HEALTH MONITORING OF ANIMALS IN THE EXPERIMENTAL UNITS

MONITORIZAREA SĂNĂTĂȚII ANIMALELOR ÎN UNITĂȚILE EXPERIMENTALE

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ABSTRACT | **REZUMAT**

Procedures of research projects using animals are run under an *authorization* of the research project advanced (mandatory starting January 2018) in authorized and highly specialized units, so that the required biosecurity level and health monitoring may be granted.

The aim of the paper is to promote the *Experimental Units* of *"Horia Cernescu" Research Unit* to veterinarian research field.

Monitoring microbiological status of the animals used in procedures is a requirement testifying to reproducibility of experimental results; such monitoring is needed, as microbiological status has a bearing on health, well-being, experimental variability and results of research projects. Monitoring program requires usage made of materials and of human resources, also analyses run, and a report drawn that covers all of the aspects concerned by monitoring representative samples. Microbiological status monitoring program will be individualized for each microbiological unit and samples drawn for each microbiological unit must be representative, accurate and clear; with the clinically healthy animal stock, determining of the minimum number of animals is mandatory, for the monitoring sample and for the randomized drawing thereof.

This study describes the practices of sampling and screening associated with microbiological status monitoring in *experimental units of "Horia Cernescu" Research Unit*.

Keywords: research, health monitoring, sampling, experimental units

Cercetările care utilizează animale în proceduri se desfășoară după prealabila autorizare a proiectului de cercetare (obligatoriu, începând din ianuarie 2018) în unități specializate și autorizate, astfel încât să se poată susține nivelul de biosecuritate și să se permită monitorizarea stării de sănătate a animalelor.

Scopul lucrării este acela să promoveze *Unitățile experimentale din cadrul Complexului de Laboratoare de Cercetare „Horia Cernescu"* către cercetătorii din domeniul medicinii veterinare.

Utilizarea animalelor în proceduri impune considerarea *statusului microbiologic* al animalelor, deoarece acesta poate influența sănătatea, bunăstarea, variabilitatea experimentală și rezultatele proiectelor de cercetare științifică. Reproductibilitatea rezultatelor experimentale se asigură prin utilizarea animalelor ale căror caracteristici biologice și status microbiologic sunt cunoscute prin monitorizarea unor eșantioane reprezentative. Programul de monitorizare a statusului microbiologic se particularizează pentru fiecare unitate microbiologică iar eșantioanele extrase pentru fiecare unitate microbiologică trebuie să fie reprezentative - precise și exacte; la efectivele clinic sănătoase este obligatorie stabilirea unui număr minim de animale pentru eșantionul destinat monitorizării și repartiția aleatorie a acestora.

Lucrarea de față prezintă practicile de eșantionare și screening asociate monitorizării statusului microbiologic al animalelor din cadrul *Unităților experimentale ale Complexului de Laboratoarelor de Cercetare "Horia Cernescu".*

Cuvinte cheie: cercetare, monitorizare sănătate, eșantionare, unități experimentale

Usage of animals in procedures requires considering the animals' *microbiological status*, as such status may have a bearing on health, well-being, experimental variability and research projects results.

Reproducibility of experimental results is granted by usage made of animals whose biological features and microbiological status are known.

Microbiological Status Monitoring Program (MSMP) of animals used in procedures requires employing personnel skilled for the veterinary epidemiology sanitary field (i.e. experienced and having their expertise certified); such personnel will determine the specific microbiological units, manage a valid samples collection, analyze results of examinations run, as also apply, report and interpret reports drawn.

Study describes MSMP practice, facilities in *Horia Cernescu Research Unit Experimental Units (Fig. 1, 2),* and activity of the researchers involved in the projects

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making use of animals in procedures.

Fig. 1. Activities run in the experimental units
Usage of the swine barn circulation alley for running the required weighings, as per research project provisions (*upper*); experimental swine unit insides, tarp rear ends raised for the research project run time, in spring (*lower*).

MATERIALS AND METHODS

Monitoring program sets out by delimiting *unit*, and *microbiological units*.

In a microbiological view, the animals' spaces and facilities must be structured and organized as *microbiological units* - defined as autonomous microbiological entities, with separate circuits and spaces for the animals, respectively for the personnel and the materials. In the case of the *experimental animals lab unit*, the microbiological unit may be the keeping hall, the species-specific locations, or even one only cage, in the case of the individually aired cages. With farm animals, the microbiological unit is actually the home-farm, or the experimental unit wherein the research project is run.

