurveillance components do not include testing for this disease in their routine. However, surveillance of suspected cases of hemorrhagic syndrome, from any components, will be tested for both PSC and PSA, in order to promote early detection of possible occurrence of PSA.

Testing for PSA or changing the sampling strategy will depend on assessments of possible changes in the risks of PSA occurrence in Brazil or the region.

10.1. COMPONENT 1 - RISK-BASED SEROLOGICAL SURVEILLANCE

The following information explains how the sampling design for this component is calculated and optimized based on the risk to PSC. Although not the primary objective of the component, serological tests will be concurrently performed for PRRS and interpreted in light of the same evaluation methods, providing results that achieve satisfactory sensitivity.

For the purposes of characterization and outlining the sampling plan, the PSC-free zone was divided into three geographic areas (Figure 3). For each of these areas, a sampling plan was developed in which different probabilities of previous absence of PSC were considered, according to the history and evaluations of the previously applied surveillance system and proximity to non-PCS-free areas.

In order to calculate the sensitivity of the component in each area, higher risk groups were selected based on the category "type of establishment".

The number of farms and pigs to be sampled in each geographical area was then determined so that the sensitivity of the surveillance component remained above 95% and the probability of the absence of PSC in the area, if all samples were negative to diagnostic tests, remained above 99%.

Figure 3. *Geographical areas of application of the integrated SURVEILLANCE SYSTEM.*



10.1.1. Probability of absence of diseases (Areas 1, 2 and 3)

Geographic Areas: Considering the great diversity of ecosystems, production systems, social realities and geographical peculiarities that influence the application of the surveillance system, the area corresponding to the PSC-free zones has been divided into three areas, as shown in Figure 3, with the aim of adequacy to epidemiological realities and challenges.

For each of the three geographic areas, the surveillance component was outlined separately by applying different levels of "Prior", i.e., the probabilities of prior absence of the disease. These different levels were established according to the history and evaluations of the previously practiced surveillance system. The more intensive and validated the previous surveillance system, with satisfactory results in the population, the higher this considered probability.

- **Area 1:** comprising the Southern Region states (PR, SC and RS), which concentrate most of the country's industrial swine production, with 50.6% of the swine population. Tech supported production in an integrated system predominates in this region. The last occurrences of PSC were in 1997. In this area, the surveillance system was first structured and achieved quite robust results in the evaluation of demonstrating the absence of infection in swine herds (MOTA, A.L.A.A, 2016). Such a condition allows, still conservatively, the value of 90% to be used for the probability of absence of PSC.
- **Area 2:** comprising the federative units of the Southeast Region and part of the Midwest Region (SP, MG, ES, RJ, GO, DF and MS), with about 23% of the swine population. There is a significant amount of swine production in integrated systems in this region. However, independent producers predominate. The proportion of non-tech supported production systems is higher than in Region I. In this Area 2, the last occurrences of PSC were in 1998. Area 2 also has a long history of surveillance for PSC with satisfactory results, but less robust and consistent than those of Area I. Therefore, for this area a probability of absence of 70% was estimated.
- **Area 3:** comprising the states bordering the ZnL of PSC (AC, RO, MT, TO, BA and SE, besides the municipalities of Guajará, Boca do Acre, south of the municipality of Canutama and southwest of the municipality of Lábrea, belonging to the state of AM, with 11% of the swine population. In this Area 3, there is a presence of technologic, integrated, and independent production systems, as well as a higher proportion of non-technologic production systems, whether commercial or not. The last occurrences of PSC were in 1995. In this Area there is also a surveillance system for PSC with many years of activities and favorable results, but with weaknesses in some states. For Area 3 a probability of absence of PSC of 50% was established, a value used when the epidemiological situation is unknown. This decision was made in view of the borders with the ZnL and the higher proportion of non-technologic production systems.

It should be noted that these prior probabilities may be adjusted to higher values depending on the results of the implementation of the components of this integrated surveillance plan. If the targets for the established indicators are reached and no cases of the diseases are detected, these adjustments can be made, implying reductions in the sampling plans for these areas.

10.1.2. Risk Attributed to Establishment Types

The swine establishments had their risks categorized according to their characteristics. This categorization considered the heterogeneity of the swine farms and the consequent impact that handling, sanitary, and biosecurity practices have on the risk of introducing or spreading diseases such as PSC.

The risk assigned to each type of technologic swine facility was based on the "Estimation of Relative Risks for Introduction of PSC" (MOTA, A.L.A.A, 2016), which also followed the guidelines proposed by the FAO's "Manual for Risk-Based Disease Surveillance." Among the main types of establishments, four were selected to make up the serological surveillance system.

- Certified Swine Breeding Farm (GRSC): This type of establishment, despite being the category of establishments with the highest biosecurity and lowest risk of PSC occurrence, was introduced in the component since testing for PSC is a requirement for certification of any GRSC. These establishments have direct or indirect links with the entire production chain, and thus ensuring the absence of PSC and PRRS in this type of establishment is of major relevance to the sector. Thus, the Risk assigned to GRSC is 1.
- Piglet Producer Units (UPL): This type of establishment represents the integrated sector of the production chain and has the highest concentration of adult pigs. The Risk assigned to this type of establishment is 3.4.
- Complete cycle establishments (CC): Complete cycle establishments are identified as those with the highest risk among technologic swine farms (MOTA, A.L.A.A., 2016). This is due, among other factors, to lower adherence to sanitary and biosecurity measures. The Risk assigned to this type of establishment is 4.5.
- Non-tech Swine Farming Establishments for Local Trade: They represent a type of high-risk establishment and are therefore responsible for a large contribution to the sensitivity of the surveillance system due to the precariousness of the biosecurity conditions and their potential for infection and spread of PSC, especially when trading. Considering that there are no scientific references in the country on the subject, the Risk attributed to this type of establishment was 7.

The "Nursery" and "Termination" type establishments were not included in the sampling due to the lower risk attributed to these categories, and especially the expectation that only young animals would be found. These animals have a shorter time of possible exposure to the pathogens, if they are present, and therefore a lower chance of showing immunological responses or presence of the investigated agents. In addition, nurseries and terminations are housed and emptied approximately every 45 days (nurseries) and 90 days (terminations), and after each animal removal, cleaning, disinfection, and sanitary break are carried out.

The non-tech subsistence swine farms were not prioritized for sampling because they were considered less risky than the non-tech swine farms for local trade.

10.1.3. Sampling

Seroepidemiological surveillance is intended to support certification of absence of transmission of the PSC and PRRS viruses. Risk-based sampling, which targets herds and individuals most likely to be infected, is more appropriate, as it provides a better level of probability of disease absence when compared to a representative sample of the same size.

The sampling design was defined in order to maximize the probability of detecting PSC. For this reason, it selects establishments from the 4 types chosen, reflecting the distribution in the 3 areas and states. As described earlier, although the model is based on the characteristics of PSC, sampling will also be satisfactory for proof of the absence of PRRS and, if necessary, also for PSA.

As epidemiological parameters, the minimum prevalence of infected establishments of 1%, the prevalence in animals within an infected herd of 15%, the sensitivity of serological tests (ELISA) of 94.4% and for PCR of 99.0% were considered. The specificity of the diagnostic system is treated as 100%, considering the complementary clinical, laboratory, and epidemiological investigations for case confirmation by the SVO. The number of samples was calculated to meet a minimum sensitivity of 95% for the surveillance system and 99% probability of absence of PSC.

The GRSC will follow the current legislation for certification with a sampling definition. With regard to the temporal aspect of the Integrated Surveillance Plan, the sampling of swine farms with and without technology for local trade must be spread over all the months of the year and may vary according to the indicators in Table 6 (Indicators for the evaluation of temporal representativeness), but must not be concentrated in a few months in order to ensure the temporal representativeness of the components.

10.1.4. Characterization of sampled establishments

The list of municipalities with the establishments to be sampled will be indicated annually by the DSA, in conjunction with the OESA of each State. However, when selecting the establishment, the OESA technician in charge of collection should make sure that it has the necessary characteristics to fit into the "establishment type" category that was indicated by the DSA, as described in Item 8 of this Plan and in Table 1 of Annex I.

Those states that develop studies to identify areas or properties at higher risk can add this information to support the selection of properties to be sampled.

10.2. COMPONENT 2 - INSPECTIONS IN BREEDING ESTABLISHMENTS

In this component, clinical and zootechnical index inspections should be targeted at other establishments where serological surveillance has not been conducted, with the presence of risk factors for the introduction, maintenance or spread of the PSC virus, PSA and PRRS. The protocol of activities and information to be checked during inspections of establishments and the list of risk factors to be considered are available in Annex II.

The surveillance of inspections in higher risk breeding farms is of great importance as it provides updated information on the farm and the swine herd, in addition to the interaction of the SVO with those responsible for the handling of pigs for the development of animal health education activities.

Complementary and non-targeted, other SVO inspections and controls in swine establishments with different purposes may be considered in the production of data and information for disease surveillance. Again, states that develop studies to identify areas or properties at higher risk can add this information to support the selection of properties to be inspected. These studies can be conducted by each state in partnership with educational and research institutions and validated by the DSA.

With regard to the temporal aspect inspections should be distributed over all the months of the year and may vary according to the indicators in Table 6 (Indicators for the evaluation of temporal representativeness), but must not be concentrated in a few months in order to ensure the temporal representativeness of the components.

10.2.1. Sampling

For the purposes of characterization and design of the sampling plan, the PSC-free zone was divided into three geographical areas (areas 1, 2, and 3), according to the criteria adopted in component 1, probabilities of absence of PSC virus infection.

Sampling for PSC detection was calculated with the goal of having confidence greater than 95% that PSC is not present on farms, and an inter-herd prevalence greater than 1% and intra-herd prevalence greater than 15%, assuming that 1% of animals would be showing symptoms at the time of inspection, and that "diagnostic sensitivity" - in this case the veterinarian's ability to detect clinical signs when performing a herd inspection - would be 15%. Similarly, to "Component 1 - Risk-Based Serological Surveillance", the relative risks for PSC by type of swine farm (UPL - 3.4; Complete cycle - 4.5; non-technologic - 7.0), and the probabilities of no previous PSC (Area 1 - 90%; Area 2 - 70%; Area 3 - 50%) were incorporated into the sampling design. These parameters were described more extensively in component 1.

The risk-based sampling design was defined in order to maximize the probability of detecting clinical signs of PSC, in the PSC-free zone, and to select establishments belonging to the types UPL, complete cycle, and non-tech swine farms, reflecting the distribution in the three sampling areas and federative units.

The veterinarians or inspectors of the official service must inspect the herd as a whole (visual inspection), proceed to individual examination (clinical inspection) of animals that they deem clinically outstanding from the others, recording the total of visually inspected and clinically inspected in the electronic form.

The list of municipalities with technologic and non-technologic establishments to be sampled will be indicated by the DSA, based on data from the records sent by each OESA. However, in selecting the establishment, the OESA technician must ensure that it has the characteristics necessary to fall into the "establishment type" category that has been indicated by the DSA, as described in Item 8 of this Plan and Table 1 of Annex I, and that it has at least one of the risk factors listed in Annex II of this Integrated Plan.

10.3. COMPONENT 3 - INVESTIGATIONS OF SUSPECTED CASES

The investigation of suspected cases, in domestic or wild swine, is the most common and most important form of the surveillance system. Notification by farmers, other professionals in the swine chain, and wild swine population handling agents who are properly trained and aware of the signs of disease is essential for early detection of outbreaks.

The procedures standardized by the DSA, both the flow of notifications and records of animal health information, and the technical procedures for handling of suspected cases and laboratory diagnosis of PSC, PSA and PRRS, are available in the "Manual of the National Animal Health Information System - SIZ" and in the datasheets of each disease".

In case the notification of suspected cases is considered a probable case of PSC, PSA or PRRS by an official veterinarian (MVO), the establishment must be immediately banned and entered in the e-Sisbravet. The clinical and epidemiological investigation of probable cases must be complemented with laboratory tests performed by the LFDA for the confirmation or discarding of cases, as presented in the datasheets for the diseases. When probable or confirmed cases occur in wild swine, there will be no banning of the establishment.

