



**Ministry of Agriculture, Livestock and Supply
Secretariat of Animal and Plant Health
Department of Animal Health**



BRAZIL- Brazilian Foot-And-Mouth Disease Eradication Program

**Veterinary Surveillance for Vesicular Diseases
General Guidelines**

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TABLE OF CONTENTS

1. Introduction.....	1
2. Background..	2
3. Information registration and consolidation ...	5
Frequency..	7
Structure of Official Veterinary Service	8
Animal demography	11
Assistance to suspicion of vesicular disease ...	14
Animal movement	15
Inspection of rural holdings ...	16
Inspection of slaughterhouses	17
Inspection in plant/animal related event	17
General actions ...	17
4. Serological surveillance ...	19
Organization and basic structure to carry out serological surveillance ...	20
Sampling method ...	23
Diagnostic method ...	27
Procedure for information registration	30
Procedures for sample identification and collection ...	36

FIGURES

Figure 1. Graphic representation of the main components of the veterinary surveillance system, proposed by OIE.....	6
Figure 2. Graphic representation of the Brazilian Veterinary Surveillance System for vesicular diseases.....	7
Figure 3. Representation of existing control levels and flow of information and samples	21
Figure 4. Representation of the main stages related to surveys and monitoring	21
Figure 5. Concentration of disease in gathering of animals (herds or others)	24
Figure 6. Graphic representation of diagnostic method to be used	29
Figure 7. Graphic representation of distribution result of I-ELISA 3ABC tests, referring to FMD- free zone without vaccination (Santa Catarina state) and regions with FMD occurrence in Brazil.....	30

ANNEXES

Annex 1 Basic list of material, equipment and forms for assistance to notification of vesicular disease suspicion	39
Annex 2. Procedures for selection of animals to be sampled in each holding.....	40
Annex 3. Example of Table of Random Numbers	41
Annex 4. Example of previous-visit forms	42
Annex 5. Example of sample collection form - Stage 1 ...	43
Annex 6. Example of sample collection form - Stage 2 ...	44

1. Introduction

The objective of this document is to define principles and general guidelines for the veterinary surveillance of vesicular diseases providing advice to standardize official veterinary service activities and reports, thus contributing to the quality assessment of veterinary assistance for vesicular diseases in all instances of the Unified System for Animal and Plant Health (SUASA)

Its elaboration comply with the Brazilian Foot-And-Mouth Disease Eradication Program (PNEA), which has been reformulated by Normative Instruction No. 44 dated, October 2, 2007 and also guidelines that are present in the Terrestrial Animal Health Code (Terrestrial Code) of the World Animal Health Organization (OIE), especially Annexes 3.8.1 (“General Guidelines for Zoonitary Surveillance”) and 3.8.7 (“Guidelines for Foot-and-Mouth Disease Surveillance”).

According to OIE, animal health surveillance represents the sum of all resources, structures and procedures that are organized with the objective of demonstrating the absence of disease/infection or establishing its outbreak and spread in the animal population. Therefore, it represents an essential component to detect diseases, monitor historical series, establish control or eradication programs of endemic or exotic diseases, support requests for national or international certificates for zones that are free from the disease or infection, provide information for risk analysis and support sanitary measures that have been adopted in zoonitary programs. In the specific case of foot-and-mount disease, it should contribute to the development of prompt notification capabilities by the official veterinary service and immediate reaction of all instances of the Brazilian Plant and Animal Health System upon the occurrence of any emergency case of vesicular disease anywhere in Brazil.

Taking into account the above-mentioned definition, it can be stated that in Brazil there is an animal health surveillance system. Its establishment started to occur with more emphasis in the 60s and its main objective was to fight and eradicate FMD. Since then, the system has become more consolidated every year including activities such as: expansion and strengthening of structures of the official veterinary service, use of sanitary education and social communication strategies, inspection and supervision of rural establishments, control and supervision of vaccination campaigns, control and supervision of animal movement, assistance in case of suspicion of animal disease and the performance of serum-epidemiological surveys and monitoring.

The group of actions carried out until now allowed, in the case of PNEFA, the progress of the sanitary condition in most parts of the country, represented by the implementation and maintenance of FMD-free zones, with real perspectives of progress in the eradication and prevention of the disease in Brazil in the years to come.

However due to the current FMD epidemiological condition, there is the need to adopt more controlled and standardized procedures so that prevention and eradication efforts against the disease can progress on a faster and more consistent fashion.

This document became part of other documents prepared by the Department of Animal Health – DSA/SDA/MAPA and the Pan-American Center of Food-and-Mouth Disease (PANAFTOSA) in the last couple of years with the

objective of standardizing and improving quality of animal health information systems. In relation to the veterinary surveillance system, the following documents can serve as complementary reading:

- Procedural Manual on Suspicion of Vesicular Disease, from PANAFTOSA;
- Procedural Manual on Auditing and Assessment of FMD Programs, from PANAFTOSA;
- Operation Manual from the Continental System of Epidemiological Surveillance –Siv-Cont, prepared by PANAFTOSA as well as forms and instructions prepared by the Epidemiological Division – DEP/DSA/SDA/MAPA;
- Guidance to inspect the trade of FMD vaccines as well as control and assessment of vaccination stages created by the Coordination Agency for FMD- CFA/DSA/SDA/MAPA, and
- Standards Manual: organization of information on the structure of plant and animal health state services, issuance and control of Animal Movement Permits (GTAs) and maintenance of registration of rural establishments, farming systems and farmers, created by the Coordination of Animal Movement and Quarantine-CTQA/DSA/SDA/MAPA.

This document contains three main parts: definitions and general guidelines for veterinary surveillance, organization and standardization of information considered important to surveillance system and operationalization of serum-epidemiological surveys and monitoring. Its content should be regularly reviewed and updated and be part of training activities for professionals, who work at the Brazilian Veterinary Service.

2. Background

The objective of this item is to, especially, introduce and discuss terms that are traditionally used by professionals from the Official Veterinary Service in their routine of veterinary surveillance activities. There are different classifications for epidemiological surveillance with definitions that are admittedly obscure. The current document does not have the intention of solving these contradictions; on the contrary, its goal is to make them more evident so that everyone is aware of the limits of their use. In this fashion, despite existing conceptual problems it is expected that, at the end, veterinarians from the official service, especially those who work with local veterinary units, become aware of the importance of their work and the need to properly register, organize and evaluate information generated in their every-day routine.

According to OIE, epidemiological surveillance means the continuous investigation of a given population to detect the occurrence of a disease/infection, with the objective of prevention, including the test (clinical or laboratorial) of part of that given population.

In South-American veterinary services, it was common to see the use of the expression “ system of animal health surveillance and assistance”, assuming that there are two different systems: one of assistance and another of surveillance. In the case of Brazil, this terminology is especially present in technical and normative documents issued until the 90s, pointing out the state’s classification system according to FMD risk levels. These two systems

are actually complementary and, many times, are taken for one another, because one can only bear the existence of a good surveillance or epidemiological system when there is a structured system of veterinary assistance.

In a nutshell, it can be said that the veterinary assistance system is the backbone of epidemiological surveillance activities. Thinking along these lines, the term “assistance” covers information related to physical resources (offices, means of communication, consumption material, laboratories etc...), human resources (quantity, quality and distribution of personnel) and those associated to political and legal fields (lines of command, political will, and legal tools), whereas the term “sanitary surveillance” covers information related to operations such as, number of visits to rural establishments, number of inspected animals, results of serum-epidemiological studies, amongst others.

In this text, the term “veterinary surveillance” will be used to mean epidemiological surveillance activities in the field of animal health, including aspects related to structure or assistance.

The sanitary surveillance structure covers each instance of the plant and animal health system in both federal and state levels and the coordination of information related to animal health, labs, procedures standardization, human, material and financial resources. The surveillance system is also responsible for the establishment of a suitable legal and administrative framework, the application of all the required resources to improve controls efficiency performance, including personnel, equipment, vehicles and financial resources. It is also responsible for staff training and the development of mechanisms to involve the services of other public and private sectors, and farmers in the veterinary surveillance system.

Based on what was described and disregarding the terminology used, there is somehow a technical consensus in considering that the system of veterinary surveillance is the set of elements and activities used with the objectives of: (1) preventing the entry of infection and contamination sources, (2) detecting these sources when they have been established in its territory, (3) promptly notifying its location to veterinary authorities and, at last, (4) coordinating an immediate reaction for total eradication of the threat to herd or for outbreak control and re-establishment of a free zone for zones or regions affected, according to the specie and farming system involved.

Some authors classify surveillance actions as primary, secondary and tertiary and others use first, second and third barriers.

Even though it is instructive, this classification poses some problems and limits, since one single action, depending on its objective, can fit in different categories. For example, FMD vaccines can be classified as tertiary when employed to stop an outbreak or as a primary type when employed with the same objective, but in neighboring country or in an infected zone inside a free-zone country. Still in relation to the vaccine, its employment during mass campaigns can fit three categories, since it is not intended to prevent the entry of infection sources, but to cut the transmission cycle and reduce the spreading speed of the disease when the primary barrier has been violated. Another example is animal movement control, which can be used as a tertiary barrier to meet the needs of a sanitary emergency, or as a primary barrier in border areas with zones of mixed sanitary conditions.

Another commonly used classification is the definition of actions taken as passive or active measures. The term “passive surveillance” has, generally, been used for routine activities involving assistance to suspect disease outbreaks or description of a given animal population of one region. However, it can also represent any use of information that has been obtained without the specific objective of surveillance, that is, situations where there is no program or planning for specific actions in order to prove the occurrence or lack of a determined sanitary event.

According to FAO, active surveillance involves intensive efforts to detect the presence or to prove the absence of disease or infection. This term is usually used for data collection that is produced especially for epidemiological survey of herds including the deliberate and detailed search for evidence of the disease in the animal population, with the objective of confirming its presence or absence. Clear examples of this surveillance are monitoring and serum-epidemiological surveys.

According to OIE, components of a successful active surveillance program should take into account, at least:

- a) Integration between field activities and lab services;
- b) Regular visits to establishments to interview farmers and rural workers, recall technical information, perform clinical tests and necropsies and collect samples for lab tests including blood serum. The visit should always be justified by the active surveillance plan or any other epidemiological estimate which has identified critical areas or higher-risk establishments;
- c) The use of production indicators, socio-economic and cultural studies, which have some relation to the livestock farming system related to species susceptible to FMD; and
- d) Serological surveillance to evaluate the presence of FMD viral circulation.

According to strategies, which have been employed, FMD sanitary surveillance can also be grouped into the following categories: clinical surveillance, serological surveillance and virological surveillance.

Clinical surveillance, as the name implies, has the objective of detecting clinical signs of vesicular disease by inspecting susceptible animals. Serological surveillance, on the other hand, has the objective of detecting specific antibodies against FMD virus. These two categories should actually be complementary. Additional lab tests should be used to differentiate vesicular diseases, which have been clinically detected, and likewise, clinical surveillance should be used to complement serological surveillance.

In the case of serological surveillance, OIE considers that one positive reaction in detection tests for antibodies against FMD virus can have four causes: infection, vaccination, presence of maternal antibodies transmitted by immune parent, which can persist, on average, until 6 months of age or crossed reactions (heterophylia). This requires that the analysis of lab results is carried out as complement to the clinical, epidemiological plant-livestock context of the investigated population and, within technical possibilities and requirements, confirmed by means of a virological surveillance.

The former is used, according to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, to isolate and identify FMD virus in risk populations, to confirm clinical cases of vesicular disease or cases of seropositive animals. It should be considered, however, that because virological tests are highly specific they are enough to confirm a FMD outbreak. On the other hand, due to their low sensitivity, they are not enough to discard the occurrence of FMD for confirmed cases of vesicular diseases.

Clinical surveillance is the least specific, whereas virological surveillance is the least sensitive. Serological surveillance, which has an intermediate performance due to the fact that it has more specificities than clinical surveillance and more sensitivity than virological surveillance, becomes a very functional surveillance tool. Zones where mass vaccination has been used for many consecutive years should combine all three surveillance forms to minimize the hyper-immunization effects in serological surveillance.

Regardless of the type employed, epidemiological surveillance applied to animal health or veterinary surveillance comprises the following activities: (1) collection and registration of relevant epidemiological information, (2) consolidation and analysis of collected data, (3) decision and establishment of preventive procedures, (4) performance of emergency operations and (5) notification and dissemination of bulletins with information on diseases and the results of measures applied, through all means available to reach most of those involved in the surveillance system (back-feeding). These activities should be subject to constant evaluation in order to assess service quality in veterinary surveillance.

Among the characteristics of the veterinary surveillance system, sensitivity, specificity and opportunity parameters can be highlighted, according to PANAFTOSA. They are understood as::

- Sensitivity: the ability to detect the suspicion of diseases, with clinical signs or evidence that are common to a group of diseases, in the case of FMD, vesicular diseases. Assistance provided by the official veterinary services to the community by means of notification, is one of the main indicators to evaluate classification parameters. The lack or reduction in the number of notifications for a long period of time poses doubts regarding the quality, reliability and sensitivity of the surveillance system;
- Specificity: the system's ability to give a definite diagnosis. It is very important for FMD veterinary surveillance, taking into account objectives established by PNEFA for zones that do not have virus circulation, to keep strict monitoring of occurrences of similar cases by means of a follow-up for all cases of vesicular diseases; and
- Opportunity: defined as the ability to present data and information in time to ensure the promptness in the application of sanitary actions as a response to the epidemiological situation identified.

Next item addresses activities related to the collection and registration of basic information for the surveillance system of vesicular diseases,. This is the basic information, which should be collected, registered, consolidated and communicated periodically by the official veterinary service, according to management rules of PNEFA.

3. Information registration and consolidation

As highlighted, information is fundamental for the veterinary surveillance system. It can be stated that the quality of this system is directly related to the quality of information generated. Therefore, the collection, organization and analysis of information represent the system's basic procedure and should be part of the daily duties and concerns of official veterinary services in all instances of the national system of plant and animal health.

Veterinary surveillance information ensures the quality of bulletins on the sanitary conditions of herds and should comply with the minimum accuracy requirements demanded for national decision-making and also for the maintenance and expansion of international trade.