Examinations for determining microbiological status of the animals may be required as early as after animals acquisition. Matrices and analysis methods (*ELISA* – enzyme linked immunosorbent assay, *MICR* – microscopy, *IFA* – immunofluorescence assay, *CULT* – culture, *PATH* – gross pathology, *PCR* – polymerase chain reaction, *HIST* – histopathology, *NT* – not tested) differ from one microbial agent to the next and from breed to breed (see Tables 1 - 8).

Selection of microbial agents to undergo monitoring is determined by contextual factors, such as: effect thereof upon the animals' health and upon biomedical research;intricacy of research results; prevalence, specificity and zoonotic potential thereof; immune status; and target state of the microbiological unit and results of previous examinations. Noteworthy,identifying a microbiological agent does not impose its elimination.

Fig. 2. Keeping locations
Keeping location for Guinea pigs (*upper*) and keeping location in individual aired cages (*lower*).

The animals used in microbiological status monitoring program (MSMP) can be resident animals and direct or indirect sentinel animals.

Sampling of the animals for monitoring must be adequate and representative for the microbiological unit considered, which is the reason why sampling will be randomized for i) actual animals ii) number of animals, computed by standard relations.

Relation used to compute the number of animals

sampled in microbiological unit *(1.1)* was advanced by ILAR Committee on Long-Term Holding of Laboratory Rodents:

Number of animals = log (1 - confidence interval) / log (1 - prevalence) *(1.1)*

Sample composition is based on the number of factors, as further detailed:

Number of animals existent in each microbiological unit for assessment, which depends on the relationship of etiologic agent with species, breed, age and sex.

Accepted confidence interval (statistic confidence level, 1-α).

Frequently, in biology and experimental medicine,

Table 1.

Recommendations for monitoring etiological agents and monitoring frequency, in mice *(Mus musculus)*

Type of etiological agent *	***at 3 months***	***yearly***
Viruses:		
*Adenovirus type 1 (**MAdV-1**) and 2 (**MAdV-2**)*		x
*Parvoviruses (**MVM** - minute virus of mice, **MPV-1, MPV2, MPV-3** - parvovirosis virus)*	x	
*Reovirus type 3 (**REO-3**)*		x
*Lymfocyter choriomeningitis virus (**LCMV**)*		x
epizootic diarrhea of infant mice virus (MRV/EDIM, rotavirus)	x	
Theiler mouse encephalomyelitis virus (TMEV)	x	
Hemagglutinating virus of Japan (PI-1, SV - Sendai virus)		x
Mouse hepatitis virus (MHV)	x	
Murine norovirus (MNV)	x	
Pneumonia virus of mice (PVM)		x
Bacteria:		
Citrobacter rodentium		x
Clostridium piliforme		x
Corynebacterium kutscheri		x
Helicobacter spp**; in case of positive result, identification continues for **Helicobacter hepaticus, Helicobacter bilis and Helicobacter typhlonius	x	
Mycoplasma pulmonis		x
Pasteurella pneumotropica	x	
Salmonella spp.		x
Streptobacillus moniliformis		x
***Streptococci** β-hemolytic (no enterococci, group D)*	x	
Streptococcus pneumoniae	x	
Parasites:		
Specific endoparasites and ectoparasites **	x	

* *Optionally, and dependent on time/space circumstances, testing may be run for: hantaviruses, herpes viruses, lactate-dehydrogenate elevating virus, polyomaviruses; and for bacteria type **Klebsiella oxytoca, Klebsiella pneumoniae, Pneumocystis murina, Pseudomonas aeruginosa, Staphylococcus aureus,** and other such like.*

** *Enteric protozoa: **Chilomastix bettencourti, Cryptosporidium muris, Cryptosporidium parvum, Eimeria spp., Entamoeba muris, Giardia muris, Hexamastix muris, Spironucleus muris, Trichomonas muris, Tritrichomonas muris**); mites: **Myocoptes masculinus, Myobia Musculi, Rafrordia affinis;** pinworms (**Syphacia Obvelata, Aspiculuris tetraptera, S. Muris),** and other such like.*

Source: bibliography compilation (2) and practices (1).

statistic confidence level 1-α is accepted, i.e. the probability that a confidence interval should contain the true parameter value of 95%, 99% and 99.99%, at interval confidence thresholds of (α) 5%, 1% respectively 0.1%.