In situations where there is confirmation of cases of PSC, PSA, or PRRS, the actions must follow what is set forth in the respective contingency plans.

10.3.1. The role of educational or research institutions and private diagnostic laboratories

It is especially important for the early detection of outbreaks to include educational or research institutions and private diagnostic laboratories in the SVO notification system. These laboratories, professionals in the field and university professors are routinely consulted by producers and veterinarians in charge of farms for the production of autogenous vaccines and the diagnosis of health problems other than officially controlled diseases.

In the event of an outbreak of one of the diseases listed in this plan in technologic facilities, these laboratories may inadvertently receive unofficial samples of suspected cases before the SVO is notified. As the samples are taken from pigs showing clinical signs, which may include cases of PSC, PSA or PRRS, they have a high surveillance value for early detection. Therefore, under conditions that characterize suspected cases of these diseases, these laboratories must immediately notify the official animal health service for investigations and differential diagnosis.

The SVO of each federative unit must have updated contacts with diagnostic laboratories, both private and university, and make periodic visits to exchange information, raise awareness, clarify obligations regarding the notification of diseases, and make contacts available for the immediate notification of suspected cases.

10.4. COMPONENT 4 - SLAUGHTERHOUSE INSPECTION

The objectives of inspection in slaughterhouses are mainly related to public health, such as ensuring the safety, wholesomeness and hygienic quality of products of animal origin, as well as reducing the risks of disease transmission or contamination by residues. The representativeness of slaughter data has limitations and has a large sampling bias, which limits the interpretation of the data produced for animal health measures, emphasizing that:

- The swine sent to slaughter in establishments under official inspection are considered a biased sample for several reasons, ranging from commercial and geographical, to epidemiological, sanitary, and seasonal, and are not representative of the general population.
- Swine sent to slaughter tend to be younger and healthier than the rest of the population, and exclude or under-represent animals that are too young, underdeveloped, or diseased.
- Diseases that cause high mortality are underestimated in slaughterhouses, since the animals don't even get to be slaughtered.
- occurs at the end of the chain, so it is a late detection within the surveillance system.

Hence, slaughter data are of limited value for the assessment of diseases that have injuries that are difficult to detect or whose occurrence reduces the likelihood of animals being sent to slaughter, but the large numbers of animals involved and the standardization of ante- and post-mortem animal inspection procedures for the early detection of suspected cases of target diseases provide a counterbalance that makes this component relevant to the surveillance system.

Inspections carried out on a regular basis can detect the presence of clinical signs and pathological injuries and target surveillance actions at the swine's establishments of origin.

Surveillance in slaughterhouses is commonly interpreted as a form of active surveillance. The main advantages are: (a) low cost, since pigs are already inspected for other purposes; (b) large number of pigs inspected; (c) constant supply of data; (d) it allows the collection of data and materials, in a few slaughterhouses, from a large number of rural holdings of origin of the pigs and with a standardized method to detect clinical and pathological signs, which are generally more specific than the observations of the owners; (e) is a way of monitoring the other components of the surveillance system, since in the event of detection failures at field level, it is at this last stage that probable cases of the disease can be detected.

In case of detection of injuries compatible with PSC, PSA and PRRS the official inspection service must communicate the animal health service to carry out the clinical and epidemiological investigation.

10.5. COMPONENT 5 - SEROLOGICAL SURVEILLANCE IN WILD SWINE

The domestic pig or wild boar (*Sus scrofa*) in all their forms, strains, races, and their different degrees of crossbreeding, living in the wild in this component of the surveillance system, without human supervision and control.

The SVO should have up-to-date data on the populations and habitat of wild swine that can be obtained from official sources, such as the Wildlife Management Information System (SIMAF/IBAMA), SVO perception maps, possibly supplemented with other governmental and non-governmental sources related to the environment and wildlife, wildlife research institutes, hunting clubs and the like. Serological surveillance in wild pigs has a complementary function to validate the condition of absence of the diseases listed in this Plan in the swine population.

The collection of data on wild swine and their correlation with domestic swine populations is of paramount importance to enable the SVO to take action to prevent the introduction of PSC, PSA and PRRS and to respond rapidly in the event of an introduction. Wild swine have epidemiological importance in maintaining PSC, PSA, and PRRS as reservoirs for the virus and possible sources of infection for domestically farmed pigs.

Serological surveillance in the wild swine population will be carried out through a partnership between authorized Population Handling Agents and the SVO, according to the procedures described in Annex III.

11. LABORATORY DIAGNOSIS

11.1 DIAGNOSES OF PROBABLE CASE INVESTIGATIONS

For probable cases of PSC, PSA and PRRS, you should proceed as recommended in their respective datasheets.

Diagnostic testing of samples from probable cases of PSC, PSA, and PRRS is only allowed in official MAPA laboratories - the Federal Laboratories for Animal Health (LFDAs).

Figure 4. Laboratory Diagnosis Flow of samples from SURVEILLANCE of probable CASES of PSC.

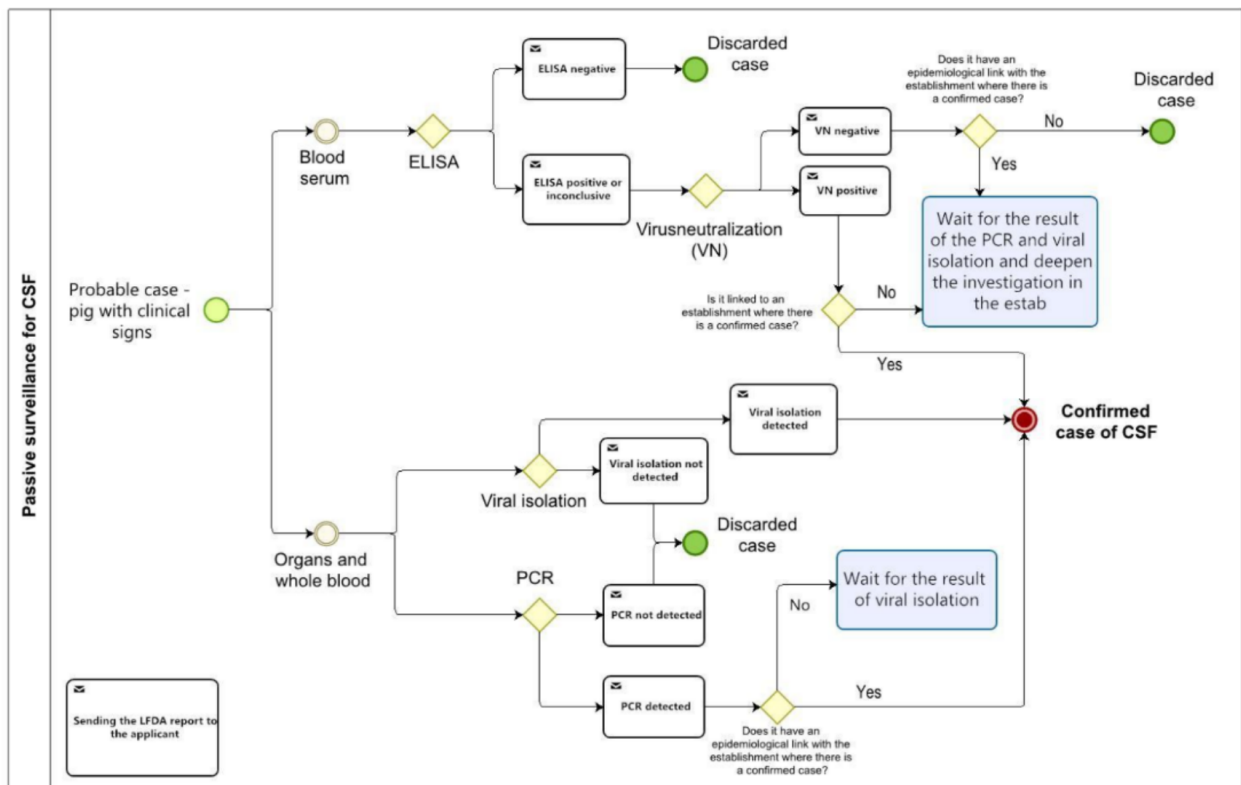


Figure 5. Laboratory Diagnosis Flow of samples from SURVEILLANCE of probable CASES of PSA.

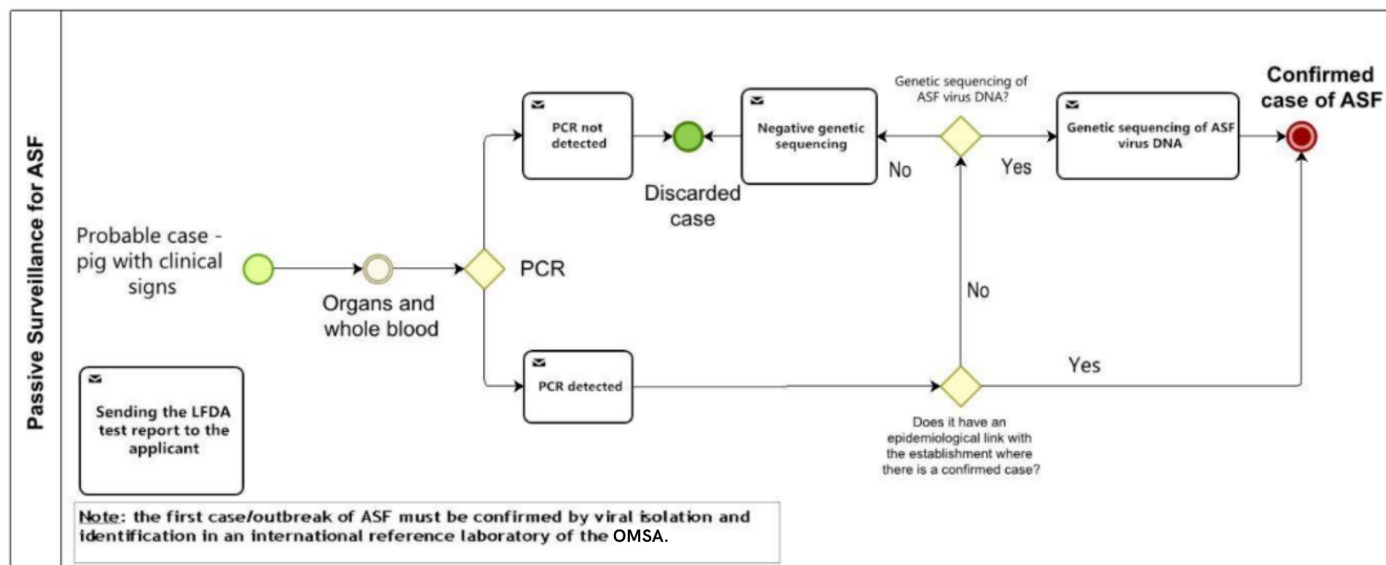
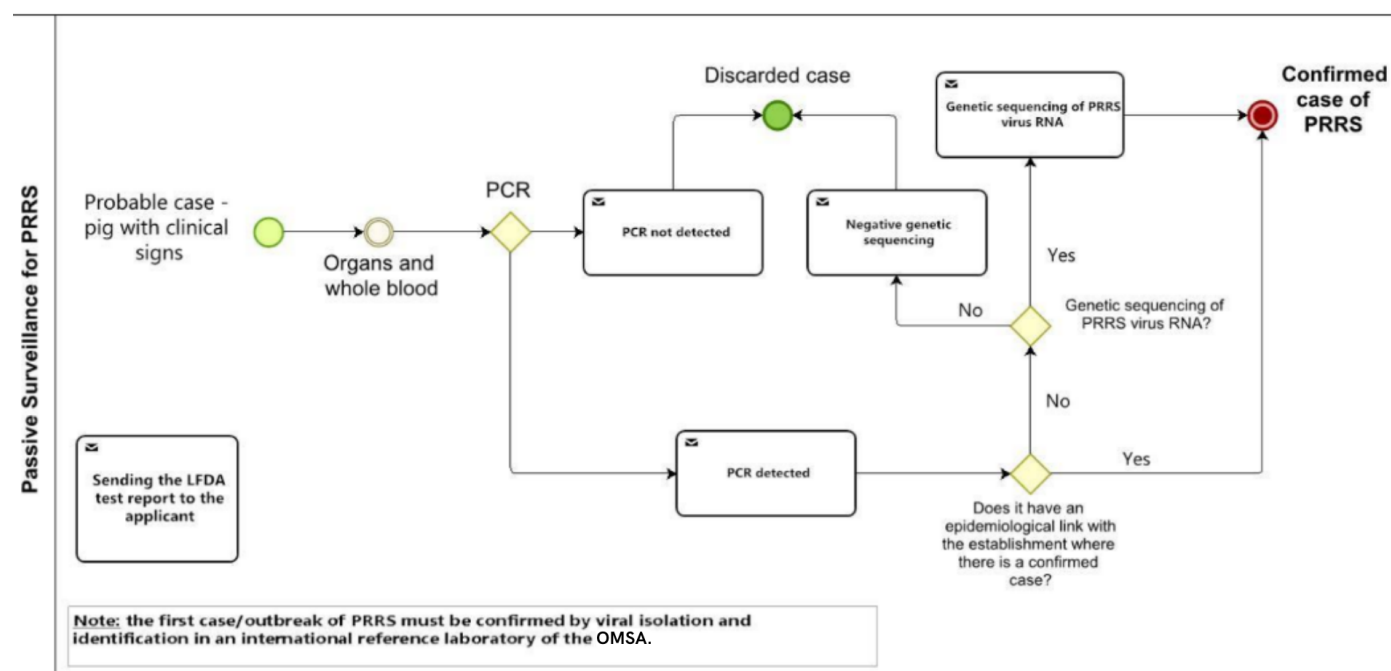