Reinforcing some points discussed previously, the five main components of information system in veterinary surveillance are highlighted below:

- a) Information collection: appropriate data source for the information system in animal health includes the monitoring system for diseases of mandatory notification: labs, slaughterhouses and industries of animal origin products; records of operation control and inspection for animal movement and animal origin products: records control for livestock farming systems; and disease control program reports as well as records monitoring;
- b) Information consolidation: includes all administrative procedures that are required to bring collected data from establishments to animal health authorities. Primary information should be organized in pre-established spreadsheets and carefully examined for inconsistencies and non-compliances.
- c) Information filing and recovery: is one of the main components of the system, since it should establish standards so that information collected by different sources is consolidated in a database, providing different levels of information clusters, comparison and required analysis. It also represents the archive for primary files, an important part of the system providing guarantees and evidences of all registered actions during the stage of data consolidation performed by professionals of the official veterinary service. It is the component which proves, under the format of document, the accuracy, and quality of the information, that is, under appropriate conditions;
- d) Information analysis: includes different levels of complexity, starting from direct observations and comparisons performed at system's database, until more sophisticated procedures including electronic and mathematical processing. The analysis result depends on the level of details and standards of information collected
- e) Communication of conclusions: includes the preparation of specific reports, as for example, results of vaccine campaigns, result of activities of animal movement control, results of monitoring and serum-epidemiological surveys performed, among others.

These five components are connected by a series of administrative procedures and the graph,, including the geographic information system, proposed by OIE, which can be evaluated in Figure 1. In Figure 2, we present the simplified graph related to the model of information and activities of the veterinary surveillance system for vesicular diseases in operation and under the coordination of CFA/DSA/SDA. The objective of this item is to show details of this model, especially, aiming at defining standards of related information.

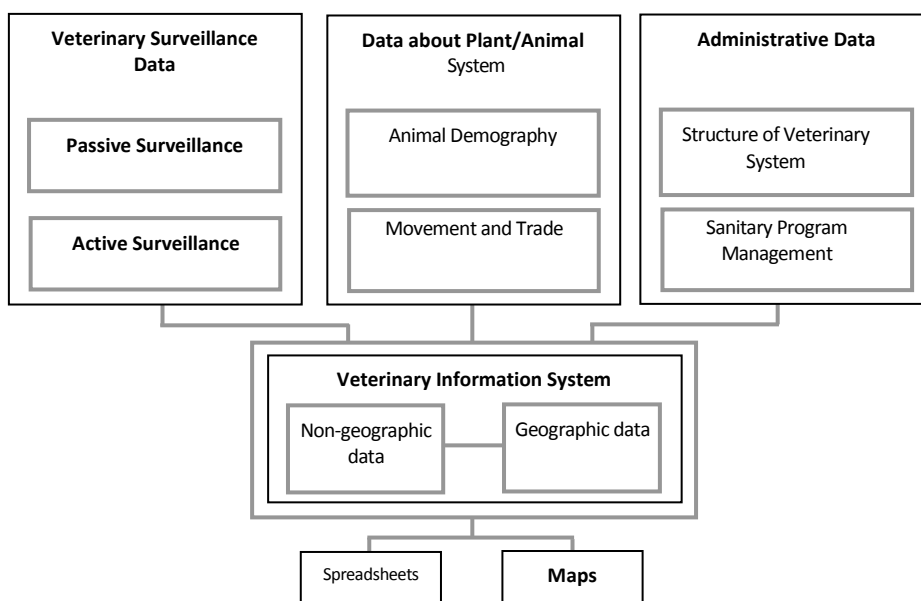


Figure 1. Graphic representation of the main components of the Veterinary Surveillance System, proposed by OIE

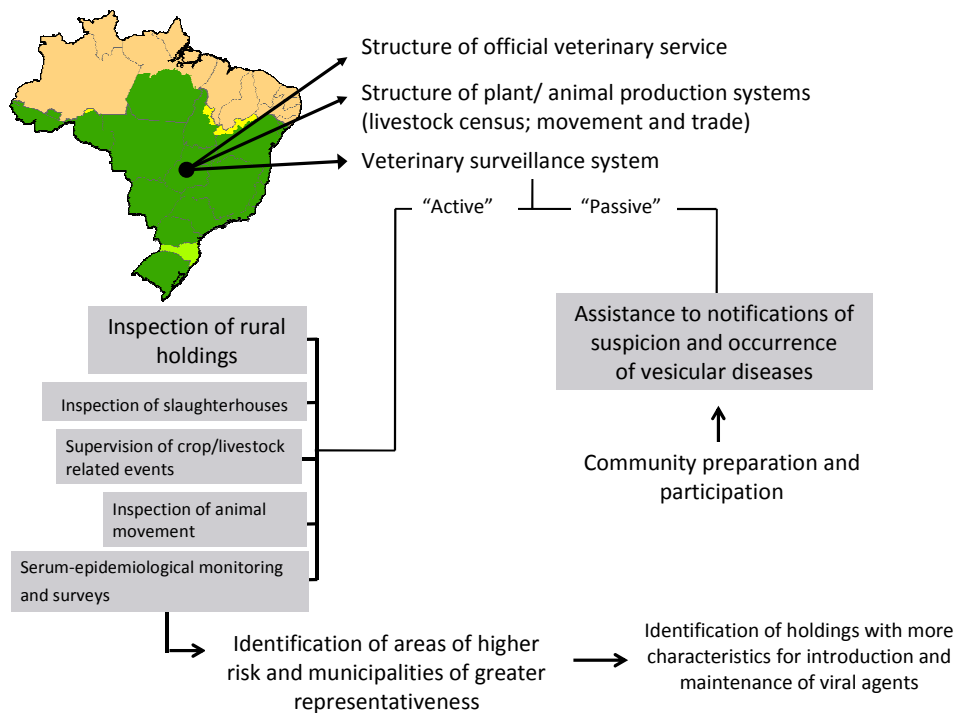


Figure 2. Graphic representation of the Brazilian Veterinary Surveillance System for vesicular diseases

For at least the last five years, CFA/ DSA/ SDA has been trying to implement a systematic procedure to collect information required for evaluation and management of PNEFA. The current procedure used was initiated after meetings held with official veterinary services from different states with the definition of the questionnaire called “Information survey to prove the maintenance of sanitary conditions of FMD free zones”. As a complement to this questionnaire, there is also the report on FMD vaccination stages, which is part of the PNEFA information system. It is based on the document “Guidance for FMD vaccine trade control and for control and evaluation of vaccine stages”. Its first version was published in 2005.

Initially, the questionnaire was completely annually and limited to those states within FMD free zones. Currently, the questionnaire is used in all states and focuses on three main points: i) structure of official veterinary service, ii) information about the amount of species susceptible to FMD and iii) activities and veterinary surveillance indicators. Information collection should take into account the recommendations and definitions presented below.

Frequency

- the collection, consolidation and information completion by the official veterinary service represent ongoing processes which should allow the consolidation and preparation of specific reports to be submitted to CFA, according to the following steps:
 - States with areas included in FMD free zones: semiannual frequency;

- first semester: report should be submitted to SFA by July 25 for check and analysis, after this date it can be submitted to CFA/DSA by July 31, with information related to the previous year, from January 1 to June 30; and
- second semester: report should be submitted to SFA by January 25 for check and analysis, after this date it can be submitted to CFA/DSA by January 31, with information related to the previous year, from January 1 to December 30.
 - States not included in FMD free zones will follow an annual frequency. The questionnaire should be submitted to SFA by January 25 for check and analysis, after this date it can be submitted to CFA/DSA by January 31, with information related to the previous year.
- In states with buffer zones, information should be submitted separately for free zones and buffer zones.
- Especially for FMD- free zones, a database should be created for the specific information, which will be listed below and may be requested at any time, and more often than what was established above.

Structure of Official Veterinary Service

- Only structures that are in use should be informed.
- For SFAs the graph below should be kept updated :

Level considered	Offices	Official Veterinarians	Other Under-Graduate Professionals	Technical Assistants	Administrative Assistants	Vehicles and Trucks	Vessels	Motorcycles	Trailers	Computers	Telephone lines	Fax Machines	GPS Devices or Alike	Certified Veterinarians
SEDESA														
Central Unit														
Regional Unit														
Sub-total														
VIGIAGRO														
Central Unit														
Regional Unit														
Ports, posts and airports														
Sub-total														
SIPAG														
Central Unit														
Regional Unit/ Establishments														
Sub-total														
SEFAG														
Central Unit														
Regional Unit/ Establishments														

are newly-created municipalities in certain states that have not included in IBGE's database, they should be disregarded and their information submitted with municipality of origin. This should also be explained in the e-mail sent to DSA;

- b) In the cases of capitals where LUVs are located, information should only be related to the local veterinary unit, not including information related to regional or central units.
- c) An electronic spreadsheet template will be available at CFA/DSA including the columns below with the respective explanation and instructions for completion:
 - i. the four first columns are related to the identification of municipalities, according to official standards by IBGE: Cod_UF (state code); Nome da UF; Cód_mun (Name of state; and code of municipality) . These columns cannot be altered;
 - ii. EAC (Community Service Office): to be completed with the number of offices of each municipality. These offices are considered the physical and structural basis in each municipality. Their files hold sanitary records of rural establishments and places where vaccination records and GTAs have been issued, among other activities. One of these offices should be the headquarters of a local veterinary unit. It represents the direct community assistance service structure. There may be more than one municipal office.
 - iii. LVU (Local Veterinary Unit): to be completed with the name of municipality where the LVU headquarters is located, (the name of municipality should be written in full). If one specific unit covers more than one municipality, all municipalities that are covered should use the name of municipality where the LVU headquarters is located. This allows for the geographic division of municipalities according to the LUVs, making it possible to prepare maps with lists of municipalities according to LVUs.

LVU can be defined as the structure for veterinary surveillance management, associated to one geographic position, under the responsibility of one or more official veterinarians. It may cover one or more municipalities and Community Service Offices. LVUs, management structure should make use of physical, financial, human and legal resources that are required for the development of activities related to animal health within its geographic boundaries. The presence of an official veterinarian is a mandatory requirement for the establishment of LVUs. For this purpose, the number of these units should not be superior to the number of veterinarians available for field activities;

- iv. UR (Regional Unit): represents the intermediary managing structure, which group LUVs. In the spreadsheet, the UR column of all municipalities that are part of one specific regional unit should be completed with the name of the municipality (in full), where the UR headquarters is located. This structure is not present in all states. In those states where this is the case, the column should remain blank.
- v. The next three columns refer to servants who work at the local veterinary unit: "Veter." (Veterinarians); "Aux. de campo" (Field Assistant) and "Aux. admin." (Administrative Assistants). This field should be completed with the number of servants involved in animal health activities for each LVU;
- vi. Telephone line: field to be completed with the total number of telephone lines available for each municipality (only telephone lines with free access, from the state veterinary service).
- vii. FAX: this field refers to the number of fax machines available in each municipality (only fax machines with free access, from the state veterinary service).
- viii. Computers: field to be completed with the total number of computers in each municipality (with free access, from the state veterinary service);
- ix. Common vehicles: field to be completed with the number of ordinary vehicles available for activities related to animal health;
- x. Light trucks or trucks: field to be completed with the number of larger vehicle (with or without 4x4 traction) available for activities related to animal health;

- xi. Motorcycles: field to be completed with the number of motorcycles available for activities related to animal health;
- xii. Vessels: field to be completed with the number of vessels or similar vehicles;
- xiii. Vans: field to be completed with the number of collective vehicles;
- xiv. Veterinarians from public institutions: field to be completed with the total number of veterinarians from other public institutions in the municipality;
- xv. Private veterinarians: field to be completed with the number of veterinarians from the private sector living in the municipality;
- xvi. Internet access: field to be completed with YES in municipalities with internet access in one or more Community Service Offices;
- xvii. Assistance kit to vesicular diseases suspicions: field to be completed with “1” for municipalities that have a kit of equipment and instruments to be used in assistance to suspicion of vesicular diseases, according to list available in Annex 1. In case the kit is not available in a given municipality, the field should be completed with “0”;
- xviii. GPS (Global Positioning System): field to be completed with the number of devices available for LVU use.

Animal Demography

- Standards used for registration, codes and organization of information related to rural establishments, farming systems and products should comply with guidelines of the Manual available at CTQA/DSA/SDA/MAPA.
- State veterinary services should keep a database that allows for consolidation of livestock herds that are susceptible to FMD, according to the graph below.

For bovine, buffalos and small ruminants farming systems:

		Bovine Animals										Buffalos	Ovines	Caprines
Holdings	Farmers	< 12 m		13 a 24		25 a 36		> 36		Total				
		M	F	M	F	M	F	M	F	M	F			

For swine farming systems:

Category	Total of farms	Breeder		Total Herb
		M	F	
Certified Pig Breeding Farms (GRSC)*				
Swine Trading Farms *				
Estimate of Non-Technified Herd				
Total				

* Total herd should include the sum of breeding stock, piglets and other existing animals.