Depending on research project requirements and specific pathogenic agents, confidence level is selected and, in function of prevalence of the microbial agent (see first column in Table 9), number of animals to undergo monitoring is determined.

Prevalence of pathogenic agents.

In principle, high prevalence is riskier than high incidence: in size terms, prevalence varies in function of the intrinsic characteristics of the animals and of the biological materials, also being influenced by the correct implementation of biosecurity and prevention procedures, the diagnosis tests used and the efficiency of the therapies run. The prior data regarding etiologic agent prevalence and incidence may greatly help the selection of the monitored agents.

Table 2.

Recommendations for etiological agents monitoring and monitoring frequency, in rats *(Rattus norvegicus)*

Type of etiological agent *	***at 3 months***	***yearly***
Viruses:		
*Adenovirus type 1 (**FL/MAdV-1**) and 2 (**K87/MAdV-2**)*		x
*Sialodacryoadenitis, or rat coronavirus (**SDAV**)*	x	
*Hantaviruses (**HANT**)*		x
*Parvoviruses: **H-1** (Toolan's rat virus) **RPV-1, RPV-2, RMV** (Rat minute virus), **RV** (ex-RKV –Kilham's rat virus)*	x	
*Reovirus type 3 (**REO-3**)*		x
*Theilovirus (**RTV** – rat theilovirus)*	x	
*Sendai virus (**PI-1, SV**)*		x
*Pneumonia virus of mice (**PVM**)*	x	
Bacteria and fungi:		
Clostridium piliforme	x	
***Helicobacter spp.**; in case of positive result, **Helicobacter Bilis** typization is recommended*	x	
Mycoplasma pulmonis	x	
Pasteurella pneumotropica	x	
***Streptococci** β-hemolytic (no enterococci, group D)*	x	
Streptococcus pneumoniae	x	
CAR bacilli (CARB – Cilia Associated Respiratory Bacillus)		x
Streptobacillus moniliformis		x
Pneumocystis spp.		x
Salmonella spp.		x
Parasites:		
*Endoparasites and ectoparasites (related to breed level)***	x	

* *Optionally, and depending on time/space circumstances, testing may be run for: bacteria type **Bordetella bronchiseptica, Corynebacterium kutscheri, Encephalitozoon cuniculi, Klebsiella oxytoca, Klebsiella pneumoniae, Pasteurellaceae, Pseudomonas aeruginosa, Staphylococcus aureus** and other such like.*

** *Enteric protozoa: **Chilomastix bettencourti, Cryptosporidium muris, Cryptosporidium parvum, Eimeria spp., Entamoeba muris, Giardia simoni, Hexamastix muris, Monocercomonoides spp, Spironucleus muris, Trichomonas muris, Tritrichomonas muris**; mites (**Ornithonyssus bacoti, Rafrordia affinis**), pinworms: **S. muris, Syphacia obvelata, Aspiculuris tetraptera,** and such like).*

Source: bibliography compilation (2) and practices (1).

If the 95% confidence level is taken as acceptable, the number of animals will be as indicated in Table 9, drawn preferably randomized, especially in the case of the clinically healthy animals, so that presence of microbial agent in the microbiological unit considered can be revealed. With rodents, at the 95% confidence level, and for the 40% infection prevalence, sample will be six animals; if the 99% confidence interval is selected, 10 animals will be drawn. In practice, in microbiological units planned for dogs, cats and pigs, layered samples are drawn, based on animals' age: at least two young animals, and at least four animals for each of the categories 2 to 7 months of age and over 8 months of age; while in units where all age categories are covered, layered sample will be at least 10 animals drawn, randomized.

RESULTS AND DISCUSSION

The health monitoring examinations results are recorded and used in *Report for veterinary sanitary status;* lab examinations results may fall under classes as further detailed.

Negative are the results in the case where the etiological agent was detected neither upon current screening, nor upon prior monitoring.

The results are *positive* where the etiological agent was detected upon both current testing, and previous examinations. In farm animals, the presence of antibodies in animals which were not vaccinated is an index for infection in the microbiological unit, vaccinated animals exempted.

The report may express epidemiological indices as *incidence* and *prevalence* - at microbiological unit considered level.

In specific cases, the index may be NE (not examined), where the microorganisms were examined neither in the past, nor currently or not included in the monitoring program.