Figure 6. Laboratory Diagnosis Flow of samples from SURVEILLANCE of probable CASES of PRRS.



11.2 SEROLOGICAL SURVEILLANCE DIAGNOSES

To perform the serological surveillance tests for PSC, PSA and PRRS, the network of public laboratories accredited by MAPA is used, depending on their scope of accreditation:

- Centro de Diagnóstico de Sanidade Animal - CEDISA, Santa Catarina;
- Centro de Diagnóstico Marcos Enrietti - CDME, Paraná;
- Instituto Biológico - IB, São Paulo;
- Instituto Mineiro de Agropecuária - IMA, Minas Gerais;
- Instituto de Pesquisas Veterinárias Desidério Finamor - IPVDF, Rio Grande do Sul;
- Laboratório de Análise e Diagnóstico Veterinário – LABVET, Goiás; and

- Laboratório de Diagnóstico de Doenças Animais - LADDAN, Mato Grosso do Sul.

If there are any inconclusive or positive samples in the screening tests, further investigations continue and confirmatory tests are performed at the reference LFDAs.

The simplified flow and laboratory tests for active surveillance can be seen in the figures below.

Figure 7. Laboratory Diagnosis Flow of samples from ACTIVE SURVEILLANCE of PSC.

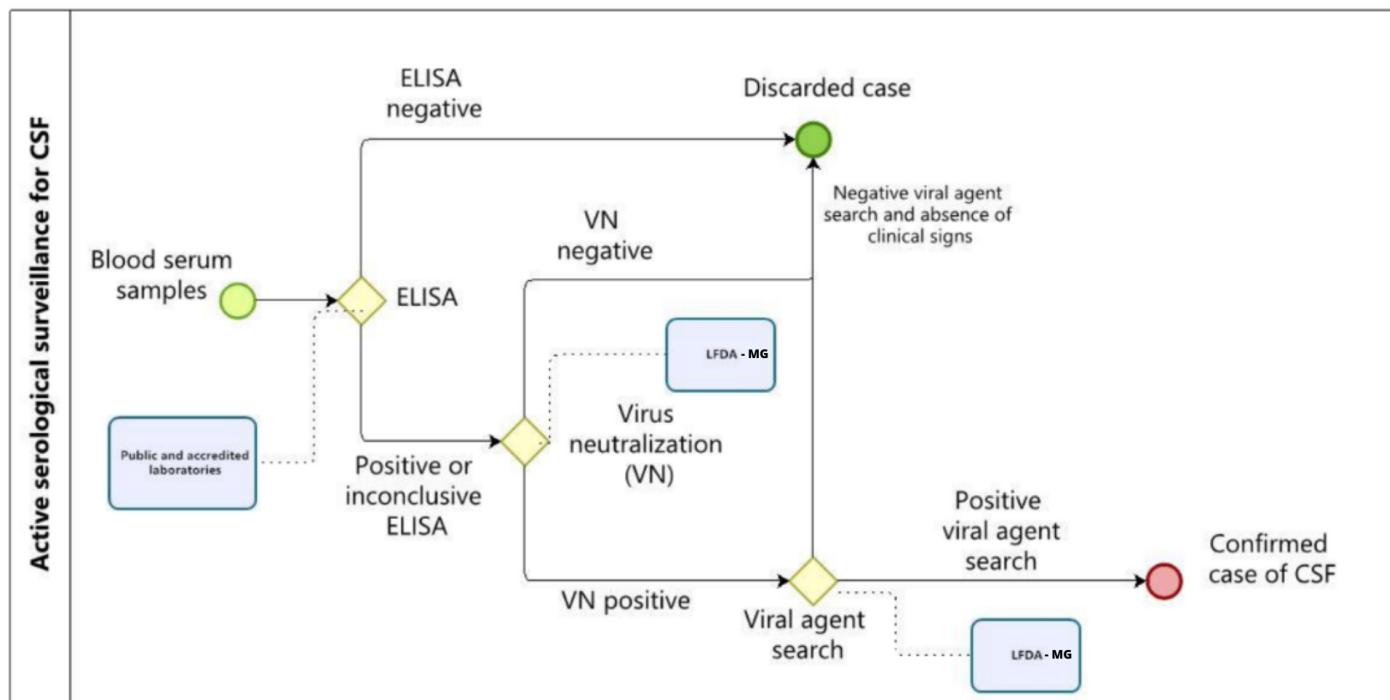


Figure 8. Laboratory Diagnosis Flow of samples from ACTIVE SURVEILLANCE of PSA.

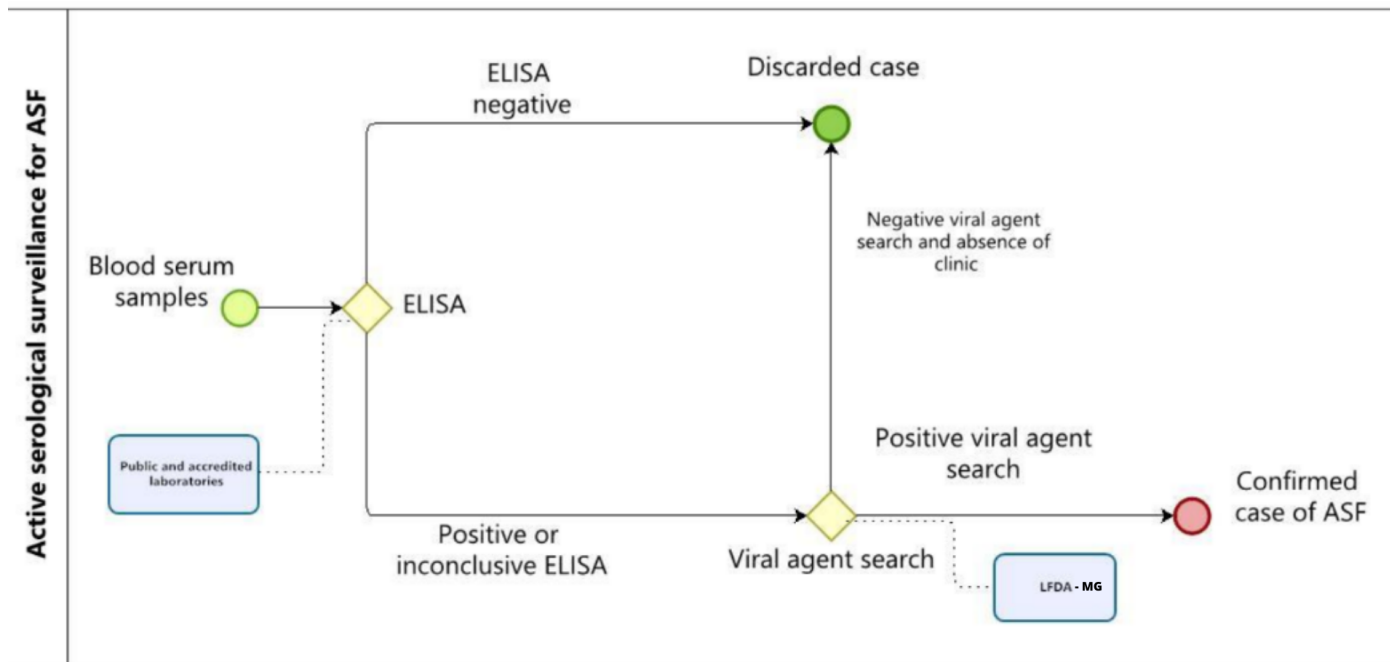


Figure 9. Laboratory Diagnosis Flow of samples from ACTIVE SURVEILLANCE of PRRS.

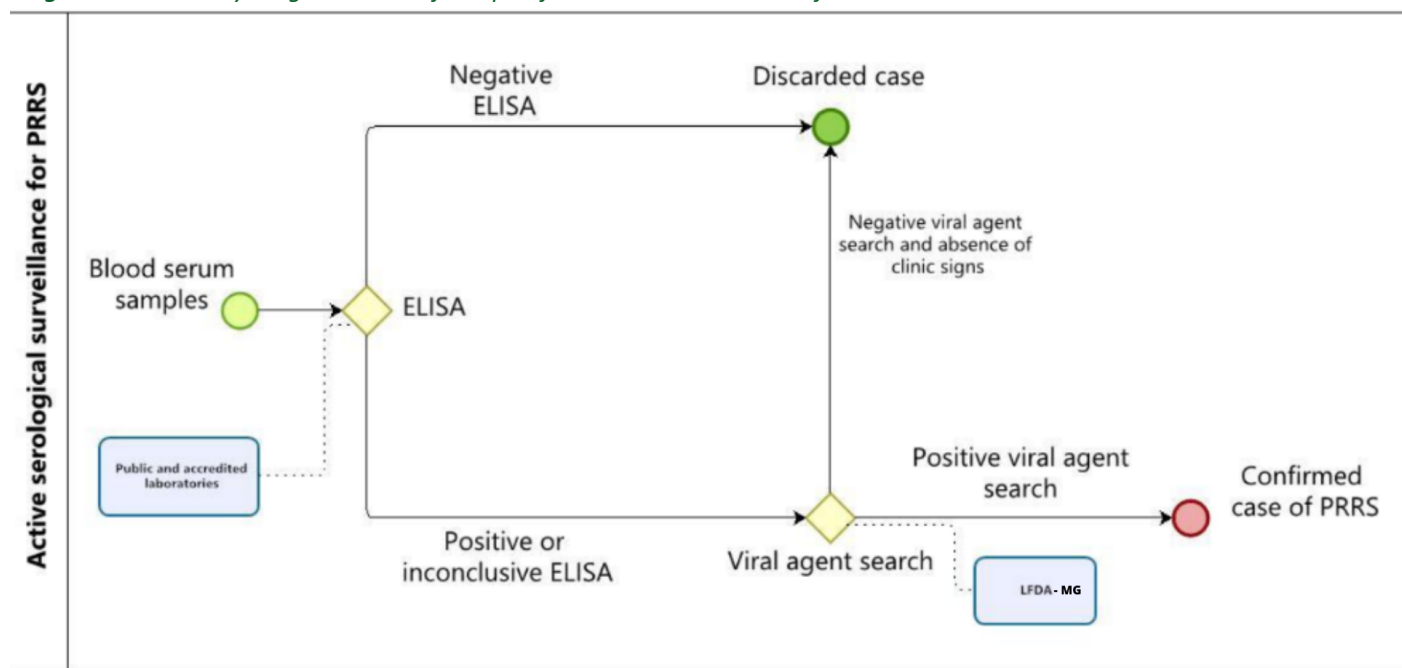


Table 3. PEOPLE IN CHARGE, sample types, laboratories, registration systems for SURVEILLANCE ACTIVITIES and data entry frequency.