- In the specific cases of swine farming, information should be in compliance with guidelines and definitions set by the National Program of Swine Health (PNSS).
- For bovine and small ruminant farming, states should provide information consolidated by municipalities with spreadsheets containing the following information:
 - a) UF: state identification code
 - b) Municipality: municipality name according to IBGE, in full (the same spreadsheet standard related to LVUs structure)
 - c) Cod_Mun: municipality code, according to IBGE
 - d) Prop_tot: total number of holdings in official veterinary records
 - e) Prop_bov: total number of holdings with bovine animals

- f) Prod_bov: number of farmers with livestock farming in holdings with bovine animals
 - g) Bov_12_F: total number of bovine animals of up to 12 months, female
 - h) Bov_12_M: total number of bovine animals of up to 12 months, male
 - i) Bov_13a24_F: total number of bovine animals from 13 to 24 months, female
 - j) Bov_13a24_M: total number of bovine animals from 13 to 24 months, male
 - k) Bov_25a36_F: total number of bovine animals from 25 to 36 months, female
 - l) Bov_25a36_M: total number of bovine animals from 25 to 36 months, male
 - m) Bov_36_F: total number of bovine animals older than 36 months, female
 - n) Bov_36_M: total number of bovine animals older than 36 months, male
 - o) Bub_12_F: total number of buffalos of up to 12 months, female
 - p) Bub_12_M: total number of buffalos of up to 12 months, male
 - q) Bub_13a24_F: total number of buffalos from 13 to 24 months, female
 - r) Bub_13a24_M: total number of buffalos from 13 to 24 months, male
 - s) Bub_25a36_F: total number of buffalos from 25 to 36 months, female
 - t) Bub_25a36_M: total number of buffalos from 25 to 36 months, male
 - u) Bub_36_F: total number of buffalos older than 36 months, female
 - v) Bub_36_M: total number of buffalos older than 36 months, male
 - w) Ovine: total number of ovine animals
 - x) Caprine: total number of caprine animals
- For bovine farming, states should report the list of holdings on a spreadsheet, containing the following information every year:
 - a) Cod_prop: code of holding
 - b) Nome_pro: full identification of holding
 - c) Mun_loc: full name of municipality where holding is located, according to IBGE
 - d) Mun_cont: full name of municipality of holding control, according to IBGE (for states where holding can be controlled by an LVU at a location that is different from place where the holding is located)
 - e) Prod: number of existing farmers
 - f) Area_total: total area of holding in ha
 - g) Area_pasto: total grazing area in ha
 - h) Bov_12_F: total number of bovine animals of up to 12 months, female
 - i) Bov_12_M: total number of bovine animals of up to 12 months, male
 - j) Bov_13a24_F: total number of bovine animals from 13 to 24 months, female
 - k) Bov_13a24_M: total number of bovine animals from 13 to 24 months, male
 - l) Bov_25a36_F: total number of bovine animals from 25 to 36 months, female
 - m) Bov_25a36_M: total number of bovine animals from 25 to 36 months, male
 - n) Bov_36_F: total number of bovine animals older than 36 months, female
 - o) Bov_36_M: total number of bovine animals older than 36 months, male
 - p) Bov_total: total number of bovine animals, male and female
 - q) Bub_12_F: total number of buffalos of up to 12 months, female
 - r) Bub_12_M: total number of buffalos of up to 12 months, male
 - s) Bub_13a24_F: total number of buffalos from 13 to 24 months, female
 - t) Bub_13a24_M: total number of buffalos from 13 to 24 months, male
 - u) Bub_25a36_F: total number of buffalos from 25 to 36 months, female
 - v) Bub_25a36_M: total number of buffalos from 25 to 36 months, male
 - w) Bub_36_F: total number of buffalos older than 36 months, female
 - x) Bub_36_M: total number of buffalos older than 36 months, male
 - y) Bub_total: total number of buffalos, male and female
 - z) Lat_G: Latitude degree (negative, in case the holding is located below the Equator, and positive in case the holding is located above the Equator).
 - aa) Lat_M: Minutes of Latitude
 - bb) Lat_S: Seconds of Latitude
 - cc) Lon_G: Longitude degree (in Brazil only negative - located on the west of Greenwich)
 - dd) Lon_M: minutes of Longitude
 - ee) Lon_S: Seconds of Longitude

Note:

- Among evaluation activities concerning information consistency, state veterinary services should also analyze the animal capacity rate for each rural holding containing bovine animals $[(Bov_total + Bub_total) / Area_Pasto]$, by carrying out surveys in places where rates presented show an expressive distortion in relation to those standards expected for the region.
- Evaluation of consistency should also consider the consolidation of the number of holdings and the total number of the bovine population by municipality, for comparison with information obtained during FMD vaccination stages or with database of previous years. Expressive inconsistencies should be investigated and justified;
- By means of a gradual process, geographic coordinates and geographic location of rural holdings should be obtained, starting from those at a higher risk. Each holding should have, at least, one location point obtained at the holding's headquarters. Templates and standards for coordinates should comply with instructions forwarded by DEP and by CTQA/DSA/SDA/MAPA;
- The definition of higher risk holdings is responsibility of LVU veterinarians and should be based on the analysis of the predominant crop/ livestock system, considering the following conditions:
 - Cod_1. holdings located on international borders, or bordering states or regions with worse sanitary conditions;
 - Cod_2. holdings close to places where there is animal gathering (including those used as animal resting places for cattle in movement)
 - Cod_3. holdings close to slaughterhouses or dairy industries;
 - Cod_4. holdings close to landfills or dumps;
 - Cod_5. holdings close to ports, border posts, airports or bus stations;
 - Cod_6. holdings close to labs that are authorized to manipulate FMD infectious material;
 - Cod_7. holdings with intense flows of susceptible animals;
 - Cod_8. livestock systems located inside rural settlements, indigenous tribes or any other situation in which the livestock system requires special veterinary attention from the official service;
 - Cod_9. different holdings with livestock that belong to the same owner, especially those in different countries, states or municipalities with different animal sanitary conditions;
 - Cod_10. holdings that are very close to highways with an intense flow of animals, especially cattle trails;
 - Cod_11. livestock farming which belong to owners who did not declare FMD vaccination or showing resistance to adopting sanitary measures established by the official veterinary service, including the statement of animal movement;;
 - Cod_12. other conditions (specify).
- The risk addressed by this document, should be understood as an estimate in terms of chances of FMD viral infection sources on a given animal population or sub-population. Generally, all holdings with susceptible animals, located in a given municipality, present a certain risk to other holdings. Therefore, veterinarians from the official service should establish a risk scale, identifying higher-risk holdings by using conditions listed above and trying to answer the following question: If an FMD virus enters this municipality, in which holdings is it more likely to be identified? Among conditions aforementioned the intense flow of susceptible animals should be considered as the main parameter when defining risk levels. Then, for example, among higher-risk holdings located in international borders or on the border of areas with inferior sanitary conditions, those with more intense animal flow pose a higher risk level.
- In order to support surveillance activities and serological monitoring, those responsible for LVUs, should keep an updated list of higher-risk holdings for each municipality and establish an active surveillance plan. Higher-risk holdings should represent 15% of the total number of holdings for each municipality. The surveillance plan mentioned should consider frequency of visits and inspection to holdings with a higher risk in periods between vaccination stages and inspection during vaccination stages.

- For serological monitoring, DSA/SDA/MAPA will use the list of higher-risk holdings for distribution and application of samples. For this reason, this list should be available, upon request, and should have the same fields as the spreadsheet for bovine farming, described above, plus field “Cod_Risco” for the introduction of the code of the risk criterion that was used, according to conditions listed previously. If the same holding presents more than one risk condition, a corresponding code should be added, separated by commas. Remember that this list should represent, at most, 15% of the total number of susceptible rural holdings in each municipality.

Assistance to suspicion of vesicular diseases

According to what was already mentioned, information related to assistance to suspicion of vesicular diseases represents a fundamental parameter for evaluation, especially in relation to the sensitivity of the veterinary surveillance system for FMD.

Brazil is part of OIE’s information system for general animal diseases and PANAFTOSA, for diseases that are part of swine vesicular, nervous, neurological and hemorrhagic syndromes

For both, OIE and PANAFTOSA, FMD is a disease of direct notification, just as exotic diseases and those listed in the old OIE “A” list (currently identified by the letter A), they must, therefore, be immediately reported to DSA/SDA/MAPA.

According to Resolution V of XXX COSALFA, PANAFTOSA and South American countries have decided to promote changes to the Continental Information System, which had been in use since the 70s, by implementing the Continental System of Epidemiological Surveillance - SivCont. This new system was created by PANAFTOSA and uses a computerized structure where states report the occurrence or non-occurrence of syndromes reported to DSA/SDA/MAPA. For further information on the use of SivCont, check operation manual prepared by PANAFTOSA.

Brazil started implementation and use of SivCont in the second semester of 2004, by standardizing and communicating its usage procedures through the following communications issued by DSA/SDA/MAPA:

- Circular Letter DDA no. 65, dated August 2, 2004 - which communicates the creation of SivCont and provides standard forms to be adopted as well as instructions for completion.
- Circular Letter DSA no. 94, dated December 13 2005 - which communicates and establishes that every FMD outbreak or suspicion that is reported to DSA/SDA/MAPA should be identified by IBGE municipality code so that they are registered on a computerized database avoiding the repetition of event number; and
- Circular Letter DSA no. 122, dated August 7, 2006 - which informs and makes available access passwords to check occurrences registered at SivCont, on the PANAFTOSA website.

Procedures related to registration of information in cases of assistance to suspicion of vesicular diseases are the following:

- a) Starting from the notification of a suspect case of vesicular disease, the official veterinary service should pay a visit to the establishment within maximum 12 hours after receipt of communication, according to Normative Instruction no. 44, dated October 2, 2007. All assistance should be carried out according to the procedures established in manuals and contingency plans and properly registered at community service offices. Registration should contain basic information, such as date and time of notification, type of notification (owner, third party or surveillance), name of notifying party, in case the person wants to be identified (a) name of holding and owner involved, location of holding, a brief description of the case presented, date and time of visit. During assistance, the investigation form (FORM IN) should be used, as well as instructions from DEP/DSA/SDA/MAPA;

- b) When the suspicion of vesicular disease is discarded by an official veterinarian, (i.e., food poisoning, irritating, chemical product, foreign material, burn, disorder etc...) the official veterinarian should register this visit in FORM IN, with the respective accompanying form (FORM COM) to formalize the end of investigation. These forms should be filed at the respective LVU in specific files of easy access. It should not be sent to DSA/SDA/MAPA. It is important to highlight that the occurrence must be entered in SivCont.
- c) when the official veterinarians confirm a case of vesicular disease, which necessarily involves the collection of sample for lab testing, they must immediately communicate other levels of the official veterinary service, by submitting the FORM IN to DSA/SDA/MAPA. The occurrence should be entered at SivCont. Samples collected should be sent to national laboratories certified by MAPA. The anatomopathologic description of vesicular wound found cannot be forgotten. All follow-up actions related to the case must be registered in corresponding FROM COM:
- d) in case the FMD occurrence is confirmed, actions and emergency sanitary measures must be taken, with an immediate communication from DSA/SDA/MAPA to OIE, PANAFTOSA, other Brazilian states, commercial blocks and countries holding bilateral trade agreements. The diagnosis result should also be entered at SivCont;;
- e) in case FMD occurrence is discarded, the final diagnosis result must be entered at SivCont;, and compatible sanitary actions should be established for each case. They must also be registered in corresponding follow-up forms (FORM COM).

Animal movement record

- Animal movement records are extremely important for several animal health-related activities, including the investigation of sanitary occurrences, evaluation and definition of regions with a higher sanitary risk and the definition and characterization of livestock circuits and livestock production systems,. At DSA/SDA/MAPA, the management of this information is responsibility of CTQA, which defines and updates, when necessary, reports that will be forwarded by state veterinary services. Among reports to be forwarded to CTQA, there is an electronic spreadsheet with the following columns:
 - a) GTA: number of animal movement permit
 - b) Series: letters indicating GTA's series number
 - c) Issuance date: date of issuance of GTA, in the format day/month/year
 - d) Objective: identification of objective of animal movement
 - e) Species: identification of animal specie authorized for movement
 - f) Total_F: total number of females in movement
 - g) Total_M: total number of males in movement
 - h) Total_G: total amount of animals in movement
 - i) Mun_orig: name of municipality of animal origin, without abbreviation, (according to IBGE)
 - j) Cod_mun_orig: code of municipality of animal origin (according to IBGE)
 - k) CPF or CNPJ_Orig (individual or company taxpayer ID): information related to animal farmer or company of origin
 - l) Nome_prod_orig: name of farmer or company of animal origin
 - m) Prop_orig: name of holding or establishment of origin
 - n) Cod_prop_orig: code of holding or establishment of origin
 - o) UF_dest: code of destination state of animals
 - p) Mun_dest: name of municipality of animal destination ,without abbreviation, (according to IBGE)
 - q) Cod_mun_dest: code of municipality of destination of animals (according to IBGE)
 - r) CPF ou CNPJ_dest (individual or company taxpayer ID): related to addressee
 - s) Nome_destinatario: full name of animal's addressee
 - t) Estab_dest: name of destination holding or establishment
 - u) Transp: means of transportation employed in animal movement. In case more than one type of vehicle is used, use additional columns for each vehicle used.
 - v) Emitente: full name of person responsible for the emission
- Based on information forwarded to CTQA/DSA/SDA/MAPA, official veterinary services should communicate consolidated data about the movement of FMD susceptible species to CFA, according to the following graph:

Specie	Within State					Out of State					GTAs issued
	Slaughter	Fattening	Rep.	Exp.	Milk	Slaughter	Fattening	Rep.	Exp.	Milk	
Bovine											
Buffalo											
Swine											
Ovine											
Caprine											

- Electronic spreadsheets forwarded to CTQA will be used by CFA to re-evaluate livestock circuits for the establishment of higher-risk municipalities of FMD entry and also to support monitoring activities. The classification of higher risk municipalities will be the responsibility of CFA/DSA/SDA/MAPA according to the following criteria:
 - municipalities located close to international border or bordering other states or zones that have worse animal sanitary condition;
 - municipalities with predominance of susceptible animals; and
 - municipalities with livestock farming systems which require special care by the official service, such as rural settlements or indigenous reserves.
- Control made in permanent inspection posts or mobile inspection teams: official veterinary services, should report consolidated data according to the graph below, following the general frequency initially defined:

Inspection	Total no inspections	Total of inspected animals				Inspected vehicles	Inspected Cattle*
		Bovines	Swines	Ovines	Caprines		
Permanent posts							
Itinerant teams							
UVL*							

* itinerant actions carried out by LVUs, not related to the specific work of mobile teams

** Cattle taken on foot

Inspections of rural holdings

- Inspections to rural holdings are fundamental for veterinary surveillance activities because they represent the main moment of information collection and registration, for FMD susceptible animal herds
- Official veterinaries should register all inspections or visits to rural holdings with specific individual forms. Information collection and registration should allow its consolidation, according to the graph below and also according to frequency they should be submitted to CFA/DSA/SDA/MAPA:

Record of inspection activities to holdings with FMD susceptible animals	No, of holdings	No, of inspections	Animais existentes			
			Swines	Bovines	Ovines	Caprines
Farms						
Higher-risk holdings*						
General holdings						

* holdings which are part of the list of up to 15% of higher-risk holdings in each municipality.

Note.: In order to avoid information duplication, information related to other activities should be deleted, such as:

- inspection of FMD vaccination registered in spreadsheets at end of vaccination stages, according to specific Manual prepared by CFA/DSA/SDA/MAPA;
- assistance provided to suspicion of vesicular diseases entered in SivCont or other report submitted to DSA/SDA/MAPA

- registration should include inspections related to other sanitary programs such as: the National Program for Control and Eradication of Bovine Brucellosis and Tuberculosis, National Control Program of Herbivorous Rabbits and other brain-related diseases: National Program for Swine Health and National Program for Caprine and Ovine Health.
- specifically in relation to PNEFA, official veterinary services,, especially in the period between vaccination stages, should establish goals and inspection priorities to rural holdings for each LVU, giving preference, when possible, to those classified as higher FMD risk holdings.