Moreover, besides the animals sampled and programmed for the routine monitoring, all of the sick or

Table 3.

Recommendations for monitoring etiological agents and monitoring frequency, in Guinea pigs *(Cavia porcellus)*

*Type of etiological agent **	*at 3 months*	*yearly*
Viruses:		
*Guinea pig Adenovirus (**GpAV, GAV**)*	*x*	
*Guinea pig Cytomegalovirus (**GpCMV**)*		*x*
*Sendai Virus (**PI-1, SV**)*	*x*	
*Guinea pig parainfluenza Virus (**CavPIV-3**)*	*x*	
Bacteria and fungi:		
Bordetella bronchiseptica	*x*	
Clostridium piliforme		*x*
Corynebacterium kutscheri	*x*	
Encephalitozoon cuniculi		*x*
Salmonella spp.		*x*
Streptobacillus moniliformis		*x*
Streptococci β-hemolitici *(no enterococci, group D)*	*x*	
Streptococcus pneumoniae	*x*	
Parasites:		
*Endoparasites and ectoparasites (ralated to breed level)***	*x*	

* *Optionally, and depending on time/space circumstances, testing may be run for: bacteria and fungi type **Chlamydophila caviae, CARB, Dermatophytes, Pasteurellaceae, Pseudomonas aeruginosa, Staphylococcus aureus, Yersinia pseudotuberculosis** and other such like.*

** *Enteric protozoa: **Balantidium caviae, Chilomastix** spp., **Cryptosporidium wrairi**, **Eimeria caviae, Giardia simoni, Monocercomonoides** spp., **Tritrichomonas caviae;** mites: **Chirodiscoides caviae, Trixacarus caviae**.*

Source: bibliography compilation (2) and practices (1).

dead animals (and the probes thereof) are to undergo **anatomopathological examination**.

Necropsy allows the investigator to tell apart the effects generated by a chance experimental protocol, and the effects caused by infections. Necropsy examination results may require an enlarged sample, a specific monitoring frequency, and extra screening of other etiological agents.

The Report for the veterinary sanitary status of the animals.

It will cover the MSMP results; the report will be written by the person in charge for each microbiological unit.

For clarity and easy usage, health monitoring reports are simple, brief and comprehensible, covering:

- *unit name and type, sanitary barrier type: individually aired cages, isolator/quarantine location, keeping locations, and such like;*
- *description of all breeds and lines existent in each unit; in the case where several breeds are kept under same sanitary barrier and restrictions, the health monitoring report will be individualized for each specific breed;*
- *date of latest cross inquiry and date of analysis bulletins presented upon acquisition/population time, and the report issuing date;*
- *testing frequency for each etiological agent;*
- *list of the etiological agents for the monitoring, method and lab;*
- *retrospective results of tests run at least 18 months before, chance tests not included in MSMP bringing extra information;*
- *necropsy examinations results will cover lesions and anatomo-pathological changes, iterated separately for each breed and line;*
- *comments column, possibly indicating, e.g. treat-ments, the latest positive results, the etiological agents for which diagnosis resulted positive, and such like;*
- *contact name and data of the person in charge with the elaboration of MSMP;*
- *general informations on MSMP used (link, procedure, and such like).*

The Report on veterinary sanitary status of animals

Table 4.

Recommendations for monitoring etiological agents and monitoring frequency, in rabbits *(Oryctolagus cuniculus)*

Type of etiological agent *	*at 3 months*	*yearly*
Viruses:		
*Rabbit rotavirus (**ROTA**)*	*x*	
*Rabbit hemorrhagic disease virus (**RHDV**)*	*x*	
Bacteria and fungi:		
*CAR Bacilli (**CARB**, Cilia-Associated Respiratory Bacillus)*		*x*
Bordetella bronchiseptica	*x*	
Clostridium piliforme	*x*	
Encephalitozoon cuniculi	*x*	
Pasteurella multocida	*x*	
Salmonella spp.		*x*
Parasites:		
*Endoparasites and ectoparasites (related to breed level)***	*x*	

* *Optionally, and depending on time/space circumstances, testing may be run for: viruses (adenoviruses, corona viruses and myxoma virus), bacteria and fungi type **Clostridium spp. Dermatophytes, Escherichia coli** (enteropathogenic strains), **Pneumocystis oryctolagi, Staphylococcus aureus, Treponema paraluiscuniculi** and other such like, if the case may be.*

** *Hepatic coccidiosis (**Eimeria stedae**) and enteric (**Eimeria intestinalis, Eimeria flavescens –** the worst pathogenic, and **Eimeria irresidua, Eimeria magna, Eimeria piriformis –** moderately pathogenic; mites: **Cheyletiella parasitivorax, Psoroptes cuniculi;** pinworms: **Passalurus ambiguus**.*

Source: bibliography compilation (2) and practices (1).

will be written by the person in charge, based on afferent documents such as lab analysis bulletins, and accompanying documents received at the time of acquisition of the animals to be used in procedures.