Sampling Type	Person in charge of collection	Sample Type	Laboratory	Data Collection System	Data Entry
investigations of suspected cases/probable cases	OESA	Whole blood, serum, and organs of choice	LFDA/MG and LFDA/PE	e-Sisbravet	Immediate and continuous
Serological surveillance in establishments technologic and non-technologic	OESA	Blood Serum	IMA/MG IB/SP CDME/PR CEDISA/SC IPVDF/RS LABVET/GO LADDAN/MS	Computerized system recommended by the DSA	Continuous, as activities progress
Serological surveillance of Wild swine	Handlers	Blood Serum	IMA/MG IB/SP CDME/PR CEDISA/SC IPVDF/RS LABVET/GO LADDAN/MS	Computerized system recommended by the DSA	Continuous, as activities progress

12. PERFORMANCE EVALUATION FOR COMPONENTS OF THE SURVEILLANCE SYSTEM

The integrated plan for the surveillance of swine diseases must include objective indicators and targets to enable the performance of the system to be evaluated with a view to appropriate monitoring and correction of deviations and deficiencies.

The evaluations of the performance indicators of the surveillance plan should be part of the compilation and analysis of the results obtained in each of the components and will be carried out by the PNSS coordination, with the support of the focal points in the SFAs and OESAs in each federative unit. They should be inserted in the Semi-Annual Reports for each federative unit involved, aiming to evaluate the fulfillment of the surveillance goals, which should be forwarded to the Animal Health Department. This data will be compiled by the DSA and will support the formulation of the annual report, as described in item 15.

For the present plan, indicators were proposed for evaluating the following performance aspects, described in the following tables:

- geographic representativeness;
- production types/age group representativeness;
- temporal representativeness;
- consistency of results with case definitions;

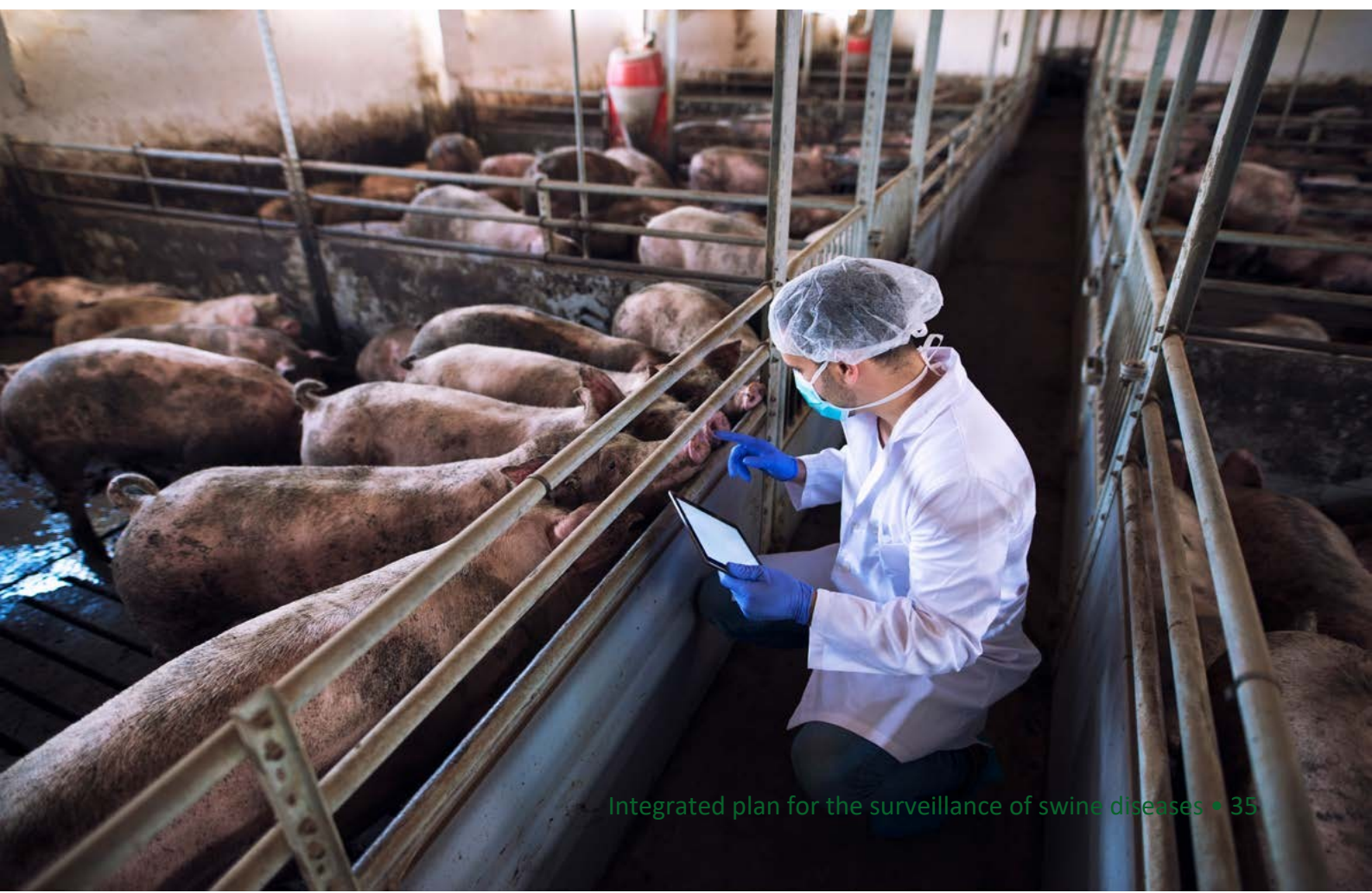


Table 4. Indicators for GEOGRAPHIC REPRESENTATIVENESS assessment

Component	Evaluated characteristics	Indicator	Target
Investigation of suspected cases	The geographical distribution of suspected and confirmed cases must reflect the distribution of the swine population	NA	NA
Serological surveillance of Swine farming Technological	The geographical distribution of the sampling should approximate the distribution of the technological swine production facilities.	% of sites within the municipalities selected by the DSA	100%
Serological surveillance of Swine farming Non-Technological	The geographic distribution of sampling in non-technological swine farming should be throughout the PSC-free zone, prioritizing municipalities in higher risk areas.	% of sites within the municipalities selected by the DSA	100%
Serological surveillance in GRSC	Sampling will involve all GRSCs	% of GRSC collected	100%
Serological surveillance in wild swine	The geographical distribution of samples of wild swine should reflect the estimated population of wild swine and be consistent with the number of Population Handling Agents trained by the SVO and active to collaborate in surveillance. Data from municipalities with population handling activities should be obtained from SIMAF.	municipalities with sample collection / municipalities with wild swine population handling	70%
Inspections in breeding establishments (technological and non-technological)	The geographical distribution of inspections should match closely the distribution of municipalities with non-technological swine production or areas classified as higher risk.	% of selected municipalities that met sampling requirements	95%
Inspections in slaughterhouses	The geographical distribution of the municipalities, with swine establishments, that sent animals for slaughter during the year.	% of municipalities with technological swine production that sent pigs for slaughter	80%



Table 5. Indicators for REPRESENTATIVENESS assessment DEPENDING ON production types/age group

Component	Evaluated characteristics	Indicator	Target
Investigation of suspected cases	The distribution of suspected and probable case notifications and their responses should reflect the production types in the respective Surveillance Area.	NA	NA
Serological surveillance in technological swine production	The number of establishments sampled in each UF should reflect the proportion of establishments by production type in the respective Surveillance Area. The distribution of samples collected should closely match the distribution of the different types of technological establishments, prioritizing those considered to be at higher risk.	% of sampled establishments that fit the production type selected by the DSA	95%
		% of collected samples from adult swine	100%
Serological surveillance in non-technological swine production	Sampling should be done on adult swine, which are more likely to have antibodies against the diseases.	% of collected samples from adult swine	100%
Serological surveillance in GRSC	All GRSC should be sampled, prioritizing sampling in adult swine. Those of type site II and site III must be contemplated, even if they do not have pigs older than 8 months, according to the specific legislation on the matter.	% of GRSC sampled	100%
serological surveillance in wild swine	It is expected that sampling will primarily be of adult pigs, which are more likely to have antibodies against the diseases. However, slaughter of young animals is representative.	NA	NA
Inspections in breeding establishments (technological and non-technological)	Establishments sampled in each municipality must have at least one of the risk criteria listed in Annex II.	% of inspected establishments that meet risk criteria	80%
Inspections in slaughterhouses	The number of establishments with swine inspected in each UF should closely match the total number of existing technological establishments.	NA	NA

Table 6. Indicators for TEMPORAL REPRESENTATIVENESS assessment

Component	Evaluated characteristics	Indicator	Target
investigations of suspected cases	Distribution of suspected and probable case notifications and responses over the defined period.	NA	NA
Serological surveillance in technological and non-technological swine production	Sampling of establishments should be distributed over the defined period.	% of establishments sampled / month	8 %
Serological surveillance in GRSC	Sampling should be distributed over the defined period.	% of establishments sampled / month	8 %
serological surveillance in wild swine	Sample collection is expected to be distributed over the defined period.	% of collected samples / month	8 %
Inspections in breeding establishments (technological and non-technological)	Sampling should be distributed over the defined period.	% of establishments inspected / month	8 %
slaughterhouse inspection	Slaughter distribution over the defined period.	NA	NA

Table 7. Indicators for ASSESSMENT of surveillance plan OPPORTUNITY

Component	Characteristic	Indicator	Target
Investigation of suspected cases	<p>Response to suspected cases must take place within 12 hours of receipt. Samples collected from probable cases should get to the LFDA as soon as possible, no later than 48 hours after collection.</p> <p>Upon arrival at the laboratory, samples must be urgently processed and laboratory test results must be made available within 24 hours of receipt.</p>	Response time;	1
		Time between collection and arrival at the LFDA;	2
		Time between sample receipt and result;	h
		Time to register treatment in e-Sisbravet	4
			8
			h
			2
			4
			h
			24h
Serological surveillance in technological swine production	<p>The samples collected from serological surveillance must be sent as soon as possible to the official accredited laboratory, not later than 15 (fifteen) days after collection.</p> <p>Upon arrival at the laboratory, samples must be processed promptly, within seven (7) days of receipt, and laboratory test results must be made available quickly after they are obtained.</p> <p>Positive samples in the screening tests must be sent to the LFDA within 72 hours of the results in the accredited laboratory.</p>	Time between sample collection and shipment;	15 days
		Time between sample receipt and result;	7 days
		Time between positive screening test result and sending the sample to the LFDA	3 days
Serological surveillance in non-technological swine production	<p>The samples collected from serological surveillance must be sent as soon as possible to the official accredited laboratory, not later than 15 (fifteen) days after collection.</p> <p>Upon arrival at the laboratory, samples must be processed promptly, within seven (7) days of receipt, and laboratory test results must be made available quickly after they are obtained.</p> <p>Positive samples in the screening tests must be sent to the LFDA within 72 hours of the results in the accredited laboratory.</p>	Time between sample collection and shipment;	15 days
		Time between sample receipt at the lab and result.	7 days
		Time between positive screening test result and sending the sample to the LFDA	3 days
Serological surveillance in GRSC	<p>The samples collected from serological surveillance must be sent as soon as possible to the official accredited laboratory, not later than 15 (fifteen) days after collection.</p> <p>Upon arrival at the laboratory, samples must be processed promptly, within seven (7) days of receipt, and laboratory test results must be made available quickly after they are obtained.</p> <p>Positive samples in the screening tests must be sent to the LFDA within 72 hours of the results in the accredited laboratory.</p>	Time between sample collection and shipment;	15 days
		Time between sample receipt and result;	7 days
		Time between positive screening test result and sending the sample to the LFDA	3 days
Inspections in breeding establishments (technological and non-technological)	<p>The inspection activities in establishments must be entered in the DSA's computer system within 7 (seven) days after they take place.</p> <p>Upon detection of probable disease cases, records related to the investigation must be entered into e-Sisbravet within 24 hours.</p>	Inspection records entered into the DSA Computer System within 7 days of the activity taking place.	100%
		Records of probable cases entered in e-Sisbravet within 24h	100%

serological surveillance in wild swine	Samples collected from surveillance on wild swine must be delivered by the Population Handling Officers urgently to OESA and sent as soon as possible to the official accredited laboratory, not later than 15 (fifteen) days after collection.	Time between sample collection and arrival at the OESA;	10 days
	Upon arrival at the laboratory, samples must be processed promptly, within seven (7) days of receipt, and laboratory test results must be made available quickly after they are obtained.	Time between OESA receiving the samples and sending them to the laboratory;	15 days
		Time between sample receipt and result;	7 days
	Positive samples in the screening tests must be sent to the LFDA within 72 hours of the results in the accredited laboratory.	Time between positive screening test result and sending the sample to the LFDA	3 days