Inspections of slaughterhouses

- Inspection activities in slaughterhouses should be consolidated according to the following Table:

Inspection Service	No. Slaughter houses	Inspected Animals			Professionals who work in meat-packing plants	
		Bovines	Swines	Small ruminants	Veterinarians	Assistants
Federal						
State						
Municipal						
Total						

Inspection of plant/animal -related event

- Plant/animal-related events with gathering of animals that are susceptible to FMD should be inspected by the official veterinary service, according to state and federal legal norms. The registration of information related to this event is extremely important and its consolidation, to be submitted to CFA/DSA/SDA/MAPA, should include the following data:

Information	No
1. Total no. of events with FMD susceptible animals	
1.1. Carried out	
1.2. Inspected by the official veterinary service	
1.3. With the presence of official veterinarians	
2. Number of certified or authorized companies	
3. Number of authorized permanent posts	
4. Total number of certified veterinarians in the State	
5. Professionals from the veterinary service official /event	
6. Total of animals that participated in the event	
6.1. Bovines	
6.2. Buffalos	
6.3. Ovines	
6.4. Caprines	
6.5. Swines	

General Actions

A series of other activities are part of the veterinary surveillance system for vesicular diseases and should be part of final reports for the evaluation of actions conducted by the official veterinary system. Next, the main piece of information to be consolidated and submitted to CFA/DSA/SDA/MAPA is presented:

- Violation/ seizure/ destruction reports that have been applied:

Inspection activities carried out by official veterinarians can generate the identification of irregularities and immediate application of legal procedures pursuant to the law. The registration of this information is fundamental

to analyze the credibility and consistency of work carried out. Its consolidation, which should be submitted to DSA/SDA/MAPA, according to established deadlines, should occur according to the graph below:

Violation records:

Type of violation	No. of violations
No FMD vaccinaion	
No communication on the accomplishment of FMD vaccination	
Transportation of animals without issuing or carrying a GTA	
Transportation of animal origin products without sanitary documentation	
Store operating without permit or approval to sell FMD vaccines	
Exhibition of veterinary products with expired expiration dates	
Holding of plant/animal -related event without a previous permit from the official veterinary service	
Other (specify):	
Other (specify):	
Other (specify):	

Seizure/Destruction(include information related to inspections carried out in posts, ports and airports):

Material or product seized and destroyed	Unity	Amount
FMD vaccine	Dose	
Bovine	Animal	
Caprine	Animal	
Ovine	Animal	
Swine	Animal	
Dairy products	Litter	
Dairy products	Kg	
Meat products	Kg	
Cattle hides ("green", without treatment)	Piece	
Other (specify):		
Other (specify):		

- Activities related to sanitary education, social communication and training:

Considering the frequency established, the following information should be submitted to DSA/SDA/MAPA:

a) Community representations and lectures/ meetings:

Information	Amount
1. Community representation	
1.1.No. of municipal animal health councils	
1.2. No. of farmers' association	
1.3. No. of rural trade unions	
1.4. No of crop/livestock cooperatives	
1.5. No. of other types of farmers representations (specify)	
2. Lectures or meetings carried out with community representatives	
2.1. No. of lectures and meetings	
2.2. Total no. of participants	

b) Training and skill-enhancement courses for professionals of the official veterinary service:

Training courses carried out*	Month	Total	Total of participants	
			Veterinarians	Assistants

* Only topics related to PNEFA or of general interest for the animal health system

• Additional information:

Official veterinary services should forward the following complimentary information to CFA/DSA/SDA/MAPA :

Information	Amount
1. Financial resources (R\$)	
1.1. Available in private funds for sanitary emergency actions	
1.2. Available in public funds for sanitary emergency actions	
1.3. Originally from partnerships with MAPA for sanitary animal health-related actions	
1.4. Related to the payroll of servants who work in animal health area	
1.5. Originally from state government for sanitary animal health-related actions	
2. List of members from the veterinary emergency groups	
3. Total of landfills (dumps) that are identified and under surveillance	
4. No. of blocked or closed clandestine slaughterhouses	
5. Auditing carried out by SFA	
5.1. Total no. of auditing carried out	
5.2. Total no. of audited LVUs	
6. Internal auditing carried out by the state veterinary service	
6.1. Total no. of auditing carried out	
6.2. Total no. of audited LVUs.	

4. Serological surveillance

The implementation of the serological surveillance at PNEFA was initiated mainly in 1997 when the first epidemiological survey was carried out in order to support the recognition requirement of Rio Grande do Sul and Santa Catarina states as FMD-free zones with vaccination. Before this date, there are records of a serum-epidemiological test in the Pantanal region in Mato Grosso do Sul for the evaluation of non-structural antibodies against the FMD virus in the period of 1995 to 1997. Starting from 1997, epidemiological monitoring and surveys have been carried out in every following year with the objective of expanding FMD free zones, providing evidences of sanitary conditions for FMD free zones or supporting emergency activities. Methodological basis used for tests conducted in the country so far has been prepared with the support of PANAFTOSA, and follows OIE general recommendations.

According to what is stated in the Terrestrial Code, there is not one single picture of serological surveillance that can be applied to all sanitary conditions, livestock sanitary system and susceptible species. Therefore, for every test carried out it is necessary to consider predominant geographic epidemiological and livestock scenarios, adapting technical and operational procedures to existing realities. For this reason, the objective of this item is to make a general description of operational and methodological aspects and variables involved in the preparation of epidemiological monitoring and surveys, so that all professionals from the official veterinary service understand the technical grounds of this type or epidemiological survey. This document, however, does not discard the need to prepare specific technical manuals for each study that will be carried out.

Before starting with specific items related to operation and theoretical fundamentals it is important to highlight that in Brazil, the expression ^epidemiological survey^ has been used to represent a transversal, non-periodic survey of population sample with the objective of establishing the presence or absence of viral circulation. Surveys are used, in the case of FMD, to support or certify the establishment or recovery of a zone free from the disease, therefore, its population sample is directed towards all livestock systems of the geographic area under investigation.

The expression ^epidemiological survey^, on the other hand, refers to the periodical verification of a given sample population, which does not need to be a representation of the target population. Surveys are used, in the case of FMD, to prove the absence of viral circulation in zones with free conditions that have been previously recognized, for purposes of maintenance of this sanitary condition and it is exclusively directed towards higher-risk livestock systems.

Organization and basic structure to carry out serological surveillance

Monitoring and surveys represent complex tasks of major dimensions. They require strictly controlled procedures in order to ensure standards, quality and reliability throughout the process. Control procedures, on the other hand, require an efficient operational structure, which should be distributed in different levels. Still, generally speaking, there are 3 basic levels of central management and control: Central/DSA, Central/LANAGRO and state level. Each of these levels have distinct and specific responsibilities, which will be summarized later. The main objectives of this organizational structure are to provide agility to the process and secure that serum collected, together with required information, get to central laboratories under good analysis conditions. The second material collection should always be avoided, especially when it has to be done because of poor quality or insufficient amount of serum. The new collection involves an increase of cost and time. It is expected that LANAGRO's only duty is to carry out serological tests, a task that requires a great deal of hard work for itself. In Figure 3 a graph of established operational levels is presented, it highlights the intention to avoid the request of complementary information or new collection of material to the state coordination from the central lab. As a complement, in Figure 4, there is a summary with the activities of serological surveillance, which can be divided into three basic stages, outline, execution and analysis/ conclusion. For each stage, there are main related activities. Activities related to the second stage will be subject to more details in the following items of this document.

All activities related to epidemiological surveys have the objective of collecting data and producing information which will become observation basis to accept or reject hypothesis under study. Accordingly, it is always important to highlight that the success of this task depends exclusively on the quality of actions carried out at all levels, from information registration and sample collection, information reporting to central instances of the official veterinary service to the issuing and interpretation of lab results.

In relation to sample collection activities, it is acknowledged that laboratories need to work with good-quality material. Practice has proven that when the process of serum collection is not carried out appropriately, all the work is compromised. On the other hand, laboratorial results lacking appropriate information do not allow for sustainable conclusions in respect to the problem investigated. In this fashion, it is necessary to establish one specific operational and management structure for the serological monitoring and surveys of the animal population.

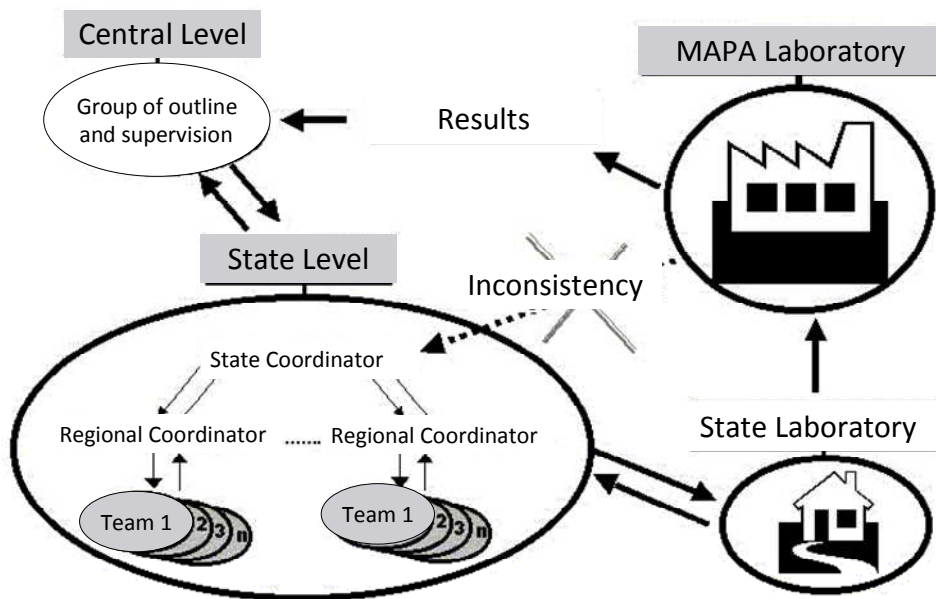


Figura 3. Representation of existing control levels and flow of information and samples

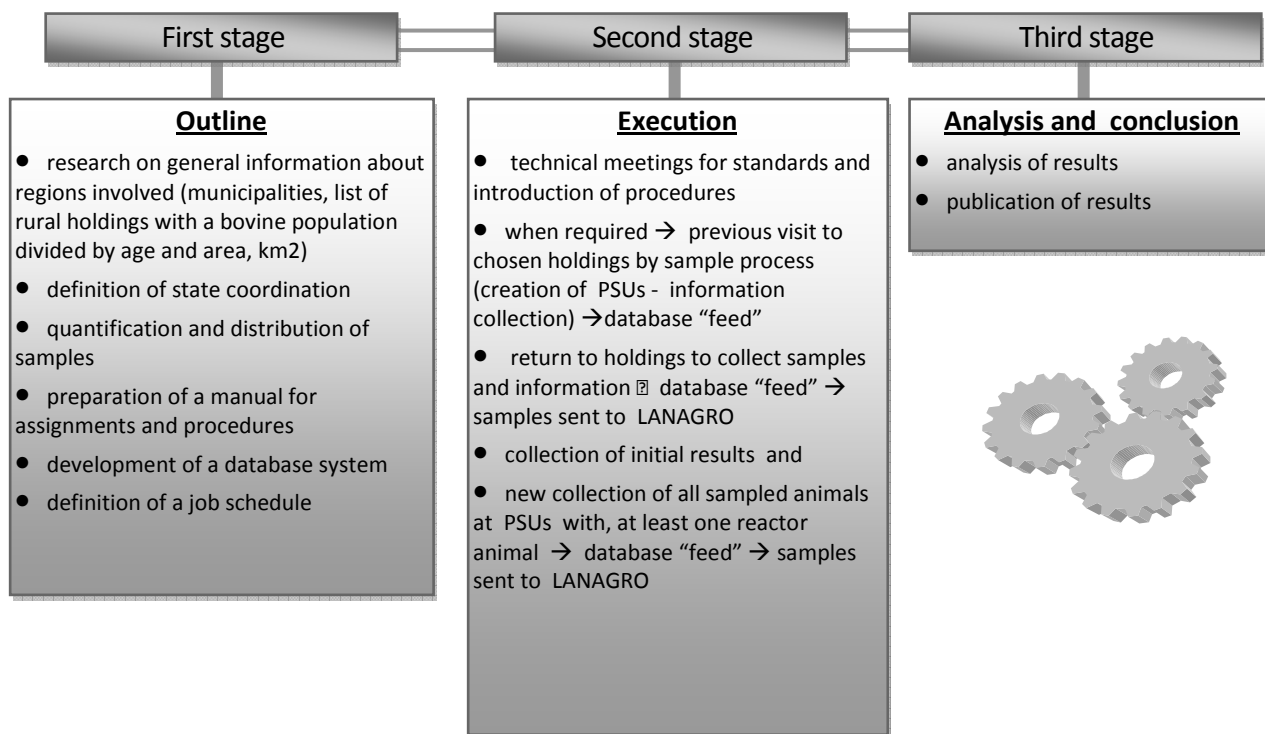


Figura 4. Representation of the main stages related to surveys and monitoring

- Constitution and responsibility of different levels of control and management:
 - a) Central Level /DSA:

The central level comprises the team responsible for the outline of the study and general supervision of activities, as well as analysis of results found. This team encompasses the General Coordination of Disease Control from DSA/SDA/MAPA in Brasilia and professionals appointed by them. Amongst its main activities it:

- support to training activities of state teams;
- development of a database system for information management;
- follow up and guidance to the state coordinator for the execution of activities and
- joint work in partnership with the General Coordination for Laboratory Support- CGAL/SDA/MAPA, to provide all required lab material..

The database, in particular, represent a computerized system whose main objective is to support the management of all data and information generated during the execution of activities, ensuring consistency and agility to process. The database, to be managed, comprises data and information produced during previous visits to selected holdings, when required, and during the procedure of sample collection. When samples are sent to LANAGRO, related data and information should be submitted together. The database management system will be installed in state veterinary services, at LANAGRO and at DSA/SDA/MAPA.

b) State Level (state services and SFAs):

The state level includes the state coordinator, regional coordinators, collection teams and those responsible for the state center for sample reception and check. The state will appoint one state coordinator who will operate as a communication connection with other considered levels. He/She will also be responsible for the execution of activities to be developed in the state, from the initial research of information on selected holdings to database “feeding”, the planning and execution of sample collection activities, submitting samples to state reception center and finally the shipment of collected material (evaluated and checked) to LANAGRO. To perform these tasks, the State Coordinator should establish state action networks, which will comprise the state reception centers and regional coordinators, to be in charge of the collection team. The number of regional teams and teams for information registration and sample collection should be calculated in order to ensure material quality in all stages as well as database consistency. Deadlines established for each activity should always be met.