The Report will allow for decision making, and strategies selection, as per the unit's specificity. *An etiological agent (bacteria, virus, parasite...),* once identified and certified to exist in one or more animals in the microbiological unit, will remain notified in the *report;* upon later testing, the etiological agents notified will be screened no more, but all of the positive results will be reported up to total ousting of such etiological agent, *e.g.* by treatments, embryo transfer, hysterectomy, and repopulation by animals from a different source.

Eradication of the infection /infections and of the etiological agent/agents will be ascertained by later testing. The decision to allow introduction of animals in a specific microbiological unit will not be based exclusively on health of monitoring report; even if the report is a basic factor, decision will be made also based on prior documents received from the origin unit, on the data for the MSMP applied in respective unit, as well as on data for the contamination risks.

Considering that over the transfer time the animals' health status may be altered, experimental units will practice quarantining the animals, as also, if needs be, testing of the animals prior to admission thereof to microbiological unit.

Table 5.

Recommendations for seasonal monitoring of etiological agents, in dogs *(Canis familiaris)*

Type of etiological agent	***Antigenic agent***
Viruses to undergo serological[a] test, and faeces[b] antigen test in:	
[a] *Canine adenovirus type 1 (**HCC**)*	*CF, NT*
[a] *Carré disease virus*	*ELISA, NT, IFA*
[a] *Canine parainfluenza virus* [a] *Canine parvovirus (**CPV**)*	*ELISA, HI*
[b] *Rotavirus*	*ELISA faeces antigen test; EM and agglutining*
[b] *Enteric Coronavirus*	
Bacteria and fungi*:	
Bordetella bronchiseptica	*culture*
***Borrelia** spp.*	*serology*
Brucella canis	*culture*
***Leptospira** spp.*	*serology*
***Salmonella** spp.*	*culture*
***Streptococci** beta-haemolytic, serogroup G*	*culture*
Parasites:**	
*All artropods: **Demodex sp.**, when signs/lesions show in dermic scraping, too, **Sarcoptes scabei**, serologic and/by dermic scraping) and all Helminth infections, eimeriosis, **Giardia spp,** and **Haemobartonella canis***	*parasitological examination, and blood smears*

* *Optionally, depending on circumstances and presence of clinical signs, there may also be tested, in bacterial cultures: **Campylobacter** spp., **Escherichia coli, Microsporum** spp., **Pasteurellaceae, Staphylococcus** spp., **Trichophyton** spp., **Yersinia enterocolitica** and **Ehrlichia** spp. by serology and/and PCR.*

** *If the case may be, blood smears: **Angiostrongylus vasorum, Dipetalonema reconditum, Dirofilaria immitis;** by smears and serology **Babesia** spp., by serology **Leishmania** spp. and by histopathological examination, **Filaroides** spp.*

Source: bibliography compilation (4) and practices (1).

The *Report* is an experimental unit home document, available to third parties. The *Report* and parts thereof will be made available to any of the interested parties, pertaining to university and various institutions interested, the *Report* final conclusions addressing the microbiological unit status.

CONCLUSIONS

Microbiological status monitoring program will be individualized for each microbiological unit;

Samples drawn for each microbiological unit must be representative, accurate and clear; with the clinically healthy animal stock, determining of the minimum number of animals is mandatory, for the monitoring sample and for the randomized drawing thereof;

All of the animals sick and dead, and probes thereof, are to be examined;

Microbiological status monitoring program will be final with the report for the veterinary sanitary status of the animals.

Acknowledgments: Present material was supported by Contract no. 6584 din 19.10.2015 carried on in *Horia Cernescu Research Unit,* established by POSCCE infrastructure project - SMIS 2669.

Table 6.