13. SENSITIVITY ACHIEVED BY THE COMPONENTS OF THE SURVEILLANCE SYSTEM

The sensitivity of each component of the surveillance system is calculated at the end of the 12-month period since the plan was implemented. Following recommended activities, such as investigating suspected cases and adhering to planned sampling, are critical to achieving satisfactory levels of sensitivity. Calculations will be done independently for each of the three defined areas, and the relative contribution of each state will be assessed.



14. NOTIFICATION SYSTEMS AND RECORDS

All of the services performed within the "component 3 - Investigation of suspected cases" must be entered in the e-Sisbravet, which aims to record, consolidate and share data from the notifications and animal health occurrences performed by local, intermediate and higher levels.

In order to allow any user to register a notification via the Internet, the web address for accessing e-Sisbravet was created. www.gov.br/agricultura/pt-br/notificacao. All registered notifications will be directed to the UVL responsible for the municipality where the property with the suspected case is located.

All activities carried out to fulfill "component 1 - Risk-based serological surveillance", "component 2 - Breeding establishment inspections", and component 5 - Serological surveillance in wild swine must be entered in MAPA's DSA computer system, which aims to record, consolidate, and share data from epidemiological studies and other active surveillance activities.

For component 4 - Slaughterhouse inspection, federal inspection records will be gathered from SIGSIF. As for the establishments under state and municipal inspection, the OESAs should seek integration to obtain the information. When suspected cases are reported and responded to, the information must be entered into the e-Sisbravet.

15. COMMUNICATION OF RESULTS

The compilation and analysis of the results obtained in each of the components of this Plan will be carried out by the PNSS coordination, with the support of the focal points in the SFAs and OESAs in each federative unit. They should develop Semi-Annual Reports, aiming to evaluate the fulfillment of the surveillance goals, which should be forwarded to the Animal Health Department. This data will be compiled by the DSA and will support the formulation of the annual report of integrated surveillance actions for PSC, PSA and PRRS.

Stakeholders should receive reports with analyses and actions taken based on the data entered in the Integrated Surveillance Plan, and it is of utmost importance that these reports reach the local level and permeate all links in this system. The DSA/MAPA will make the reports available in the Electronic Information System (SEI) to the SFAs and the OESAs, as well as making them available on the website.

The feedback of information demonstrates the transparency of the system and keeps the communication chain active among the stakeholders, properly informing and stimulating cooperation through the perception of the importance of their contribution to the system.

This ensures that it remains effective and guarantees the quality of the data obtained.



16. PLAN'S RESOURCES AND FUNDING

The Integrated Swine Disease Surveillance Plan should be jointly financed by the various stakeholders involved and interested in the promotion of animal health and the development benefits of the swine production system.

It is necessary to establish a cooperation agreement between the parties involved in the swine production chain of the current PSC-free zones in the country, in order to guarantee the financing, with complementation from the private sector or from MAPA, to sustain the costs of each component of the surveillance system in all the states involved.

The costs involve the human, physical, and financial resources, public and private, essential for the success of each of the Plan's components.

In each Brazilian state, the players involved must organize meetings to define the distribution of the costs of the surveillance system and to evaluate the application of resources and the execution of the planned activities. Table 8 illustrates an example of the distribution of costs among the main players involved, but, as previously expressed, the arrangement to be established between the public and private players must be built observing the organization of the production chain in each State.

It is important to emphasize that the surveillance system presented aims at the early detection of diseases, mitigating the economic and social impacts of eventual occurrences, and generating security to maintain and open new markets for Brazilian swine and pork products.

Thus, it can be seen that the cost of the surveillance system is much lower than the benefits it brings to the production chain, and therefore shared funding between the public and private sectors in the application of human, physical and financial resources is essential. Nevertheless, future cost-benefit analyses of the Integrated Plan for Swine Disease Surveillance should be carried out in order to be accountable to society and to improve the use of resources in its implementation.



Table 8. Proposal of a model of cost distribution in human, physical and financial resources of the Integrated Plan for SURVEILLANCE of swine diseases, among the INVOLVED PLAYERS.

Component	Field activity and sample collection				Laboratory testing			
	Human	Material	Financial	Sending samples	Human	Material	Financial	System s, Analysi s, and Reporti ng
Investigation of probable cases ¹	OESA	OESA	OESA	Funds/ association s	LFDA/ MAPA	LFDA/ MAPA	LFDA/ MAPA	DSA/ MAPA
Serological surveillance in technological swine production	OESA	OESA	OESA	Funds/ association s	Accredited Laboratori es LFDA/ MAPA2	Lab. Laboratori es LFDA/ MAPA2	Lab. Laboratori es LFDA/ MAPA2	DSA/ MAPA
Serological surveillance in non-technological swine production	OESA	OESA	OESA	Funds/ association s	Lab. Laboratorie s LFDA/ MAPA2	Lab. Laboratori es LFDA/ MAPA2	Lab. Laboratori es LFDA/ MAPA2	DSA/ MAPA
Serological surveillance in GRSC	OESA	GRSC	GRSC	GRSC	Lab. Laboratorie s	Lab. Laboratori es	GRSC	DSA/ MAPA
Serological surveillance in wild swine	Handli ng Agents For swine population s	OESA	---	Funds/ association s	Lab. Laboratori es LFDA/ MAPA2	Lab. Laboratori es LFDA/ MAPA2	Lab. Laboratori es LFDA/ MAPA2	DSA/ MAPA
inspections in breeding establishments	OESA	OESA	OESA	---	---	---	---	DSA/ MAPA

Inspections in slaughterhouses	SIF, SIE, SIM	---	----	---	---	---	---	DSA/ MAPA
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Notes: 1Sending samples and performing tests only in probable cases. 2LFDA/MAPA will perform the confirmatory tests.

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ANNEX I - PROCEDURES FOR ACTIVE SEROLOGICAL SURVEILLANCE IN DOMESTIC SWINE

1. Establishment identification

Each breeding establishment to be sampled will have a unique identification, which will be the MAPA Code. This code will be generated according to the number of breeding establishments and will be made available by the DSA.

ATTENTION: MAPA CODES WILL BE GENERATED BY THE DSA. DO NOT ASSIGN A NUMBER TO IDENTIFIED BREEDING ESTABLISHMENTS WITHOUT PRIOR CONSULTATION.

2. Selection of the breeding establishments to be sampled

The pig farms to be sampled are classified and characterized according to Table 1:

Table 1. Characteristics of THE TARGET POPULATION for the Integrated Surveillance Plan for PSC, PSA and PRRS.

POPULATION	PRODUCTION CHARACTERISTICS
Technology Supported Swine Farming	Loan for use agreement, partnership, buying and selling, genetics and nutrition
	Technical control over breeding
	Separation of pigs according to production stage
	Controlled conditions of genetics, nutrition, facilities and health
	Scale-up production
Independent	No contract, but inserted in a development program (through other companies, cooperatives and mini-integration)
	The pig producer in person negotiates the slaughter (through other companies or cooperatives and mini-integration).
	Technical control over breeding
	Separation of pigs according to production stage
	Controlled conditions of genetics, nutrition, facilities and health
	Scale-up production

POPULATION		PRODUCTION CHARACTERISTICS
Non-technological supported swine production		Own consumption, no trade or only occasional trade in surplus pigs
	Trade	No technical control over breeding
		No nutritional control (food or crop leftovers)
		Rustic and improvised installations
		No separation of pigs according to production stage
		Local trade on a small scale and in a limited manner
		Poor or non-existent reproductive, nutritional and sanitary management
Wild swine		Free-living pigs in the wild

The types of establishments that will be sampled are as follows:

Technology Supported Swine Farming

- Certified Swine Breeding Farm (GRSC): the sampling will involve all GRSCs according to the established certification protocol;
- Piglet Producer Unit ("UPL") and Complete Cycle (CC): the selection of municipalities with UPL and CC to be sampled will be done by the DSA, through the records made available by each state. The selection of the "technology supported facilities" will be the responsibility of the veterinarians in charge of the local veterinary units of the OESA.

Non-technological supported swine production: The selection of the municipalities for sampling will be carried out by the DSA and the selection of the "non-Tech supported Swine Farms" will also be the responsibility of the Veterinarians in charge of the OESA Local Veterinary Units, promoting the sampling of establishments that fit into the non-tech supported swine production. The selected establishments need to have a herd of at least five (5) adult pigs. Priority must be given to establishments with evidence of local trade practices. In addition to these characteristics, the search for establishments with one or more risk factors is of great value for the early detection of the circulation of the targeted diseases. Thus, establishments that have one or more of the risk factors described below should also be prioritized:

- Adjacent to or in close proximity to municipal solid waste landfills;
- Feeding (washing) waste to the pigs;
- Adjacent to or in close proximity to international points of entry for people or products, such as airports, ports, railroads, highways, and international border crossings and borders with non-free zones;
- Location on international borders or borders with states, countries, or areas that are not free of PSC, PSA, and PRRS;

- Belong to owners who keep pigs in different establishments, especially in other countries or area not free of PSC, PSA and PRRS;

- Belong to owners who market pigs to non-free zones;
- Proximity to nature reserves, environmental protection areas, or national parks with the presence of wild swine;
- Proximity to roads with heavy swine traffic;
- Adjacent or in close proximity to slaughterhouses or rendering plants;
- Location in rural settlements, indigenous villages, peri-urban areas, poor communities, or any other situation where biosecurity is compromised and the livestock system requires special veterinary attention by the SVO;
- Adjacent or close proximity to laboratories authorized to handle infectious material for PSC, PSA, and PRRS;
- Adjacent or in close proximity to bus stops originating in non-free zones.

Other factors can be identified as risk factors for PSC, PSA, and PRRS, depending on the characterization of the area or municipality, and be included in the surveillance component.

If there is more than one selected establishment in the municipality, there must be a **minimum distance of**

5 Km (calculated as a straight line) between the sampled establishments.

3. Swine sampling

Only adult pigs (over eight (8) months of age or already in the reproductive phase) should be considered for sampling in each selected breeding establishment, since they are more likely to have antibodies against the diseases targeted by the Integrated Surveillance Plan, as they have been exposed to the risk of infection for a longer time, if there is viral activity in the breeding establishment.

Selected pigs, which do not have individual identification, may be given long-lasting individual numerical identification, at the OESA's discretion, which should be recorded on the **Domestic Swine Sample Collection Form (Form 1)** and will facilitate further investigation, should it occur.

Owners should be advised not to move or consume the sampled animals and to report immediately to OESA in cases of disease, deaths, or disappearances of the pigs.