Among co-responsibilities of regional coordinators one can highlight: planning activities for collection teams, daily assessment and checking of day-to-day activities carried out, material delivery to state reception center.

The responsibility scope of collection teams is focused on observation and compliance with norms and instructions, which have been established in order to guide and standardize animal selection mechanisms, samples, collection material and preparation and packing of collected material, always taking biosecurity issues into account.

The state reception center represents the last state barrier for the detection of possible problems in relation to collected material. It will also be responsible for feeding and maintaining the database for samples that are received, and, finally for their preparation and submission to LANAGRO. In this sense, one can highlight the responsibility that lies upon the center to check information and samples received and also to comply with norms for material storage and shipment, as well as to fulfill biosecurity requirements considering that the material may travel through FMD free zones..

In a nutshell, it has the following responsibilities for each segment of the state level:

⇒ State Coordinator:

- ✓ establish the state cooperation network, its communication flows, distribution and shipment of equipment and material to be used, as well as samples collected and information registered;
 - ✓ identify and meet the needs in terms of material, equipment or training;
 - ✓ support regional coordinators to plan activities related to information registration;
 - ✓ feed and maintain database in relation to previous-visit forms (when used) and sample collection.
- ⇒ Regional Coordinator:
- ✓ plan activities related to the application of forms during previous visits (when used) and sample collection, observing procedures defined for the creation of UPAs; and
 - ✓ check forms completion, seeking to secure content integrity and quality (including legibility aspects).
- ⇒ State center for sample reception and check:
- ✓ store and distribute to regional coordinators material and equipment required for the execution of activities;
 - ✓ check information and samples submitted by regional coordinators;
 - ✓ feed and maintain the database, consolidating information related to samples that have been received; and
 - ✓ send samples to LANAGRO after verification, taking biosecurity procedures into account.
- ⇒ Team for information registration and sample collection:
- ✓ seek understanding and practice of actions to be carried out;
 - ✓ care for quality of material, which has been received by regional coordination, submit it to regional lab and care for reliability and consistency of registered information and collected samples; and
 - ✓ learn and fulfill all methodological requirements, which have been defined for each respective activity.
- c) Central Level/ LANAGRO:

LANAGRO will be responsible for performing the last verification of the material received, processing it and “feeding” the database with results obtained. A general supervisor should be appointed for the verification of activities carried out. He/She should establish a communication channel with state coordinators and with central level/DSA. All problems found in material, which has been received from state, should be reported to state coordinator.

Sampling methods

In studies involving infectious agents it must be considered that these agents are rarely uniformly distributed in animal population. They tend to concentrate in animal clusters, normally herds or herd groups. As a result, a small herd proportion may be affected by one disease, but within the infected herd the proportion of infected animals may be higher, according to Figure 5. In case a serological survey has the objective of detecting a disease or infection, without considering its concentration, its results may not be valid, since in random samples it's assumed that all sample units have the probability of being drawn. This could only be acceptable if all animals had the same probability of being affected by the disease. In this fashion, in the present study, the main epidemiological unit (primary unit) is represented by the primary sample unit – PSU (also known as conglomerates or clusters), which on a synthetic fashion, can be understood as a gathering of animals (elementary units) in a holding or group of holdings

that are close and under the same risk conditions. Further on, a specific item on the constitution of these PSU will be presented.

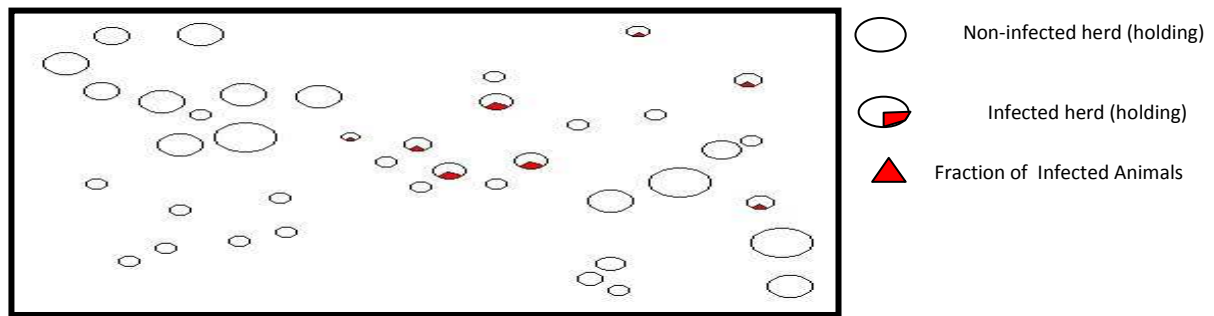


Figura 5. Concentration of disease in gathering of animals (herds or others)

There is also a logistic problem in sample surveys carried out in large areas. The simple random animal selection, which will comprise the sample of a given regional or national populations will only be executable if there is a list of millions of animals, individually identified. This condition is rarely met.

The absence of an exhausting list of elementary sample units is fixed by using a two-stage sample (1) first, a number of herd is randomly drawn or other primary sample units (for example, a group of close herds, or community villages (2) secondly herd status is established (for example, infected or not), an appropriate number of animals are randomly drawn (elementary sampling units). The herd will be considered infected when the number of seropositive animals found is above statistic epidemiological parameters defined during investigation plan.

With this method, the direct access of elementary sampling units is no longer a problem since official veterinary services from the region under study owns a record of rural holdings. On a two-stage sampling, the added nature of the disease is equally considered, because all herds of the sampling population are likely to be drawn. The choice of a herd as a primary sampling unit should consider the reality of existing animal production system in the region under study. Therefore, in regions with the predominance of small herds, it is suggested that herds that are physically close are considered as one primary sampling unit; this method is consistent with FMD epidemiology because when there is an outbreak in regions with a large concentration of small herds, the disease is likely to spread quickly, affecting several holdings. It must be highlighted that, according to OIE, one PSU can comprehend one group of animals with enough contact that all animals have the risk of being infected by an infectious agent entering the group.

- Constitution of PSUs

This is a critical point in serological surveys. Coordinators of sample collection teams are those responsible for the final establishment of PSUs. The constitution of a PSU starts with the random choice of a holding within the universe of holdings comprising the sampling population, that is, those that are registered in each community service office. In case of monitoring, the choice of selected holding in which the number of involved animals (elementary sampling units) is lower than the amount established for the PSU constitution, it will be necessary to identify holdings that are close and have the same characteristics, until a minimum amount of animals under the age group studied, is achieved. In this fashion, a PSU is then made up of a

gathering of small herds. Holdings with more animals than the minimum amount required will constitute one PSU for a single holding.

The minimum number of bovine animals for a PSU constitution is defined by DSA/SDA/MAPA for each state, considering issues of statistic relevance and characteristics of predominant production systems for each region under study, especially the average number of elementary units per holding.

It is important to highlight that the basis for a PSU constitution lies on the holding and not on the owner or breeder. Then, in case where several breeders, with different farming systems, work in one single holding with joint handling and structure, all animals will be evaluated as a whole. When the total amount of animals (of established age, according to study) of several breeders is equal or higher than the minimum animal number established, one PSU will be formed comprising all mentioned breeders. On the other hand, in case the total number of animals in the holding is smaller than the amount established, it will be necessary to gather neighboring holdings to constitute a PSU, according to what was described above.

Within every PSU, samples should be distributed) proportionally, taking the following into account:

- a) in PSUs are made up of more than one holding it will be necessary to distribute samples proportionally among involved holdings, that is, the total number of animals of a PSU will be distributed among the different holdings that are part of it, with a proportional distribution to relative weigh of each one of them. For example, if there are 15 animals, with the age that was established, in the first holding, and another 15 in the complementary holding and the amount to be collected is 20, the amount to be collected will be 10 for each holding.
 - b) In beef cattle farms, where animals are separated in groups with independent handling for each group, it will be necessary to distribute samples proportionally amongst various groups. Groups of animals whose age is above established by the study should not be considered in sampling process;
 - c) In holdings with several leaseholders two distinct situations can occur to sample distribution: (1) if animals belonging to each tenant are kept in separate herds, the same procedure above-mentioned should be applied as mentioned in letter “b” ,when animals are separated into different groups (for example, sample distribution will be proportional to relative weigh of bovine population for each tenant); (2) if animals from leaseholders are kept together in the same herd, there will be no need of sample proportional distribution among tenants.
- Target population, sampling population and elementary sample units

Target population for surveys and monitoring represent all FMD susceptible livestock systems that exist in the region which is under surveillance. Therefore, results obtained are assumed for the whole susceptible population of that specific region. Starting from the target population, a sampling population is chosen, depending on the objectives of the survey and operational feasibility of the area where animals will be chosen (elementary sample units) for sample collection.

In activities carried out in Brazil, the sampling population has been composed of bovine animals aged 6 to 24 months, divided into groups of 6 to 12 months, 13 to 18 months and 19 to 24 months. Especially in regions where systematic vaccination is applied bovine animals in the age group of 6 to 24 months are, recognizably, the population where the presence of viral circulation is best detected. The inclusion of animals younger than 6 months can lead to passive

antibodies interference in lab results, which should be avoided. On the other hand, the inclusion of older animals, with history of multiple vaccinations can result in a high percentage of reactions caused by multiple vaccination.

In the case of surveys, the database to outline studies and for selection of rural holdings, which will be part of PSUs, is made of spreadsheets, described in item 3 of this document, under sub-item “Animal Demography” referring to livestock systems divided by municipality and by holding.

For monitoring, apart from spreadsheets previously mentioned, spreadsheets of FMD higher-risk holdings should also be considered. From this list, holdings will be chosen to take part in survey. Animal movement in each municipality should also be taken into account to support the choice of holdings which will be part of the area to be surveyed.

- Random selection of primary and elementary sample units

Reliability levels achieved from the conclusion of sample survey depend on error produced by the sample size or error produced by selection method used. The understanding of reliability levels is fundamental for safety of final conclusions of serological surveys.

The error caused by sample size can be estimated, whereas the error, which accompanies sample selection method cannot be calculated but it can be avoided with the use of random procedures. Therefore, it is extremely important that records of holdings represent field reality, since they are the starting point for random selection of holdings, which will constitute PSUs. The more reliable they are, the more accurate estimates of reliability levels will be.

According to what was explained, sample selection in FMD serological surveys should be carried out in two stages: first, selection of holdings which will originate PSUs, and secondly, selection of animals, within each holding, to compose the referred PSUs.

The first stage of the survey, which is the random selection of holdings is under DSA’s responsibility, which will use the spreadsheets containing information of all holdings, made available by the official veterinary service of each state involved in the study. In case of monitoring, the spreadsheets refer only to higher-risk holdings.

Animal selection within each drawn holding, is responsibility of the person in charge of the collection team, who should use a random-sampling method. OIE Terrestrial Code highlights the importance of a random process in the choices of animals and, on its Article 3.8.1.4, it recommends that when it is not possible to select a random sample, the veterinarian should provide the best practical possibility for sample selection that represents a sampling population. In order to support this activity, Annex 2 presents instructions of two methods of simple and systematic random sampling. Note that the random choice for the number of animals for collection can be carried out before the visit to the holding for any of the methods proposed.

- Sample size

Serological surveys carried out represent viral circulation **detection** studies, since zones studied are FMD free zones or areas where the occurrence of the disease has not been reported for a long time. For calculation of sample size, different methods and programs can be used. DSA/SDA/MAPA has used software FreeCalc Version 2, recommended by OIE, using the option for hypergeometric distribution and the formula below, proposed by Cannon & Roe, 1982; Martin et al., 1992; Noordhuizen, J. P. T. M. et al., 1997*, which depends on confirmatory tests or complementary survey activities for the identification of false-positive results

$$\text{Fórmula: } [1-(1-C)^{1/(D*\text{SENS})}]*[M-(D*\text{SENS}-1)/2]$$

Where:

C= reliability level

M= no. of animals (animals/herds) at risk

D= no. of units with disease/infection

SENS= sensitivity test

Note that different elements interfere with sample number calculation, considering that the lower the expected prevalence is, the higher it will be for the same reliability level.

Statistic parameters used for sample size calculation need to be pre-established:

The minimum reliability level used in animal health surveys accepted by the scientific community is 95%, representing the level employed in surveys and monitoring by DSA/SDA/MAPA.

The prevalence of infected holdings varies according to factor related to agents, host and environment of transmissibility of infectious agent, as well as size and number of holdings etc... The detection of rare events represent a larger size of sample, that is, the rarer the event, the larger the sample needs to be to ensure minimum chances of detection. Expected prevalence of holdings with circulation used in serological surveys in Brazil has varied between 1% and 2%, that is, in case there is viral circulation, it is expected that with 95% of reliability it will be possible to detect, at least, one positive herd, considering that, at least, 1% or 2% of herds show viral circulation.

In regions with more intense history of vaccination, the expected prevalence of 1% is used, whereas in areas where the history is not as intense or is not present, like the recent case of Santa Catarina state, 2% prevalence was used. These levels are presented on a conservative fashion, because especially in regions with high concentration and animal movement, it is expected that a higher percentage of herds show viral circulation in case FMD virus is present. However, they are especially used so that a sample has appropriate levels of relevance. For example, when the formula above is used and reliability and sensitivity levels of diagnostic tests of 95% are used in areas with more than 10,000 rural holdings, the minimum total of PSUs would be 310 for a minimum expected prevalence of 1% and 155 for a 2% prevalence.

The minimum expected prevalence of infected animals within the same herd also depends on variables related to agent, host and also specific environments of each holding. The expected prevalence of infected animals within an infected herd, which has been used in serological surveys in Brazil, varies from 5 to 10%, according to epidemiological and crop/ livestock conditions of the farming system: 10% in herds with up to 500 bovine animals and 5% in herds with more than 500 bovine animals. Therefore, for each PSU, the required total of samples should be calculated by using the program or formula presented.

However, it is important to highlight that, both for PSUs and for animals within the same herd, levels of minimum expected prevalence to be used are not permanent, they can vary according to sanitary conditions and existing breeders.