Recommendations for seasonal monitoring of etiological agents, in cats *(Felis silvestris libyca)*

Type of etiological agent	***Antigenic agent***
Viruses:	
Feline calicivirus	*NT*
*Feline immunodeficiency virus (**FIV**)*	*ELISA, WB*
*Feline leukemia virus (**FeLV**)*	*ELISA*
Feline parvovirosis virus	*ELISA*
*Feline infectious peritonitis virus (**FIP,** conavirus)*	*ELISA, PCR*
Feline rhinotracheitis virus	*NT*
Rotavirus	*faeces antigen test, EM and ELISA*
Feline enteric coronavirus	
Bacteria and fungi*:	
***Bartonella** spp.*	*culture*
Bordetella bronchiseptica	*culture*
***Campylobacter** spp.*	*culture*
Chlamydia psittaci	*serology*
Microsporum spp.	*culture*
Pasteurellaceae,	*culture*
***Salmonella** spp.*	*culture*
***Staphylococcus** spp.*	*culture*
***Streptococci** β-hemolytic, serogroup G*	*culture*
***Trichophyton** spp.*	*culture*
Yersinia enterocolitica	*culture*
Parasites:**	
*All artropods and Helminth infections, **Eperythrozoon felis, Haemobartonella felis, Isospora spp., Sarcocystis** spp. **Toxoplasma gondii***	*parasitological examination serological examination*

* *Optionally, and depending on circumstances, **Helicobacter** spp. may also be tested.*

** ***Giardia** spp. and, in necropsy examination / histopathological examination, **Ollulanus tricuspis.***

Source: bibliography compilation (4) and practices (1).

REFERENCES

1. *Huțu I.,* (2017), Manual de bune practici în unitățile experimentale, vol.1., Ed. Agroprint, Timișoara.
2. *Mähler M., Berard M.M, Feinstein R., Gallagher G., Illgen-Wilcke B., Pritchett-Corning K., Raspa M.,* (2014), FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units, Laboratory Animals, Vol. 48(3): 178-192.
3. *Rehbinder C., Alenius S., Bures J., de las Heras M., Greko C., Kroon P.S., Gutzwiller A.,* (2000), FELASA recommendations for the health monitoring of experimental units of calves, sheep and goats, Laboratory Animals, 34:329-350.
4. *Rehbinder C., Baneux P., Forbes D., van Herck H., Nicklas W., Rugaya Z., Winkler G.,* (1998), FELASA recommendations for the health monitoring of breeding colonies and experimental units of cats, dogs and pigs , Laboratory Animals, 32:1-17.

Table 7.

Recommendations for seasonal monitoring of etiological agents, in swine *(Sus scrofa)*

Type of etiological agent	*Testing method*
Viruses monitored serologically* when present in geographical region **:	
Porcine cytomegalovirus (rhinitis virus), ***SMEDI***	*NT*
*Transmissible gastroenteritis (****TGE****)*	*ELISA*
Classical swine fever (hog cholera)	*ELISA*
*Porcine reproductive and respiratory syndrome (****PRRS****)*	*ELISA*
Aujeszky disease virus (pseudorabic)	*ELISA*
Encephalomyocarditis virus	*ELISA, PCR*
*Swine influenza virus (****H_1N_1****), (****H_3N_2****), swine parvovirus*	*ELISA, HI*
Encephalomyelitis hemagglutinin virus	*ELISA, HA, NT,*
African swine pestivirus	*ELISA*
Bacteria, fungi and micoplasmas*:**	
Actinobacillus pleuropneumoniae, Leptospira *spp.*	*serology*
Bordetella bronchiseptica	*culture*
Erysipelothrix rhusiopathiae, Haemophilus parasuis, Mycoplasma hyopneumoniae, Pasteurella multocida	*culture, serology*
Eubacterium (Corynebacterium) suis	*culture*
Salmonella *spp.*	*culture*
Staphylococcus hyicus	*culture*
Streptococci *β-hemolitici*	*culture*
Streptococcus suis	*culture*
Yersinia enterocolitica	*culture*
Parasites**:**	
All Helminth infections, ***Eimeria*** *spp.,* ***Isospora*** *spp.,* ***Sarcoptes*** *sp. and other type arthropods, if lesions occur.*	*parasitological examination*

* *In the case of porcine epidemic diarrhea virus, and for swine rotavirus, diagnosis is set by faeces test, by ELISA and EM tests, and by agglutining.*