The veterinarian responsible for collecting the samples must perform the general inspection and clinical examination of the sampled pigs, and report the results on the respective **Domestic Swine Sample Collection Form (Form 1)**.

Sampling of the pigs will be performed according to the table below, based on an intra herd prevalence of pigs with disease-specific antibodies equal to 15%, aiming for a herd sensitivity greater than or equal to 95%.

Table 2. Number of pigs to be sampled per establishment according to the number of existing adult pigs.

Number of adult pigs	Number of samples to be collected*
5 - 14	All
15 - 25	15
26 - 30	16
31 - 50	18
51 - 70	19
≥ 71	20

*Prevalence of animals with disease-specific antibodies equal to 15% and herd sensitivity $\geq 95\%$.

Note: Possible loss of samples in transit from the office to the laboratory of destination does not imply replacement of the establishment.

4. Sample Collection and Conditioning

The blood sample collection must be done in a way to avoid contamination, which can make the execution and interpretation of the laboratory tests unfeasible. For this reason, sterile, disposable material should be used.

After proper restraint of the pig, blood can be collected by puncturing the jugular vein or the cranial vena cava, using a set (needle, syringe, and tube previously identified) for each pig.

The collected volume should be at least 7.0 ml and should fill 60% of the collection tube capacity at the most, without applying high pressure at the time of transfer to the tube in order to avoid hemolysis.

In order to minimize possible sanitary risks, it is recommended that the inspection work at the establishments and surveillance through sample collection follow **biosecurity** procedures.

The adoption of good practices in the process of collecting, preserving, and shipping samples is one of the main factors for the success of obtaining material for laboratory diagnosis.

To obtain an adequate serum sample, the tubes with blood should be kept tilted, protected from light and at room temperature, until complete coagulation and serum release (usually 2 to 3 hours) or centrifuged in proper equipment.

After complete formation of the blood clot, serum should be transferred to a properly labeled Eppendorf microtube. The minimum amount of serum to be sent to the laboratory must be **1.5 ml**, respecting the maximum filling of 2/3 (two thirds) of the microtube, since in freezing there is an increase in volume with the risk of content overflow.

The tubes containing the samples should be **frozen** upright and preferably kept at -20°C (minus twenty degrees Celsius), avoiding thawing. Never freeze serum samples with the presence of clots, as hemolysis and impairment of laboratory tests will occur.

If no means are available for freezing the samples, they should be kept under refrigeration and arrive at the laboratory within two days at the latest.

Serum samples should be in proper condition, i.e., clear, frozen/refrigerated, legibly labeled, and well conditioned.

5. Sample Identification

The serum samples must be identified with the **MAPA Code** for the selected establishment, as stated in the instructions for filling out **Form 1**.

The identification must be on the **Domestic Swine Sample Collection Form (Form 1)** and on the microtube.

6. Form Completion

The **Domestic Swine Sample Collection Form (Form 1)** may be completed by hand, in legible handwriting and ballpoint pen, and signed by the MVO responsible for the collection. In this case, it must be subsequently entered into the DSA's Computer System.

Preferably, whenever possible, it should be filled out directly in the DSA's Computer System. In this case, the form must be printed and signed to accompany the samples to the laboratories that will perform the analyses.

One copy of the form should be forwarded with the samples to the Public Laboratories Accredited Labs that will perform sample analysis.

7. Packaging and shipment of samples to the Accredited Public Laboratory

Before forwarding to the Accredited Public Laboratory or to the state reception and checking center, the MVO responsible for the collection and for filling out the collection form must perform a last check to verify that all information and proper identifications correlate to the samples collected from the respective establishment.

The procedures regarding the conditioning of samples for shipment by air transport are established in ANAC's Supplementary Instruction (IS) No. 175-004 (link to access: <https://pergamum.anac.gov.br/arquivos/IS175-004D.PDF>). Blood serum samples with no suspicion of infection are considered "minimum risk animal specimens", whose guidelines for packaging and shipping documentation are in chapter 8 of the said standard.

8. Study management system

A computer system to be made available by the DSA will be used to manage the information about the establishments and samples coming from "Tech supported Swine Farming" or "Local Trade Non-Tech supported Swine Farming".

Entering the data from the sample collection forms into the computer system will be the responsibility of the OESA.

9. Laboratories

Samples from the "Techs supported Swine Farm" or "Local Trade Non-Tech supported Swine Farm" establishments will be analyzed by the Accredited Public Laboratories that will perform the screening serological tests:

- Centro de Diagnóstico de Sanidade Animal – CEDISA, Santa Catarina;
- Centro de Diagnóstico Marcos Enrietti– CDME, Paraná;
- Instituto Biológico– IB, São Paulo;
- Instituto Mineiro de Agropecuária– IMA, Minas Gerais;
- Instituto de Pesquisas Veterinárias Desidério Finamor - IPVDF, Rio Grande do Sul;
- Laboratório de Análise e Diagnóstico Veterinário – LABVET, Goiás and
- Laboratório de Diagnóstico de Doenças Animais - LADDAN, Mato Grosso do Sul.

10. Diagnostic Tests

For the Integrated Surveillance Plan for PSC, PSA and PRRS, the ELISA test will be used as a screening test.

Thus, when identifying a positive or inconclusive sample in the ELISA, the laboratory should send this sample to the reference LFDA for confirmatory testing.

11. Laboratory Results

All laboratory results must be entered into a computer system to be made available by the DSA as soon as they are obtained. The positive and inconclusive results, in addition to being entered into the system to be made available by the DSA, must be immediately sent to the OESA Head offices and the animal health service of the Federal Superintendence of Agriculture and Livestock (SFA).

12. Complementary epidemiological investigation

The detection of at least one positive or inconclusive sample to the screening laboratory test (ELISA) triggers a complementary epidemiological investigation, which must still be entered into the computer system to be made available by the DSA.

In this case, the procedures described in Annex IV of this manual "PROCEDURES FOR COMPLEMENTARY SURVEILLANCE" must be adopted.

If further investigation has taken place, all corresponding documents should be archived at the OESA Head offices and the UVLs that carried out the investigation.

13. Payment of shipping and analysis costs for samples

Payments for the costs of sample shipments by the SVO and laboratory testing for active surveillance under the Integrated Surveillance Plan for PSC, PSA, and PRRS may be agreed upon, preferably with the private sector.

ANNEX II - CLINICAL SURVEILLANCE IN BREEDING ESTABLISHMENTS

In this component, surveillance is active and is structured according to different risk categories, considering the factors for the introduction, maintenance and spread of the PSC, PSA and PRRS viruses. By taking into account the risk factors for a specific disease, the probability of detecting an infected animal is increased without necessarily increasing the number of animals tested compared to a non-risk-based surveillance system, i.e., this technique results in increased sensitivity of the system as well as increased efficiency and effectiveness.

For this reason, surveillance carried out in breeding establishments and higher-risk areas is of great importance, as it can detect probable cases of notifiable swine diseases. This surveillance also provides for the collection and registration of information on the pig herds and the interaction of the SVO with those responsible for the handling of animals for the development of continuous education actions in animal health.

1. Establishment identification

Each breeding establishment to be inspected will have a unique identification, which will be the **MAPA Code**. This code will be generated according to the number of breeding establishments and will be made available by the DSA.

ATTENTION: MAPA CODES WILL BE GENERATED BY THE DSA. DO NOT ASSIGN A NUMBER TO IDENTIFIED BREEDING ESTABLISHMENTS WITHOUT PRIOR CONSULTATION.

2. Selection of the breeding establishments to be inspected

Swine breeding establishments for the purposes of clinical surveillance follow the classification and characterization in Table 1 of Annex I of this Plan.

In the case of technological supported facilities, the SVO must call the veterinarian in charge, the technician or the hygienist of the facility before the inspection, so that he/she can accompany the activity and clarify the zootechnical and hygienic indexes. If the veterinarian is not available, the inspections must follow the schedule proposed by the SVO.

For non-tech supported pig farming, we should prioritize establishments that have evidence of local trade practices

evidence of local trade practices.

Priority should be given to those establishments that have one or more of the described risk factors.

Risk factors for selection of the properties to be inspected:

- Adjacent to or in close proximity to municipal solid waste landfills;
- Feeding (washing) waste to the pigs;
- Adjacent to or in close proximity to international points of entry for people or products, such as airports, ports, railroads, highways, and international border crossings and borders with non-free zones;
- Location on international borders or borders with states, countries, or areas that are not free of PSC, PSA, and PRRS;

- Belong to owners who keep pigs in different establishments, especially in other countries or area not free of PSC, PSA and PRRS;
- Belong to owners who market pigs to non-free zones;
- Proximity to nature reserves, environmental protection areas, or national parks with the presence of wild swine;
- Proximity to roads with heavy swine traffic;
- Adjacent or in close proximity to slaughterhouses or rendering plants;
- Location in rural settlements, indigenous villages, peri-urban areas, poor communities, or any other situation where biosecurity is compromised and the livestock system requires special veterinary attention by the SVO;
- Adjacent or close proximity to laboratories authorized to handle infectious material for PSC, PSA, and PRRS;
- Adjacent or in close proximity to bus stops originating in non-free zones.

3. Swine inspection

Clinical surveillance in breeding establishments should preferably be carried out by an official veterinarian. However, there is the possibility of it being carried out by an inspection agent (a mid-level agricultural technician). Therefore, on the inspection form for breeding establishments (form 4), unlike the other forms, there is a field "inspection agent's signature". It is important to emphasize that this agent should be well oriented about the procedures of clinical surveillance and how to fill out the form and, if they notice any clinical manifestation, they should immediately inform the official veterinarian.

The number of pigs that must be inspected, either clinically or visually, will be determined by the inspection agent, at the time of the visit, considering the biosecurity level of the establishment, the existing risk factors, herd composition, clinical manifestations found, and other aspects deemed relevant.

4. Form Completion

For this component, the **Breeding Establishment Inspection Form (Form 4)** must be applied, seeking to characterize the risk attributed to the breeding establishment, production and sanitary aspects of the breeding, information about the transit of swine, clinical manifestations found and reported. If the result of surveillance is a probable case of hemorrhagic syndrome or PRRS, the procedures described in the respective datasheets must be followed.

Preferably, whenever possible, the form should be filled out directly in the DSA's Computer System, at the time of surveillance. If that is not possible, you can fill it out by hand, in legible handwriting and ball-point pen, for later entry into the DSA's system.

ANNEX III - PROCEDURES FOR ACTIVE SEROLOGICAL SURVEILLANCE IN WILD SWINE

1. Population Handling agents

Serological surveillance in the wild swine population will be carried out in partnership between the authorized Population Handling Agents and the SVO.

The OESAs must register and provide training to personnel handling the population for the collection of serologic samples and on notifiable diseases.

2. Documentation and sample collection kit

The Population Handling Agents must be duly registered and trained by the SVO and present a copy of the valid Certificate of Good Standing (CR) to pick up the sample collection kit.

The list of the minimum kit for sample collection follows below and can be adapted to the reality of each Brazilian State:

- Swine Sample Collection Form (Form 2);
- latex gloves (PPE);
- Falcon tube with screw cap, capacity 15 ml;
- Falcon tube with screw cap, capacity 50ml;
- 3 ml Pasteur pipette;
- plastic bag with ziploc-type closure, 23 x 17 cm;
- 10-liter isothermal box;
- explanatory leaflet with the instructions for material collection.

3. Sample collection, reception and conditioning

Correct guidance on the procedures for collecting and conditioning samples from wild pigs is critical for sample quality and accurate diagnosis. Contamination of samples can make the laboratory tests unfeasible to perform and interpret.

The Population Handling Agent has up to 48 hours after collection to deliver to UVL refrigerated whole blood samples, which must be centrifuged at UVL and the serum frozen, and up to 7 days for frozen blood serum samples. The blood serum samples should remain frozen at UVL until they are sent to the Accredited Public Laboratory or to the OESA state center for reception and checking.