Another element, which has a significant interference on sample size is the need to sub-divide sampling population into smaller sub-populations with bovine herds that share more similar and closely related structural and production characteristics. This procedure, especially in large geographic areas and heterogeneous populations is required, since it allows a better homogeneity of population under survey, leading, as a consequence, to higher levels of representation and relevance. It also allows independent evaluation for each sub-group. The disadvantage is that there is a large increase in the number of samples since for each sub-population, an independent sampling calculation is performed. Therefore, during the process of sub-population definition one should seek a satisfactory number, avoiding excessive sub-divisions, which would lead to an unnecessary increase in the total number of samples.

Diagnostic Method

The method used consists in the research of antibodies against non-structural (NSP) and non-capsid protein of the FMD virus (NSP), complemented by clinical inspections and sample collection of oesophageal-pharyngeal fluid (OPF) for viral research. Depending on the immune characteristics for FMD sample population, tests for antibodies evaluation can also be used against viral agent structural protein.

For the identification of antibodies against NSP, DAS/SDA/MAPA has been using the diagnosis kit developed and produced by PANAFTSA. This technique is recognized by OIE and involves the application, in series, of two diagnosis tests I-ELISA 3ABC (Indirect - Enzyme Linked Immunosorbent Assay) used as screening tests and (Enzyme - Linked Immunoelctrotransfer Blot Assay) as confirmatory test. For this diagnosis system a level of accumulated sensitivity of 95% has been used. Detailed information on these diagnosis techniques can be obtained in bulletins that are available on the website of PANAFTOSA (www.panaftosa.org.br) under links Laboratorios/Actividades (labs/ activities) of vesicular references and the OIE Manual of Diagnostics, Tests and Vaccines for Terrestrial Animals.

In figure 6, a graph of the diagnosis system is presented for the evaluation of FMD viral circulation. As it can be observed, the system involves the paired collection of samples in all PSUs, with animals that have tested positive in diagnosis tests used.

Initially, all samples collected in PSUs are submitted to PANAFTOSA diagnosis kit for the detection of antibodies against NSP and all sampled animals are submitted to clinical inspection of foot and mouth. All sampled animals should receive permanent, long-lasting identification.

Because we are dealing with a paired sample, the follow up on rural holdings that are part of PSU,s is fundamental as well as the participation and engagement of those responsible for the animals. In this fashion, during activities related to previous visits, or first collection, those responsible for the animals should be notified in relation to the following points:

- they can only give vaccine or market sample animals after approval by official veterinary service and
- they should notify the official veterinary service immediately in case of any sanitary occurrence related to sampled animals.

The period of time between the first and second collection represent the critical stage of the survey. Therefore, it is important that the official veterinary service inspects holdings involved in activities related to the second collection. In the period of time between collections the service should:

- carry out, alt lest, two visits to involved herds, with a minimum 15-day break, including the clinical inspection of sampled animals; and
- reinforce the notification to those responsible on the prohibition of vaccination or marketing of animals sampled against FMD.

In case of death of sampled animal, the official veterinary service should carry out all required research to clarify and register causes involved. If, during clinical inspections carried at any stage of the survey, evidences of compatible signs of vesicular diseases are noticed, the recommended sanitary procedures should be adopted for assistance related to confirmed cases of vesicular diseases, including farm blocking.

Interpretation of results after the second collection will comply with the following OIE recommendations:

- PSUs with reduction or without increase of positive bovine animals to diagnosis system used are considered without viral circulation; and

- PSUs with a significant increase of positive bovine animals require further investigation since it is not possible to discard the possibility of viral circulation.

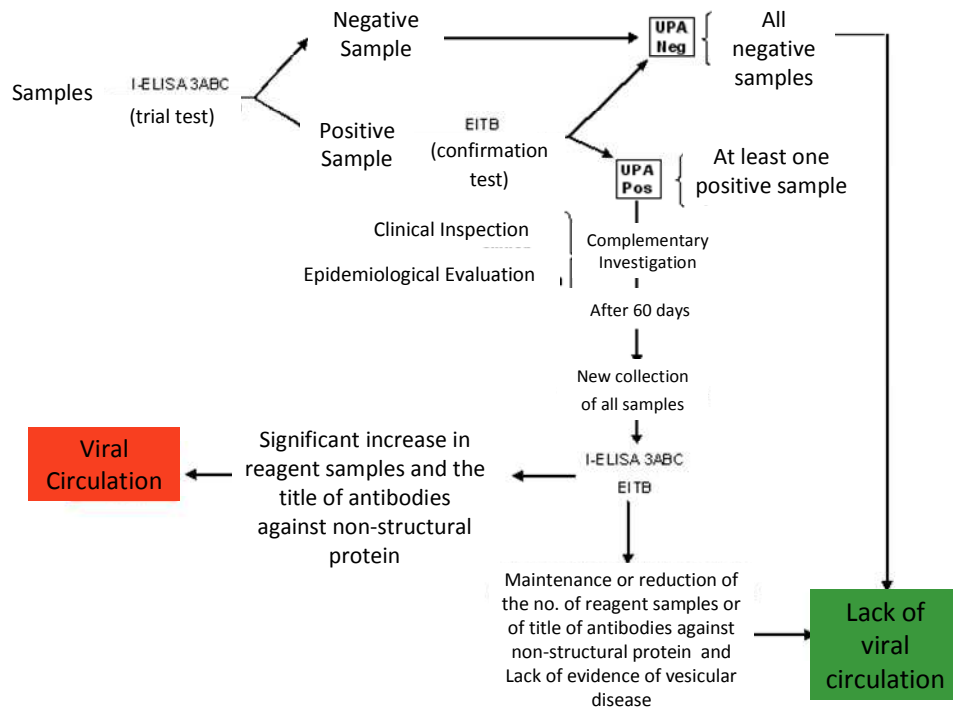


Figura 6. Graphic representation of diagnostic method to be used

Complementary activities used to research and clarify events observed in PSUs with increase of positive animals to diagnosis system may involve:

- new clinical inspection of involved animals;
- evaluation of movement of FMD susceptible animals in holdings with positive animals, investigation and inspections of other holdings involved in entry and exit of susceptible animals;
- collection of OPF for viral survey in positive animals; and
- new collection of blood serum for sampled animals or other existing susceptible animals in involved holdings.

Epidemiological evaluation also includes specific studies to support the population interpretation of diagnostic test results. Among studies carried out one can highlight:

- evaluation of positive animal distribution in relation to vaccination record and animal age. Especially in herds submitted to mass vaccination, in the case of the presence of FMD virus, it is expected to have a larger concentration of positive results in younger animals with fewer FMD vaccination events.
- analysis of population feature in relation to reactions to I-ELISA 3ABC. The behavior of sampled population in relation to I-ELISA 3ABC tests can be evaluated by preparing a frequency distribution graph related to reactivity to antibodies against non-structural proteins of FMD virus. This analysis allows for the construction of reactivity features for populations with different epidemiological situations. Figure 7 presents an example of the application of this study in Brazil, with the comparison of results obtained in areas of FMD occurrence and areas with proven absence of FMD viral circulation, like the case of the FMD free zone without vaccination represented by Santa Catarina state.
- analysis of the space location of holdings with positive animals to diagnosis system used. In the case of the presence of viral circulation the creation of clusters for positive holdings is expected. This condition has been researched by using Splancs library, R environment, version 2.4, using estimates for K function with 95% reliability level.

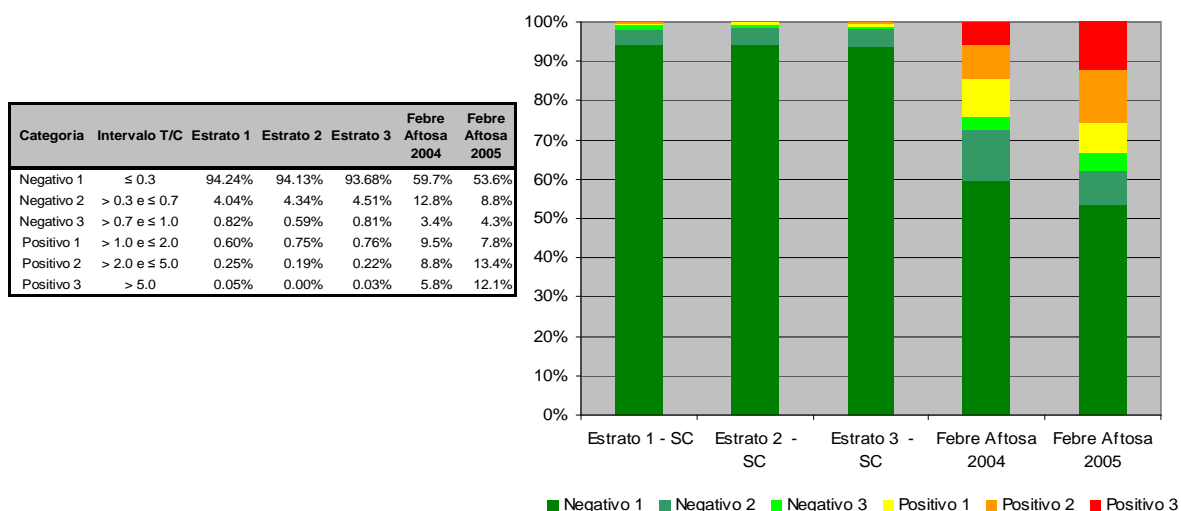


Figura 7. Graphic representation of distribution result of I-ELISA 3ABC test, referring to FMD -free zone without vaccination (Santa Catarina state) and regions with FMD occurrence in Brazil

According to what can be observed, the interpretation of lab results is complex and requires a great deal of research and complementary procedures. In order to be successful it requires the correct registration of information related to sampled animals and holdings. This will be addressed in the next item of this document.

Procedures for information registration

As it was reinforced in this document, information registration is fundamental for the success of studies. Considering the impossibility of establishing a single model, that can be applied to all sanitary, geographic and livestock conditions in which surveys and monitoring will be applied, it is important to create specific forms for the application in every stage of the activity. However, based on accumulated experience starting from 1997, it can be observed that some procedures and some information have remained constant. Next, we present the main forms used in surveys carried out in Brazil until the present date, with instruction for completion. It can be highlighted, however, that changes and adaptations will have to be made for each new study carried out.

- **Previous-visit form**

This form is especially used for epidemiological surveys, however, in some cases, it can be disregarded. Its main objective is to support the process of PSU constitution and planning for following stages of the study, especially those related to the collection of serum. It represents a set of important information for projection and preparation of state teams in relation to troubles that they might find during the sample collection process. Among the main aspects related to this information, we have a) identification of particular features of each holding which constitute a PSU and problems that they may offer for serum collection; b) support to process of PSU constitution in the field; c) establishment of the number of samples to be obtained in each PSU and its distribution among holdings or estates that are part of it; d) activity planning for collection and preparation of kits; e) characterization of holdings in terms of FMD vaccination and features of holdings and production systems. Considering all points that have been presented, the constitution of PSUx stands out, since it will be carried out simultaneously to the application of forms of previous visits. The process, as described, consists on a set of actions whose aim is to verify if visited holding has the minimum requirements for the constitution of a PSU, that is, if the holding has the minimum number of elementary units of sample population. In Annex 4, a previous visit model form is presented, as well as instructions for completion:

- Field 01: reserved for information of each holding that was randomly selected for the 1st visit. In cases that the incorporation of new holdings is required for the constitution of a PSU, the PSU number from the previous visit form should be copied into all forms without identification. Finally, put together (staple) all related forms of a single PSU, based on the initial form, which should have been given by the regional coordinators.

- Field 02: Code of holding

It should be completed with the code used by the state veterinary services for the identification of holding. It is fundamental since it represents the code used to identify holdings that are part of the same PSU.

- Field 03: Number of Estates E__ and Name _____

In case the holding, part of a PSU has separate estates, for each one of them a previous-visit form should be completed. For this purpose, use previous-visit forms without any identification. Identify the estates by copying the code of related PSU into field 1. The field "Estates" should be completed with their own sequential number, for example, if a holding owns 5 separate estates and they all have animals aged 6 to 24 months, these estates will be given code E1 to E5. For each numbered Estate, a name can be used, if there is one (note that the name of E1= headquarter estate). For each Estate, complete one previous-visit form.

Note.: Estates are defined as areas that exist inside holding, comprising a set of grazing areas/ Winter pastures with own structure (pen, farm hand, horses...) where a bovine herd can be found with handling that is independent from other herds in the holding.

- Field 04: Date of visit __/__/__

It represents the day that the visit occurred and information was collected

- Field 05: Geographic coordinates: Latitude____ - Longitude ____

Field for inclusion of information related to geographic location of holdings. To complete this field, geographic coordinates should be obtained at the headquarters of holdings or estates. This piece of information is fundamental for demonstration of the sample distribution process and for the re-evaluation related to space distribution of holdings with or without seropositive animals. The configuration of GPS devices and information on datum and type of geographic coordinates should be previously standardized.

- Field 06: Information of breeder and holding

Basic identification, containing the following information:

name of holding;

name of breeder, in case there are leaseholders, complete with the main breeder (owner);

total grazing area in Ha: this information is required so that the database system can calculate the animal capacity rate and support the evaluation of animal concentration. This supporting information is important for the interpretation of lab results

contact telephone number: information to be especially used for future contacts of sample collection preparation activities.

condition of holding: if it has electricity, restraints employees to help;

features of holding: important issue to establish risk classification for existing livestock farming and also to provide support for interpretation of lab results. Issues presented can vary among regions, as an example, the following questions are posed: if predominant activity is beef cattle raising or dairy cattle raising (in case of beef-cattle farms inform predominant production stage and for dairy farms inform predominant production system), frequency of entry and exit of bovine animals, identifying predominant purpose; FMD risk factors, total number of workers who handle animals (remember to give instructions on clinical signs compatible with FMD and the importance of prompt communication to official veterinary). In this respect it is recommended that a booklet is prepared and handed to all holdings involved in the survey.

- Field 07: Existing bovine and buffalo herds

In the example used, the animal population is aged 6 to 24 months. For this reason, age for this age group is divided into 6 to 12, 13 to 18 and 19 to 24 months. This subdivision, despite its difference from the age group definition found in GTAs and records of official veterinary services, is important to evaluate the possible

number of vaccines applied to animals analyzed. Therefore, this field should be filled out based on the interview carried out during the visit and not only based on official records. Another aspect to be observed is that there are specific fields for the total number of animals of sample population, according to what is shown in the Figure below. This field will be fundamental for decision-making in relation to the constitution of PSUs. Based on numbers notified, the decision will be made by the information collection team and later approved by the regional coordinator, who will decide whether or not neighboring holdings or those that are close will be required for the constitution of the PSU. The PSU should have a minimum amount established during the outline stage. It is important to highlight that this field should be filled out according to interview carried out and not to records of local veterinary units..