** *Detection of antigens for aftosa fever virus, swine respiratory coronavirus and swine vesicular disease virus SVDV) by method ELISA and vesicular exantema virus (VEV) and swine vesicular stomatitis virus (VSVS), by neutralizing (NT).*

*** *If the case may be, there will also be monitoring for* ***Trueperella pyogenes, Brucella suis, Clostridium perfringens, Escherichia coli, Microsporum*** *spp.,* ***Brachyspira hyodysenteriae, Trichophyton*** *spp.*

**** *Optionally, and depending on circumstances, there may also be tested* ***Cryptosporidium parvum*** *(by Ziehl-Neelsen dies, and IFA)* ***Eperythrozoon suis*** *(serology, HA)* ***Toxoplasma gondii*** *(serology) and* ***Trichinella*** *(serology).*

Source: bibliography compilation (4) and practices (1).

Table 8.

Recommendations for monitoring etiological agents, in calf *(Bos taurus)*

Type of etiological agent	*Testing method*
Viruses monitored by routine control tests[a], at foci occurrence time[b] and upon request[c]:	
[a] *Bovine Herpes Virus type 1 (**BHV 1** or **IBR/IPV**)* [a] *Bovine Leukemia Virus (**BLV**)*	*ELISA (individual and in lot probe)*
[a] *Bovine viral diarrhea virus (**BVDV**)*	*Isolation, ELISA, PCR*
[b] *Bovine adenovirus (**BAV 1-10**) /*[b] *Bovine papular stomatitis virus*	*ELISA, NT / EM*
[b] *Bovine corona virus (**BCV**), Bovine herpetic virus type 1 and 5, Bovine mammillitis virus (**BHV-2**)*	*Isolation, ELISA, PCR NT, imunohistochemival*
[b] *BSE*	*Necropsy, histology*
[b] *Foot and mouth disease/ Malignant catarrhal fever*	*ELISA, Isolation /PCR*
[b] *Bovine pest rotavirus*	*CIEP / EM, antigenic-ELISA, PAGE*
*Bovine respiratory syncytial Virus (**BRSV**), Parainfluenza virus type 3 (**PIV-3**)*	*ELISA (individual and lot serology, and milk)*
Bacteria, fungi and mycoplasmas*:	
Enterohaemorrhagic E. coli** O157, **Salmonella** spp, **M. paratuberculosis	*culture, serology, faeces, microscope, ELISA*
***Brucella (B. abortus; B. melitiensis; B. ovis), Coxiella burnetti, Haemophilus somnus, Leptospira** spp.*	*culture, serology, milk, ELISA*
***Leptospira** spp.*	*serology*
Mycobacterium bovis, tuberculosis** and **avium	*dermic test*
Parasites:**	
*Gastro-enteric Helminth infections, intestinal protozoa (**Eimeria, Cryptosporidium, Giardia**), hepatic trematodes, lung located parasites (**Dictyocaulus**), ectoparasites, hypodermosis and such like other type parasites, if lesions occur.*	*parasitological examination*

* *If needs be, there will also be monitoring for **Actinobacillus** spp., **Trueperella (Archanobacterium) pyogenes, Clostridia (Cl. chauvyi, Cl. Septicum, Cl. Sordelli, Cl. Novyi** and **Cl. perfringens), Campylobacter fetus var venerealis, Campylobacter fetus var fetus, Dermatophilus congolensis, Dermatophytes, Erysipelothrix rhusiopathiae, Mycoplasma bovis** and **Pasteurella** spp.*

** *Optionally, and depending on circumstances, there may also be tested: babesiosis (**Babesia** spp.), neosporosis (**Neosporosis caninum**), theileriosis (**Theileria** spp.) and toxoplasmosis (**Toxoplasma gondii**).*

Source: bibliography compilation (3) and practices (1).

Table 9.

Number of animals needed per sample, for detection of an etiological agent present in a microbiological unit

Prevalence set	*Sample size * for various statistical confidence levels*		
	95%	*99%*	*99,9%*
10 %	*29*	*44*	*66*
15%	*18*	*28*	*43*
20%	*14*	*21*	*31*
25%	*10*	*16*	*24*
30%	*9*	*13*	*20*
35%	*7*	*11*	*16*
40%	*6*	*10*	*14*
45%	*5*	*8*	*12*
50%	*5*	*7*	*10*

* *Number of animals drawn for the sample collection of probes.*