Samples must be received and shipped by UVL regardless of the quality they were delivered by the Population Handling Agent. When observing poor quality samples, the MVO should guide the Population Handling Agent on good collection practices to obtain a quality sample.

Tubes containing the samples should be **frozen** upright and preferably kept at -20°C (minus twenty degrees Celsius), avoiding thawing. Never freeze serum samples with the presence of clots, as hemolysis and impairment of laboratory tests will occur.

Serum samples should be in proper condition, i.e., clear, frozen/refrigerated, legibly labeled, and well-conditioned.

4. Sample identification and form completion

The Population Handling Agent must deliver the samples, at the UVL where he/she picked up the kit or at another previously determined location, at the OESA's discretion, accompanied by the completed and signed **Wild Swine Sample Collection Form (Form 2)**.

UVL should check the identification of all samples along with the collection form(s) and the information should be entered into the DSA System.

Send a copy of the Population Handling Agent's collection form along with the samples and file the original at UVL.

5. Packaging and shipment of samples to the Accredited Public Laboratory

Before forwarding to the Accredited Public Laboratory or to the state reception and checking center, the MVO responsible for the collection and for filling out the collection form must perform a last check to verify that all information and proper identifications correlate to the samples collected from the respective establishment.

In order to be forwarded to one of the Accredited Public Laboratories, the samples must be frozen, legibly identified, accompanied by the respective collection forms, and well-conditioned.

The procedures regarding the conditioning of samples for shipment by air transport are established in ANAC's Supplementary Instruction (IS) No. 175-004 (link to access: <https://pergamum.anac.gov.br/arquivos/IS175-004D.PDF>). Blood serum samples with no suspicion of infection are considered "minimum risk animal specimens", whose guidelines for packaging and shipping documentation are in chapter 8 of the said standard.

6. Study management system

The DSA will provide the OESAs with a computer system for recording activities.

7. Laboratories

The samples from wild swine will be analyzed by the Accredited Public Laboratories that will perform the screening serological tests:

- Centro de Diagnóstico de Sanidade Animal – CEDISA, Santa Catarina;

- Centro de Diagnóstico Marcos Enrietti– CDME, Paraná;
- Instituto Biológico– IB, São Paulo;
- Instituto Mineiro de Agropecuária– IMA, Minas Gerais;
- Instituto de Pesquisas Veterinárias Desidério– IPVDF, Rio Grande do Sul;
- Laboratório de Análise e Diagnóstico Veterinário – LABVET, Goiás; and
- Laboratório de Diagnóstico de Doenças Animais – LADDAN, Mato Grosso do Sul.

8. Diagnostic Tests

For the Integrated Surveillance Plan for PSC, PSA and PRRS, the ELISA test will be used as a screening test.

Thus, when identifying a positive or inconclusive sample in the ELISA, the accredited public laboratory should send this sample to the reference LFDA for confirmatory testing.

9. Laboratory Results

All laboratory results must be forwarded to the OESA Head Office and the Animal Health Service of the Federal Superintendence of Agriculture, Livestock, and Supply (SFA).

At the OESA Head Office, an organized and auditable file on all samples collected and their laboratory results must be kept. A scanned copy of the result should be entered into the DSA Computer System.

10. Complementary epidemiological investigation

The detection of at least one wild swine that is positive or inconclusive to the screening laboratory test (ELISA) triggers a complementary epidemiological investigation, which does not require registration in e-SISBRAVET.

In this case, the procedures described in **Annex IV** of this manual “PROCEDURES FOR COMPLEMENTARY SURVEILLANCE” must be adopted.

If further investigation has taken place, all corresponding documents should be archived at the OESA Head offices and the UVLs that carried out the investigation.

11. Payment of shipping and analysis costs for samples

Payment for shipping costs and sample analysis for the Integrated Surveillance Plan for PSC, PSA, and PRRS should preferably be agreed upon with the private sector.

ANNEX IV – PROCEDURES FOR COMPLEMENTARY SURVEILLANCE

This text aims to standardize the epidemiological investigation procedures to be adopted when positive or inconclusive results are detected in serological surveillance performed by the Integrated Surveillance Plan for PSC, PSA and PRRS.

In serological surveillance, positive/inconclusive results can be due to the following factors:

- Natural infection with PSC, PSA and PRRS viruses;
- Vaccination against PSC, PSA (if available and authorized by DSA) and PRRS;
- Maternal antibodies arising from immunized females;
- Cross-reaction with other Pestiviruses (Bovine Viral Diarrhea – BVD and “Border Disease” of sheep – BD), in the case of PSC;
- Non-specific responses to the test used.

Thus, we must have an effective procedure in place to indicate the presence of infection by the PSC, PSA and PRRS viruses by means of laboratory tests (confirmatory and differential) and further investigations at the establishment of origin of the samples.

The further epidemiological investigation procedure described below must be initiated after a positive or inconclusive ELISA screening test result and will be carried out by OESA, with follow-up by the SFA animal health service. Their goal is to obtain a conclusive final diagnosis on the presence of the PSC, PSA, and PRRS viruses in the pig populations of the states that comprise the PSC ZL.

1. COMPLEMENTARY INVESTIGATION FOR PSC IN DOMESTIC SWINE

A. Positive and inconclusive laboratory result on ELISA test.

Samples with positive or inconclusive laboratory results for PSC in the ELISA test should be forwarded to the LFDA reference network, by the accredited public laboratories, and will be tested in the viral neutralization (VN) test for PSC and BVD.

When the ELISA result is positive or inconclusive, OESA must conduct a detailed investigation at the establishment of sample origin.

In case of the presence of pigs with clinical signs compatible with hemorrhagic syndrome, the establishment must be quarantined and the procedures for probable cases described in the PSC data sheet (with records in e-Sisbravet) must be applied.

If there are no pigs showing clinical signs compatible with hemorrhagic syndrome, the establishment must remain under surveillance, with weekly inspections duly recorded on the **Monitoring inspection form (form 3)** available in the DSA computer system, until the outcome of the VN.

The owner should be instructed not to move or consume the sampled animals and that upon observing any changes in animal behavior or high mortality in their herd, they should notify OESA immediately.

At this time, there is no need to ban the establishment, since the animals do not show clinical signs compatible with hemorrhagic syndrome and false-positive results to the ELISA screening test are expected.

If swine remain without clinical signs compatible with hemorrhagic syndrome, the investigation will be closed when the result of the VN for PSC is negative.

B. Positive laboratory result in the viral neutralization test - VN.

If the outcome of the VN for PSC is positive, the current investigation will continue. All information from the investigation must still be recorded in the DSA computer system using **Form 3**.

Again, a clinical examination of the swine herd should be performed, as well as a new epidemiological investigation, always looking for signs of the occurrence of porcine hemorrhagic syndrome.

If clinical signs compatible with PSC or PSA are found, the procedures for probable cases described in the datasheets (with records in e-Sisbravet) must be adopted.

If no pigs with clinical signs or injuries suggestive of PSC are observed, **whole blood samples (with EDTA)** must be collected from the pigs in the establishment in order to perform the PCR test, with individual identification of the swine and completion of the **Complementary Sample Collection Form (Form 5)** in the DSA computer system.

For the sampling we considered the following age groups:

- Adults: breeding stock (males and females) and castrated males and females over eight months old.
- Piglets: pigs that have not yet entered reproductive age (under eight months old).

To calculate the sample, the total number of pigs in the establishment must be considered, and the number of samples will be calculated according to Table 1.

For the choice of pigs to be sampled, prioritize the age range of piglets. If there are not enough pigs in the piglet age group, supplement the sampling with pigs in the older (adult) age group.

To determine the number of pigs to be sampled at each establishment, the following parameters were considered: 95% sensitivity of the diagnostic system (PCR), 95% confidence, and a minimum expected prevalence of 5% of infected pigs at each establishment.

Table 1. Number of pigs to be sampled per establishment

Number of pigs	Number of samples to be collected*
5 - 10	All
11 - 20	11
21 - 30	15
31 - 40	19
41 - 50	21
51 - 60	25
61 - 70	27
71 - 100	33
101 - 200	44
201 - 400	50
401 - 600	55
>600	56

**Prevalence of animals with disease-specific antibodies equal to 5% and herd sensitivity $\geq 95\%$.*

REFERENCES Dohho I, Martin W, Stryhn H (2003). Veterinary Epidemiologic Research. AVC Inc, Charlottetown, Prince Edward Island, Canada, pp. 47 and pp 102-103.

The proposed calculations were performed with the Epi Tools - Epidemiology Toolbox App., version 6.0, using the finite population correction factor option.

All pigs sampled must be individually identified. Those that do not have earrings should have them put by OESA at the time of collection. The identification on the blood vial must be traceable to the animal in your sample.

The samples must be sent to LFDA/MG, upon prior contact, accompanied by a copy of the

Complementary Sample Collection Form (Form 5).

Establishments that have all results negative and the pigs remain without clinical signs of disease will have the complementary investigation closed. These establishments should be included in the list of establishments under active surveillance of the respective UVL, i.e., higher risk establishments.

C. Toxic or inconclusive laboratory result on viral neutralization test

When the result of the VN is toxic or inconclusive, we may have two situations:

- when the sampled animal has identification: a new serum sample must be collected from this animal by filling out the **Sample Collection Form for Domestic Swine Samples (Form 1)**. The sample must be sent to LFDA-MG for repetition of the VN test. If the toxic or inconclusive result remains, collect whole blood samples (with EDTA) from existing pigs according to the sampling described in Table 1 of item "B. Positive laboratory result in viral neutralization test – VN" for PCR with completion of the **Complementary Sample Collection Form (Form 5)**. Surveillance records should be made on the **Monitoring Inspection Form (Form 3)**.
- when the sampled animal does not have identification: whole blood samples (with EDTA) must be collected from existing pigs for PCR, following the described sampling

in Table 1 of the item “B. Positive laboratory result in the viral neutralization test – VN”, and with **individual identification** of the sampled pigs. Surveillance records should be made on the **Monitoring Inspection Form (Form 3)** and whole blood samples should be identified on the **Complementary Sample Collection Form (Form 5)**.

D. Positive PCR laboratory result

In cases of positive PCR results, the positive pig(s) must be euthanized for organ sampling (20 to 50 grams of: tonsils, spleen, lung, lymph nodes, and distal ileum) for identification of the PSC virus. The collected material and the **Complementary Sample Collection Form (Form 5)** must be sent to LFDA-MG, after previous contact. Surveillance records should be made on the **Monitoring Inspection Form (Form 3)**.

The final and conclusive diagnosis of the investigation will take into consideration all laboratory results and clinical-epidemiological investigations carried out in the establishment involved.

E. Procedures in case of absence of pigs at the suspected establishment

If, for any reason, there are no more pigs in the establishment where ELISA or VN positive results occurred, it will be necessary to collect serum from pigs in neighboring establishments or those linked epidemiologically for serological testing.

This investigation will include the collection of serum from the pigs in these establishments, by sampling, as shown in **Table 2 of Annex I** of the Integrated Surveillance Plan.

All information regarding these establishments should be recorded on the **Monitoring Inspection Form (Form 3)** and the **Domestic Swine Sampling Form (Form 1)**, noting in the remarks field of Form 1 that this is an investigation of the original establishment where there were no longer any pigs.

F. Documentation and data records in MAPA’s computer systems.

All the actions carried out in the complementary investigation must be recorded on forms available in the DSA’s computer system. Records and their organization must be carefully maintained to provide evidence of actions taken for future audits and international missions. It is also important to keep a photographic record of the clinical examinations and necropsies performed.

The complementary investigation from active surveillance does not require registration in e-SISBRAVET.

2. COMPLEMENTARY INVESTIGATION FOR PRRS IN DOMESTIC SWINE

When the screening-test (ELISA) result is positive or inconclusive), OESA must conduct a detailed investigation at the establishment of sample origin. The herd must be inspected for clinical signs and zootechnical and sanitary indicators consistent with the presence of PRRS. Information about possible epidemiological links should also be collected, aiming at an expansion of the investigation, if necessary.