07 - Rebanho bovino e bubalno existente:

Espécies	6 a 12 meses		13 a 18 meses		19 a 24 meses		Total	24 a 36 meses		> 36 meses	
	M	F	M	F	M	F		M	F	M	F
Bovina											
Bubalina											
TOTAL											

Total to constitute a PSU

- Field 08: Two last FMD vaccines given to breeders' livestock herds./ day/month/year, lab code and vaccine batch..

This field has significant importance for the process of lab result interpretation. It is known that there is a possibility of multiple vaccination interference on lab results, both in relation to the number of vaccines applied and vaccine used. Another important aspect to be considered is the period of time between the last vaccination and blood serum collection. Therefore, the establishment of the sample collection schedule should take into account the date of last vaccination. The completion of spaces, from left to right, should start by the most recent date (last vaccination). The field for lab code should be filled out with defined codes and those that can be found in the form itself (on the right of the field).

- Field 09: Other domestic susceptible species.

The information on the existence of other susceptible species has a major epidemiological value to support the final evaluation of lab results, these animals can be taken into account in research activities, when required.

- Field 10: Have animals been ear-tagged yet? Is there resistance, on behalf of breeder to eartag animals?

The objective of this information is to provide support to the planning process of serum collection activities, with the projection of possible troubles that might be found. Animal identification is fundamental for the success of the survey, for this purpose, special ear tags should be acquired, according to the projection of total elementary sample units. It is important to reinforce the importance of use of special survey ear tags. In case it is not possible and animal has already got its own identification, this identification can be used, with its number associated to survey ear tag.

- Field 11: is there an intention to sell bovine animals younger than two years of age in the next 90 days? How many?

This question has the objective of helping the process of PSU constitution. In case breeder is a cattle trader and has the intention of selling bovine animals younger than 2 years of age in the next 90 days, these amount projected to be sold should be deducted from total amount in field 07 (only for bovine species). In this fashion, if the difference between the total number of bovine animals aged 6 to 24 months of field 07 and the projection of animal sales is inferior to the amount established, other holdings should be incorporated until the minimum number condition of animals of target population is achieved. On the other hand, if sales forecast represent the total elimination of animals in the age group considered, this holding should be replaced (remember that for holdings to be incorporated

or replaced they should be close and have a similar livestock farming system to the holding chosen by sample process. The replacements have to be approved and checked by regional coordinators.

- Field 12: Total number of bovine animals aged 6 to 24 months to be considered for the PSU constitution and calculation of sample number..

This field should register the result of calculations with the objective of a PSU constitution, just as described in previous item,

- Field 13: Evaluation result, if holding has or not conditions to constitute a PSU.

It should be highlighted that the considered condition represents the existence of bovine animals of sample population in the minimum amount that was established. **This is the only condition for elimination of holdings in the PSU constitution process.**

In case the holding is not used for a PSU constitution, the field should be filled with NO, moving on to neighboring holding to continue the activity of PSU constitution. The initial form should be forwarded (stapled together) with other forms used in the PSU constitution.

- Field 14: Statement of owner or person responsible for animals, in case the herd is used to constitute a PSU.

In case field 13 has been filled out with YES,, it must be necessarily signed by owner or person responsible for animals. This field has been included so that the official services can have more guarantees.

“I declare that I am aware that the holding will take part in a survey of FMD viral circulation and that FMD vaccination for animals in the farm can only occur with approval and acknowledgment of the official veterinary service. I have also been informed that the movement of animals involved in the survey, as well as the occurrence of any clinical signs compatible to vesicular diseases must be immediately notified to the official veterinary service”

- Field 15: people responsible for information collection

This completion of this field is mandatory. Forms without the identification of those responsible will not be accepted.

The form should be completed in three copies. The first copy should be forwarded to state coordination, the second shall be sent to regional coordinators and the third stays with the team responsible for information collection

- Collection report - Stage 1

In Annex 5 an example is presented of the sample collection form used in the first stage of the study. This form does not require the use of the previous-visit form but requires a more complete and updated database in relation to holdings and the respective number of bovine animals according to age group. Its application considers at the same time PSU constitution and sample collection and therefore requires more participation from in charge of collection teams. It should be used in cases where time to carry out activities is reduced, making it impossible to have previous visits.

This form was chosen because it includes the possibility of PSU constitution and presents all required information for identification and qualification of collected samples. In the sequence, guidelines and instructions for completion are presented:

- Forms to start the process of PSU constitution should be submitted to field teams with items 1,4,5,6 and 7 filled out by state coordination, based on the list of holding randomly chosen by DSA/SDA/MAMA. Other non-completed forms should be delivered to teams for use in case of replacement or complement.

Based on visit to holdings, those responsible for form completion, should classify the rural establishment marking an X, in options available on item 2:

- Complete: holding with enough number of elementary units, which can comprise a sample population (age group defined at study outline).
- Replaced: holdings without bovine animals of the age group established as sample population (only condition to be considered for holding replacement).
- Incomplete: holdings with amounts that are lower than the amount of elementary units established for the PSU constitution.
- Complimentary: holding chosen to complement the minimum condition of elementary units established for a PSU constitution. The box should only be ticked in case of complementary holdings, for holdings of origin, which have been randomly chosen. Only the box incomplete” should be ticked.

In cases of replaced and complementary holdings, field teams should transcribe to field 1 of the new form, the same PSU number of origin for both. The correct completion of this field is very important since the division of holdings into PSUs will be based upon it.

Forms of holdings that have been randomly chosen and that have been replaced because they do not have bovine animals of the age group defined should be enclosed to forms of replacing holdings. Further fields of the form do not have to be filled out.

Item 3 should only be filled out for complementary holdings, letter A should be used for the first complementary holding and further alphabet letters, sequentially, for other holdings of a PSU. For the initial holding, which originated the PSU constitution, this item should be left blank.

- Item 8, Coordinates: remember to standardize configurations of GPS devices.
- Item 9, Existing bovine herd: herb should be updated based on information provided by owner or representative during visit to holding. The item cannot be filled out based on figures found in local veterinary units.
- Item 10, Existing herds: inform the existing total number of other FMD susceptible species.
- Item 11, Last vaccination against FMD. Fill out according to guidelines given for the previous-visit form
- Field to register information related to bovine animals with sample collection, fields for up to 60 samples:
 - Animal identification: write number of survey or monitoring ear tag in column “survey”. If animal has a permanent identification and the owner does not approve the use of survey ear tags, the number of survey ear tags should be written in column “survey”, and the number of permanent identification in column “Owner”.
 - Sex: M for male and F for female.
 - Age (months): field to be completed with the estimated age of sample animal.
 - Vaccine dose: field to be completed with the estimated number of FMD vaccines given to sample animal, according to owner or those responsible for the animals. The person in charge of the collection team should make a technical analysis between the age of sample animal and the stated number of vaccines applied. He/ She should make a note at the back of the form, when necessary, cases where the number of vaccines is very different from that expected.
 - Born in holding (Y or N): field to identify animals that have or not been born in the holding. In case animal was born in the holding, complete the field with Y (yes), if it came from other holdings, write N (no).

- The veterinarian from official veterinary service, in charge of the collection team should carry out a clinical evaluation of sample animals, with inspection of feet and mouth, as well as a general check of other susceptible animals in the holding. When there is no evidence of vesicular disease occurrence, the veterinarian should sign the certification field corresponding to item 13. The presence of clinical signs suggesting the vesicular disease should be addressed as confirmed case of vesicular disease with the application of due procedures. In this case, the certification field should be filled with the term ENCLOSED ANNEX. Remember biosecurity requirements, the team should immediately go back to local veterinary unit of holding municipality location for the immediate notification to the veterinarian in charge and adoption of corresponding sanitary measures.
- The owner or person responsible should be notified in relation to responsibilities described in item 13, and sign the corresponding field

“I am aware that the animals above identified cannot be vaccinated against FMD, sold or transported without approval of the official veterinary service. I am also aware of the need to immediately inform the official veterinary service of any problems that might occur with sample animals such as death, loss of ear tag, theft, among other things”

- The signature of the form is mandatory. It must be signed by the state coordinator (item 14).
 - In the period of time between the first and second collections, the official veterinary service should program at least two inspections to holdings that constitute PSUs.
- Collection form - Stage 2 (example Annex 6)

Since it represents a sequential activity, most information collected in the first stage of study should not be obtained again. The stage-2 form should be only used to identify PSU, its location, register collected samples in second stage of study, identify the reasons of a possible lack of collection, check the result of clinical inspection carried out in sample animals, as well as the general inspection carried out in the rural holding, register collection dates and sample shipment and collect signatures of those in charge of collection and sample check and shipment to LANAGRO.

Forms should be made available for collection teams with the following fields already filled out, provided that data was available in database from the first stage of study:

- Identification of PSU;
- Name of municipality;
- Name of rural owner;
- Name of owner;
- No. of registration at local veterinary unit, and
- Identification (ear tag number) of bovine animals to carry out new collection

Printed forms should be forwarded to field teams so that they can carry out inspection activities and sample collection. If complementary information is required, the official veterinary service should prepare specific forms, which should be later forwarded to DSA/SDA/MAPA.

The “N” field of the lab form should be filled out by the state coordination, according to what was scheduled with LANAGRO.

On the table for bovine identification for new collection, as mentioned previously, the field for animal identification (ear tag) should be filled out by the system. Further fields should be filled out during collection activities, taking into consideration the following guidelines:

- Vaccinated after 1st collection: write “Yes” if the person responsible for herd informed that animals have been vaccinated (in this case samples should be collected anyway) or “No”, if animals have been kept without vaccination. In case vaccination occurred, the official veterinary service should adopt and present to DSA/SDA/MAPA actions that are pursuant to law to punish the violators.

- Reason for lack of collection: if animal has not been found, the professional in charge of collection should mark an X in only one of the options available: death, loss of ear tag, not found or other.
- As it was highlighted on the Table for sample identification, in case of death, not found or other, complementary information should be provided in appropriate field, always referring to the number of line related. In case of movement or undue trade, adopted punitive actions should be presented.

The completion of fields for registration of collection dates and sample shipment to LANAGRO is mandatory.

It is mandatory that the official veterinarian in charge of sample collection carry out the clinical inspection of animals and epidemiological evaluation of animal herds susceptible to FMD in the holding and sign the corresponding certification field, in case evidence of vesicular diseases is not identified.

After checking samples and form completion, it is mandatory that the state coordinator write his/her name and sign the corresponding fields.

Procedures for sample identification and collection

1. Material preparation

The regional coordinator is in charge of preparing and delivering material for blood serum collection. The amount of material that each veterinarian should receive depends on the number of holdings to be visited and the number of animals involved in collection. The material should be divided into kits for each holding. Remember to include ear tags with sequential numbering for each prepared kit.

2. Pre-collection

Each person in charge of a collection team should check all material received carefully. Pay attention to biosecurity procedures, for example, all teams must wear clean overalls and rubber boots, all individual protection material must be cleaned and sanitized after activities in each holding.

3. Animal identification

Animals submitted to blood collection must be identified individually. Ear tags should be, preferably, applied to the left ear. The location for ear tag application should be sanitized with iodophor at concentration 1.200. The ear tag should be applied to ventral midline of ear. The front part of ear tag with identification number should be exposed to make reading easier.

The number of ear tag applied to animal should be registered in the label of the vacutainer tube and the sample collection form. The person in charge of collection should check the accuracy of these transcriptions. No tubes can have a repeated code.

Ferreira & Meirelles (2002)*, observed 336 bovine animals of the nelore breed under grazing conditions, divided into two holdings located in Mato Grosso do Sul state and realized that, even with recent improvements of the ear tag application (male and female), it is expected to have the loss of approximately 1.2% of ear tags after 6 months..

In case the owner does not allow the application of survey ear tags (which should be always avoided) and the animal already has an identification ear tag or other permanent identification, write down in sample collection form, in proper field, both the number of identification ear tag in serological survey and the number the animal has already been identified with at holding. The identification ear tag for serological survey, which has not been used, should be given to regional coordinator in order to avoid its use in another holding generating samples with similar number. This ear tag should be forwarded to Regional Coordinator together with samples of holding.

The sample collection form should be carefully completed, with a blue ball-point pen and legible handwriting. Ear tag number for animals selected and identified in sample collection form should correspond exactly to the number of

corresponding serum flasks. Repeated numbers for animals or flasks should not occur in collection form. Note that in order to make it easy for typing, consolidation and recovery of data, activities should be organized so that the entry of numbers in form is sequential.

4. Sample collection

Blood collection should be made under aseptic conditions, with the use of clean boots and overalls, sterile disposable material as well as antiseptics of site of puncture.

A special vacutainer needle appropriate for tubes should be used for each animal. This needle, which should be previously adjusted to the applicator, will be inserted into the bovine jugular vein, with the vacuum collection tube placed on the free end of the needle, pressing and piercing the rubber cap. Blood will flow into the tube. Blood amount should represent approximately 60% of total tube volume.

If there is obstruction or breaking of needle, the collection can be performed with an ordinary disposable injection needle (one for each animal) using the traditional method and the same tube, the veterinarian should only remove rubber cap during blood dripping process.

After collection, tubes should be placed on specific shelves or isothermal boxes and supported for example, by newspaper sheets. Collection teams should wait clot retraction and transfer blood plasma to centrifuging flasks with the same label of the flask used in collection. Plasma transference should be performed by using disposable pasteur pipettes (use one pipette per sample).

5. Material discarding

All disposable needles used should be discarded in recipient with disinfectant solution (Biocid 1:200, calcium carbonate at 4% or citric acid at 0.2%) All material used should be disinfected, packed in garbage plastic bags and taken from holding to community service office. The final destination of this material should be burial or incineration in appropriate location. It is the duty of regional coordinators to identify this location and consider landfills, boilers for industrial incineration in universities, hospitals local government buildings etc...

6. Collection and preparation of serum sample

Tubes containing serum should be centrifuged at a speed of 3000 to 5000 rpm, for 5 to 8 minutes. Serum obtained should be transferred to an "eppendorf"- type tube, already labeled with the same tube number of centrifuging. This labeling should be made with a blue ball-point pen on a white adhesive tape, horizontally placed on tube or directly on flask, with appropriate pen. Both centrifuging and transference of serum to tube should be performed on a clean and closed place, so that the possibility of infections is minimized.