The diagnostic tests for complementary investigations will be carried out at LFDA/MG, and the samples sent with the **Complementary Sample Collection Form (Form 5)**.

Sample collection

After a positive or inconclusive result for PRRS in the screening test (ELISA), collection of material for confirmatory laboratory diagnosis is mandatory. In this first moment, the quarantine of the investigated establishments will depend on the sanitary condition found by OESA in the investigation of clinical signs and zootechnical and sanitary indicators consistent with the presence of PRRS.

Criteria for making a decision on banning the investigated establishments:

- In the presence of clinical signs consistent with PRRS accompanied by a POSITIVE laboratory test: Interdiction/ban of the establishment and collection of material for diagnosis according to the "probable case" of the PRRS Data Sheet (registered in e-Sisbravet);
- In the absence of clinical signs consistent with PRRS accompanied by a POSITIVE laboratory test: Follow-up and investigation, with completion of the **Monitoring Inspection Form (Form 3)**, without interdiction/ban of the establishment, and with the collection of whole blood (with EDTA) or oral fluid, as described below. The choice of the type of material to be collected will be at the OESA's discretion, based on the clinical-epidemiological evaluation, sanitary conditions, biosecurity, and facilities of the technological supported swine farm. For non-technological supported swine establishments, the samples to be collected are mandatorily whole blood samples.

A. Whole blood with EDTA:

Whole blood collection (with EDTA) should be performed considering the total number of pigs in the establishment (as per Table 2) and taking into account that if an establishment is infected with PRRS virus, the intra herd prevalence would be at least 5% of infected pigs. The sensitivity of the diagnostic system (PCR) was considered to be 95%. Thus, the samples to be collected at each establishment should be as shown in Table 2. Sampling per establishment defined in the table would be sufficient to detect at least one positive pig, if infection exists in the establishment at the prevalence considered and with a 95% confidence level.

The choice of whole blood collection eliminates the need for oral fluid sampling. Must be filled in
o **Complementary Sample Collection Form (Form 5)**.

For the choice of pigs to be sampled, prioritize pregnant or lactating sows that have a history of reproductive failure.

Table 2. *Number of pigs to be sampled per establishment*

Number of adult pigs	Number of samples to be collected*
5 - 10	All
11 - 20	11
21 - 30	15
31 - 40	19
41 - 50	21
51 - 60	25
61 - 70	27
71 - 100	33
101 - 200	44

Number of adult pigs	Number of samples to be collected*
201 - 400	50
401 - 600	55
>600	56

**Prevalence of animals with disease-specific antibodies equal to 5% and herd sensitivity $\geq 95\%$.*

The proposed calculations were performed with the Epi Tools - Epidemiology Toolbox App., version 6.0, using the finite population correction factor option.

REFERENCES Dohho I, Martin W, Stryhn H (2003). Veterinary Epidemiologic Research. AVC Inc, Charlottetown, Prince Edward Island, Canada, pp. 47 and pp 102-103.

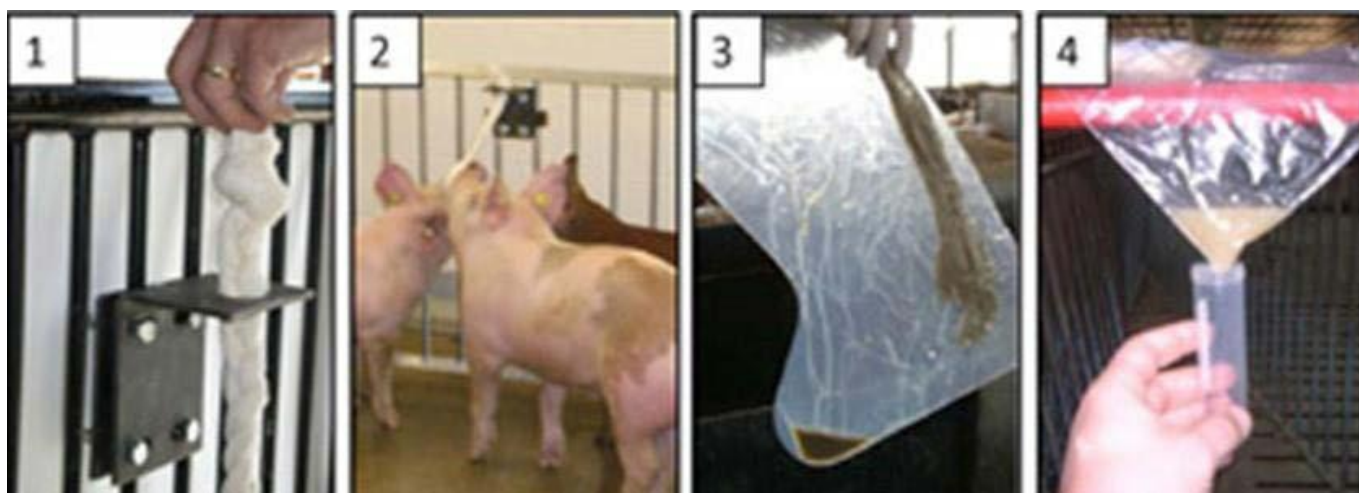
B. Oral Fluid:

Collecting oral fluid samples eliminates the need to collect whole blood samples. Must be filled in **o Complementary Sample Collection Form (Form 5)**. Oral fluid collection should only be performed in technological supported swine farms, in the weaning piglet phase or in group gestation stalls.

Oral fluid collection should be done in three pig stalls of at least 10 pigs per stall. If the number of pigs per stall is less than 10, the collection should be done in 4 stalls so that a minimum of 30 pigs per farm are exposed to oral fluid with the ropes.

Instructions for oral fluid collection are provided below:

- a) Cotton ropes (1.3 m) will be suspended in the pig stalls or tied tightly at the sides so that they do not come loose from the contact and force of the pigs;
- b) Care must be taken that the rope does not come into contact with the floor, feeders, and drinkers;
- c) Use 1 rope/10 pigs or one rope/stall, exposed for 20 - 30 minutes;
- d) Cut the end of the rope that the pigs have chewed into a properly labeled plastic bag with a Ziploc-type closure, making sure that the portion containing the oral fluid does not come in contact with anything to avoid contamination;
- e) By manual compression, oral fluids will be collected in plastic bags (Ziploc type), decanted into 5 ml plastic vials and stored at -20°C until shipment to LFDA-MG; f) The material must be sent frozen to the laboratory;
- f) Material identification: identify on **Form 5** the individuals in the sampled stalls, and when there is no individual identification of the pigs (dent, earring, or tattoo), this information should be recorded under Notes on **Form 5**.



Source: Oral Fluids | Iowa State University (iastate.edu)

C. Documentation and data records in MAPA's computer systems.

All the actions carried out in the complementary investigation for PRRS must be recorded on forms available in the DSA's computer system. Records and their organization must be carefully maintained to provide evidence of actions taken for future audits and international missions. It is also important to keep a photographic record of the clinical inspections.

The complementary investigation from active surveillance does not require registration in e-SISBRAVET.

3. COMPLEMENTARY INVESTIGATION FOR PSC IN WILD SWINE

A. Positive or inconclusive laboratory result on ELISA test.

Samples with positive or inconclusive laboratory results for PSC in the ELISA test should be forwarded to the LFDA reference network, by the accredited public laboratories, and will be tested in the viral neutralization (VN) test for PSC and BVD.

When the ELISA result is positive or inconclusive, OESA must conduct a detailed investigation at the establishment of origin of the sample, where the wild swine was slaughtered, in order to obtain a final conclusive diagnosis on the presence or absence of PSC virus in the wild swine population.

The investigation will be closed when the result of the VN for PSC is negative.

Actions in the sample's establishment of origin with domestic pig farming:

- i. clinical inspection in domestic pigs from the sample's establishment of origin with completion of the **Monitoring Inspection Form (Form 3)**;
- ii. If there are no pigs showing clinical signs compatible with hemorrhagic syndrome, the establishment must remain under surveillance, with weekly inspections duly recorded on the Monitoring inspection form (form 3);
- iii. survey of the sanitary history of livestock with a focus on investigation of diseases that may present cross-reactivity in serological tests to detect antibodies against the PSC virus;

iv. guidance to domestic pig farmers to provide measures to avoid contact between domestic and wild pigs;

v. awareness and guidance to pig farmers to notify the SVO of any changes or clinical signs consistent with PSC in domestic and wild pigs.

In wild swine

i. awareness of Population Handling Agents of wild pigs to detect and report to the SVO any animal with clinical signs and/or injuries consistent with porcine hemorrhagic syndrome or wild pigs found dead.

ii. investigate, together with the population handling agents, the destination of the carcasses of reactive wild pigs slaughtered from the same source.

iii. wait for the VN's result.

B. Positive laboratory result in the viral neutralization test (VN).

Actions in domestic swine breeding establishments:

i. carry out epidemiological investigation of possible contacts between domestic pigs and wild pigs in the establishment of origin of the sample;

ii. conduct clinical-epidemiological investigation of the pigs at the establishment of origin of the sample, observing signs of the disease and changes in the zootechnical and sanitary indices of the establishment, completing the **Monitoring Inspection Form (Form 3)**;

iii. guide the enhancement of biosecurity in technological and non-technological swine establishments.

iv. reinforce the orientation for pig farmers to notify the SVO about any changes in zootechnical or sanitary indices and the detection of clinical signs consistent with PSC in domestic and wild pigs.

In wild swine

i. meeting to seek the cooperation of Population Handling Agents for surveillance;

ii. reinforce guidance to all Population Handling Agents on biosecurity procedures and notifiable diseases.

4. COMPLEMENTARY INVESTIGATION FOR PRRS IN WILD SWINE

A. Positive or inconclusive laboratory result on ELISA test.

Samples with positive or inconclusive laboratory results for PRRS in the ELISA test should be forwarded to the LFDA reference network, by the accredited public laboratories, and will be tested in the PCR test.

When the ELISA result is positive or inconclusive, OESA must conduct a detailed investigation at the establishment of origin of the sample, where the wild swine was slaughtered, in order to obtain a final conclusive diagnosis on the presence or absence of PRRS virus in the wild swine population.

The investigation will be closed when the result of the PCR for PRRS is negative.

Actions in the sample's establishment of origin with domestic pig farming:

- i. clinical inspection in domestic pigs from the sample's establishment of origin with completion of the **Monitoring Inspection Form (Form 3)**;
- ii. If there are no pigs showing clinical signs compatible with hemorrhagic syndrome, the establishment must remain under surveillance, with weekly inspections duly recorded on the **Monitoring inspection form (form 3)**;
- iii. guidance to domestic pig farmers to provide measures to avoid contact between domestic and wild pigs;
- iv. awareness and orientation for pig farmers to notify the SVO about any changes in clinical signs consistent with PRRS in domestic and wild pigs.

In wild swine

- i. awareness of Population Handling Agents of wild pigs to detect and report to the SVO any animal with clinical signs and/or injuries consistent with PRRS or wild pigs found dead.
- ii. investigate, together with the population handling agents, the destination of the carcasses of reactive wild pigs slaughtered from the same source.
- iii. wait for the PCR's result.

B. Positive laboratory result in the PCR test

Actions in domestic swine breeding establishments:

- i. carry out epidemiological investigation of possible contacts between domestic pigs and wild pigs in the establishment of origin of the sample;
- ii. conduct clinical-epidemiological investigation of the pigs at the establishment of origin of, observing signs of the disease and changes in the zootechnical and sanitary indices of the establishment, completing the **Monitoring Inspection Form (Form 3)**;
- iii. guide the enhancement of biosecurity in technological and non-technological swine establishments.
- iv. reinforce the orientation for pig farmers to notify the SVO about any changes in zootechnical or sanitary indices and the detection of clinical signs consistent with PSC in domestic and wild pigs.

In wild swine

- i. meeting to seek the cooperation of Population Handling Agents for surveillance.
- ii. reinforce guidance to all Population Handling Agents on biosecurity procedures and notifiable diseases.



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