Please note that the serum obtained, to be submitted to regional laboratory should be clear, have maximum 1.5 ml of volume. Do not fill tube (use only $\frac{3}{4}$ of total tube), because by freezing there will be an increase in volume with risk of content leakage.

7. Check of serum quality, number of tubes and sample collection form

The veterinarian in charge of blood serum sample collection should check all fields of the "Form of sample collection". Ear tag numbers should be associated to only one "eppendorf"-type tube. After checking is finished (including the legibility of information), the person in charge should sign the appropriate field of form, according to instructions.

8. Samples packing for each holding

In order to be shipped to laboratory, samples should be packed in appropriate plastic bags containing samples of only one holding. It is vital that samples are kept under refrigeration and are appropriately identified. Label for each bag should contain the following information:

- a. number of survey identification;

- b. code of holding;
- c. name of municipality and
- d. number of the first and last flask of samples.: 30.000 to 30.050.

9. Transportation to state reception and check center

Field teams are in charge of handling serum packaging with ice and corresponding checked collection sample forms to regional coordinator according to the schedule established (we suggest that collections are performed in the morning, and material checking is done in the afternoon).

The regional coordinator should make sure that serum packages are kept under refrigeration until the moment they are shipped to the state center.

10. Activities of state coordinator at reception and check center

The State Coordinator is in charge of structuring activities to check information of Sample Collection Forms and also tubes received for each holding. Packages should be opened in the laboratory and tubes should be checked in relation to:

- a. quality (there should be no hemolysis or contamination);
- b. amount (minimum volume of 1,5 ml);
- c. equivalence between the number of tubes and ear tags of Sample Collection Form; and
- d. storage and conservation (or refrigeration) of samples.

Activities of tube checking should be performed as fast as possible since serum packages, properly identified, should be kept under refrigeration.

Note: For an appropriate completion of adhesive tape, the piece of adhesive tape should be placed into a smooth surface, information should be written on it with a blue ball-point pen in legible handwriting. The label should then be placed on the upper front side of package.

For shipment of samples to MAPA laboratories, Eppendorf flasks should be placed into a Styrofoam plate, prepared according to the distribution of ELISA's plates. State veterinary services have the model, which should be used. It is important that the shipping of serum follows the total number of samples for a given PSU. In this aspect, activities carried out between 2003 and 2004 have showed appropriate levels of control. The experience of professionals who worked in this area should also be taken into account. It is extremely important that samples are shipped with the proper identification. Each Styrofoam plate put together should have, at least, the following information:

- a. identification number of survey;
- b. code of holding;
- c. name of municipality; and
- d. sample numbers according to their distribution on plate.

Annex 1 - Basic list of material, equipment and forms for assistance to notification of vesicular disease suspicion

1. Notice for farm blocking and release from blocking
2. FORM-IN and FORM COM
3. Small board for note making
4. Paper for note making
5. Rubber boots (or shoe protector)
6. Overall (aprons)
7. Plastic and surgery gloves
8. Nose tongs
9. Mouth opener
10. Rope
11. Thermometers
12. Scissors and a bladed scalpel
13. Gauze
14. Tweezers
15. Syringes and needles
16. Brush
17. Collection flasks with Vallée liquid
18. pH indicating paper or Vallée liquid with pH indicator
19. Test tubes or vacutainer
20. Adhesive tape
21. Ear tags and applicator for animal identification
22. Antiseptic
23. Sanitizers
24. Sprayer
25. Absorbent paper (paper towel)
26. Plastic bags
27. Plastic bucket
28. Styrofoam-type cooler
29. Farm blocking sign
30. Box to store and transport material
31. Sterile swabs for material collection to support differential diagnoses (IBR and DVB)

Annex 2. Procedures for selection of animals to be sampled in each holding

Observe the number of samples to be collected, which will be informed by the state coordination team for serological study. The same number of animals should be randomly selected from the total number of animals participating in sample population (age group). For such, make sure to check the current number of animals of the age group, which constitute the sample population for the HOLDING. **Attention, this number cannot be inferior to that indicated for sample collection.** Then, divide the total number of age group by the number of animals to be sampled, if result is inferior than 2, we suggest the use of the simple random method indicated in item A of the table below. On the contrary (results > 2), should be processed as indicator of item B for the systematic random method. The following examples demonstrate both sample methods:

a) Simple random sampling:

In order to draw a sample of X animals, start anywhere at Table of Random Numbers (Annex 2) from 1 to 30 and draw numbers along columns and lines. If a repeated number is drawn, or one that is not included in the interval of intended numbers, continue choosing the next number on Table.

Example (check **Table of Random Numbers** - Annex 3) to draw a random sample of 20 animals in a holding with 30 animals belonging to a PSU:

- (1) Start anywhere at the Table
- (2) Select, in column below the chosen point, one number at a time, writing it down on a separate piece of paper. Reject repeated numbers.

For example, in the sequence of numbers presented below (starting at number 9, on the first line of Table and going down vertically) the 20 underlined numbers would be drawn:

Random numbers:	9	25	26	22	7	28	14	29	11	25	28	6	2	8	11	9	11	28	16	9	16	4	11	9	20	19	2	17
Chosen numbers:	<u>9</u>	<u>25</u>	<u>26</u>	<u>22</u>	<u>7</u>	<u>28</u>	<u>14</u>		<u>11</u>			<u>6</u>	<u>2</u>	<u>8</u>					<u>16</u>		<u>4</u>				<u>20</u>			<u>17</u>

Random numbers:	11	21	10	20	4	26	4	15	3	24	3	16	4	6	16	2	10	17	11	8	2	16	8	15	13	16	5	26
Chosen numbers:		<u>21</u>	<u>10</u>						<u>15</u>	<u>3</u>																	<u>5</u>	

The sample will be made up by animals: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 17, 20, 21, 22, 25, 26 e 28
 In order to draw samples, make all animals go through restraint or gate until the corresponding animal, which was drawn comes by. In farms where animals are kept separately (according to ear mark, for example), the numeric order will simply be the animal sequence.

b) Systematic random sampling:

In systematic random sampling we have the drawl of animals belonging to a population made up of **N** animals. The **n** animals are drawn in regular intervals, which can be = N/n . First a random number that is inferior to N/n is drawn (check Table of Random Numbers presented in ANNEX 2). Then animals in interval equal to N/n are drawn.

Example: in order to obtain a sample of 30 animals in a beef cattle herd made up of 300 animals:
 (1) draw a random number between 1 to 10 ($N/n = 300/30 = 10$), checking Table of Random Numbers, for example 9 (first line of table);
 (2) make all animals go through restraint; sample animal no. 9; then sample in intervals of each 10 animals ($N/n = 300/30 = 10$): 19, 29, 39, 49, 59, 69, 79, 89, 99, 109, 119, 129, 139, 149, 159, 169, 179, 189, 199, 209, 219, 229, 239, 249, 259, 269, 279, 289, 299

Annex 3. Example of Table of Random Numbers

27	25	15	23	23	11	9	15	15	23	18	27
22	21	10	1	4	22	25	3	28	11	19	15
14	20	26	24	7	22	26	24	24	3	24	9
16	26	18	15	24	23	22	3	2	24	10	20
11	23	25	29	13	28	7	16	6	26	13	18
8	14	20	25	27	5	28	4	28	18	28	15
18	10	6	12	5	5	14	6	26	1	3	15
25	1	2	11	6	9	29	16	18	14	27	17
2	25	4	5	23	2	11	2	1	7	18	9
3	5	20	15	1	12	25	10	29	11	4	16
13	12	15	3	6	19	28	17	9	28	16	19
8	10	28	12	3	23	6	11	22	7	12	8
12	27	26	2	10	20	2	8	14	16	11	3
2	19	3	5	7	6	8	2	17	24	13	16
5	4	26	19	1	23	11	16	5	3	14	7
16	2	2	23	27	8	9	8	15	21	29	10
26	5	7	26	6	5	11	15	12	13	12	28
29	22	9	10	14	1	28	13	20	19	29	9
17	28	29	27	7	16	16	16	16	23	7	28
12	5	17	17	22	26	9	5	17	19	15	25
16	25	29	6	28	18	16	26	22	19	24	11
10	20	2	10	18	26	4	16	19	27	21	3
21	17	8	26	15	22	11	9	5	3	29	19
2	7	17	28	15	2	9	28	28	19	1	19
7	15	26	2	11	17	20	2	8	27	19	16
5	7	23	1	3	8	19	14	19	11	21	26
19	6	12	2	18	28	2	19	26	17	19	11
13	9	22	11	14	25	17	4	6	14	23	10
21	3	28	2	4	8	11	27	8	14	14	9
3	17	3	13	26	6	21	13	7	20	15	25
10	23	11	13	12	29	10	1	6	28	26	18
12	3	29	2	16	7	20	3	3	13	16	16
13	4	25	3	15	13	4	26	20	17	19	17
20	3	11	17	13	21	26	27	4	12	28	6
11	21	26	15	19	19	4	29	4	12	4	9

Annex 4. Example of previous-visit form

BRAZILIAN FOOT-AND-MOUTH DISEASE ERADICATION PROGRAM				→ FORMULÁRIO DE VISITA PRÉVIA										
Inquérito para avaliação de circulação viral - ANO														
01 – Identificação da propriedade a ser visitada		02 – código da propriedade		03 - Retiro										
Município:				R _____										
Propriedade:				Número _____										
Proprietário:		04 – data da visita		05 – Coordenadas geográficas										
Identificação da UPA:		____/____/____		Latitude _____										
				Longitude _____										
06 – Informações do produtor e da propriedade														
Nome da propriedade				Área (em ha)										
				Total _____										
Nome do produtor				Pastagem _____										
				Telefone para contato _____										
Condições na propriedade														
Luz elétrica: Não <input type="checkbox"/> Sim <input type="checkbox"/>		Curral para contenção: Não <input type="checkbox"/> Sim <input type="checkbox"/>		Trabalhadores para ajudar: Não <input type="checkbox"/> Sim <input type="checkbox"/>										
Características da propriedade	Sistema de produção principal: Corte <input type="checkbox"/> → Cria <input type="checkbox"/> Recria <input type="checkbox"/> Ciclo completo <input type="checkbox"/> Terminação <input type="checkbox"/> Leite <input type="checkbox"/> → Produção familiar de subsistência <input type="checkbox"/> Produção empresarial <input type="checkbox"/> Produção familiar comercial <input type="checkbox"/>													
	Frequência de ingresso: mensal <input type="checkbox"/> trimestral <input type="checkbox"/> semestral <input type="checkbox"/> anual <input type="checkbox"/> raramente <input type="checkbox"/> Finalidade principal Engorda <input type="checkbox"/> Cria/recria <input type="checkbox"/> Reprodução <input type="checkbox"/>													
	Frequência de egresso: mensal <input type="checkbox"/> trimestral <input type="checkbox"/> semestral <input type="checkbox"/> anual <input type="checkbox"/> raramente <input type="checkbox"/> Finalidade principal Abate <input type="checkbox"/> Engorda <input type="checkbox"/> Cria/recria <input type="checkbox"/> Reprodução <input type="checkbox"/>													
	Fatores de risco: Parada de animais <input type="checkbox"/> Empréstimo de curral/brete <input type="checkbox"/> Próximo a laticínio <input type="checkbox"/> Próximo a aglomerações <input type="checkbox"/> Criador possui propriedade em país ou zona infectada Estrada boiadeira <input type="checkbox"/> Próximo a abatedouro <input type="checkbox"/> Abate informal <input type="checkbox"/> Próx. estradas movimentadas <input type="checkbox"/>													
	Total de trabalhadores que lidam com os animais: _____ → Atenção: repassar orientações sobre os sinais clínicos da febre aftosa e os procedimentos de notificação no caso de suspeitas de ocorrência da doença													
07 - Rebanho bovino e bubalino existente:														
Espécie	< 6 meses	6 a 12 meses		13 a 18 meses		19 a 24 meses		Total	24 a 36 meses		> 36 meses			
		M	F	M	F	M	F		M	F	M	F		
Bovina														
Bubalina														
TOTAL														
08 - Últimas duas vacinações contra febre aftosa realizadas no rebanho do criador:														
Dia/mês/ano	Cód. Lab.	Partida	Dia/mês/ano	Cód. Lab.	Partida	Códigos a serem empregados para Laboratórios:								
						Bayer	BA	Intervet	IN	Vallée	VA			
						Coopers	CO	Merial	ME	Pfizer	PF			
Mais recente			Anterior a mais recente											
09 - Outras espécies domésticas susceptíveis:														
		Ovinos		Caprinos		Suínos								
		< 6 meses	≥ 6 meses	< 6 meses	≥ 6 meses	< 6 meses	≥ 6 meses							
		Total												
Data da última vacinação (dia / mês / ano)														
10 – Os animais já estão brincados? Não <input type="checkbox"/> Sim <input type="checkbox"/> Existe resistência do criador em brincar os animais? Não <input type="checkbox"/> Sim <input type="checkbox"/>														
11 – Pretende vender animais abaixo de 2 anos nos próximos 90 dias? Não <input type="checkbox"/> Sim <input type="checkbox"/> Quantos? _____														
12 – Total de bovinos com idade entre 6 e 24 meses a ser considerado para constituição da UPA e para cálculo do número de amostras (= Total Campo 7 - Campo 11) _____														
13 – Resultado da avaliação → propriedade contempla as condições para constituir uma UPA? Não <input type="checkbox"/> Sim <input type="checkbox"/>														
14 – Declaração do proprietário ou responsável pelos animais, no caso do rebanho ser empregado para constituir uma UPA														
Declaro estar ciente de que a propriedade irá participar do estudo de circulação viral para febre aftosa e de que a vacinação contra a febre aftosa dos animais existentes na propriedade somente poderá ocorrer mediante autorização e conhecimento do serviço veterinário oficial. Também fui informado de que a movimentação dos animais envolvidos no estudo bem como a ocorrência na propriedade de quaisquer sinais clínicos compatíveis com doença vesicular deverão ser imediatamente notificadas ao serviço veterinário oficial														
Nome (legível) _____														
Assinatura _____														
15 - Responsáveis pelo levantamento das informações:														
Chefe da equipe de colheita:		Nome legível _____		Assinatura _____										
Coordenador Regional:		Nome legível _____		Assinatura _____										
1.ª VIA: Coordenação Estadual --- 2.ª VIA: Coordenador Regional --- 3ª VIA: Equipe de colheita														

