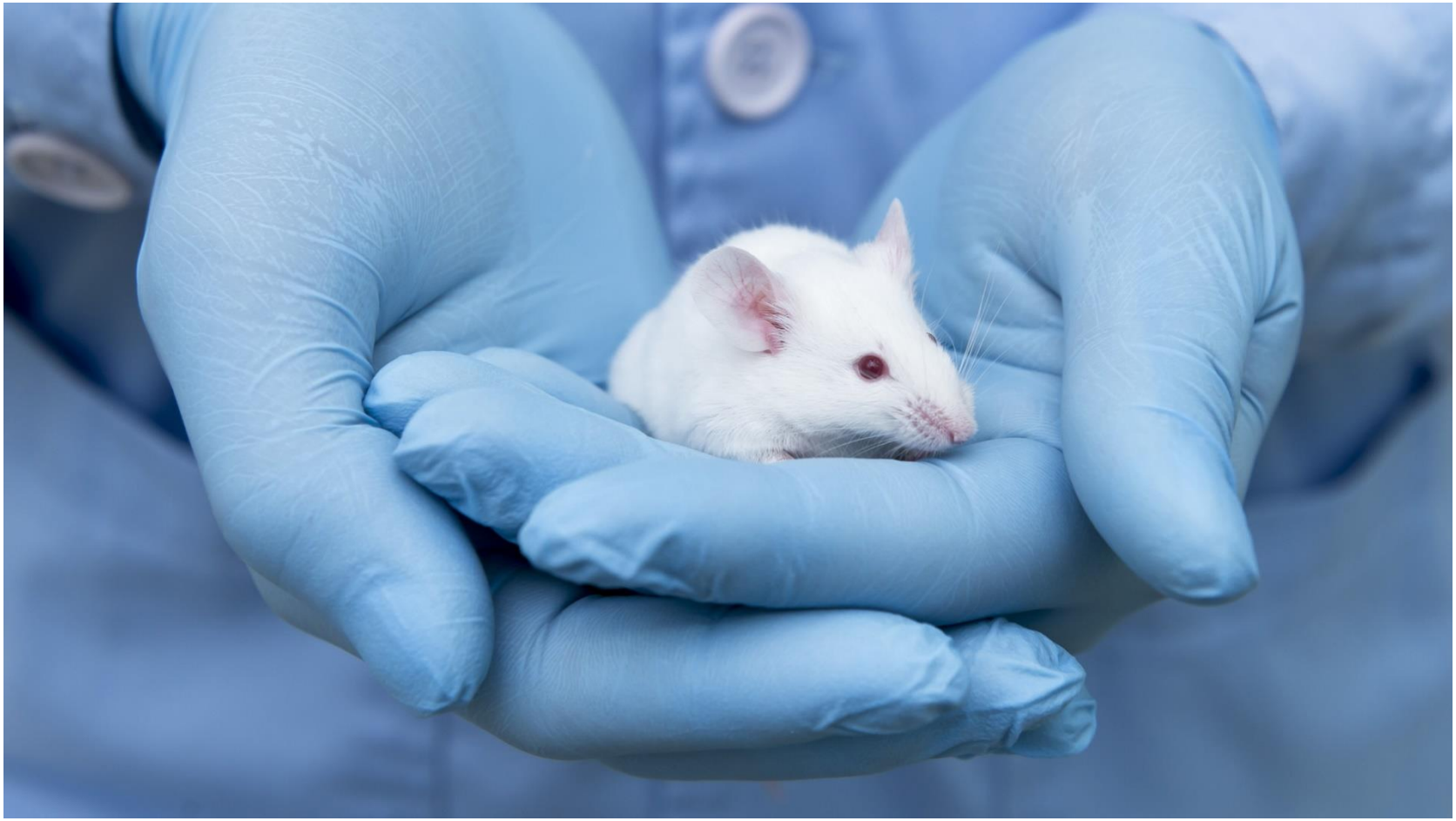
Almazov National Medical Research Centre

Laboratory animals health monitoring: from Danio Rerio to mini-pigs

Bredneva O., Egorova N., Murashova L., Burzak N., Vasyutina M.



Laboratory animals quality components

Environment standardisation

Controlled genetic status

Controlled microbiota

Laboratory animals status is determined by the tasks and directions of activity. There are multiple options of laboratory animals classification based on their microbiological status.

Classification of laboratory animals according to microbiological status

	Control	Barrier	Use
Conventional	Zooanthroponotic causative agents + agents, absolutely pathogenic for animals	None, open system	Training, acute experiments
Improved conventional, minimal diseases (MD)	Same but augmented list of agents	Incomplete	Routine research, short-term experiments
Specific pathogen free (SPF)	Regular, serological and bacteriological tests (PCR, ELISA)	High reliability barrier system	Preparation of cell cultures for vaccine production (GMP), testing of new drugs, maintenance of transplantable tumors, etc.
Gnotobionts (germ-free)	Regular, serological and bacteriological tests (PCR, ELISA)	Film box isolator	

Example: laboratory rodents in Taconic facility

Taconic produces mice and rats of various health standards, determined by research requirements, from germ free gnotobionts to Murine Pathogen Free animals with a simplified list of excluded pathogens. The methods of animals housing differ to maintain the specified standards.

HTTPS://WWW.TACONIC.COM/QUALITY/ANIMAL-HEALTH/HUSBANDRY-METHODS/

Examples of the effect of rodent microorganisms on the results of the experiment

- Altered immune response (Ectromelia virus, mouse hepatitis virus)
- Altered physiological response in pharmacological and toxicological studies (Bacillus piliformis, Salmonella enteritidis)
- Carcinogenesis or spontaneous neoplasia (Lymphocytic choriomeningitis virus, Sendai virus)
- Transplant tumor contamination (Mycoplasma arthritidis, Mycoplasma pulmonis, Kilham rat virus)

Laboratory animals health status monitoring

Internal monitoring (vivarium personnel):

- initial detailed examination and weighing
- daily examination
- periodic detailed clinical examination
- unscheduled examination

External monitoring (sending animals and materials received from them to certified centers):

- Sentinel animals program
- Pathology
- Parasitology
- Bacteriology
- Serology

Regional recommendations, international recommendations,

FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units

FELASA working group on revision of guidelines for health monitoring of rodents and rabbits, M Mähler (Convenor), M Berard, R Feinstein, A Gallagher, B Illgen-Wilcke, K Pritchett-Corning and M Raspa

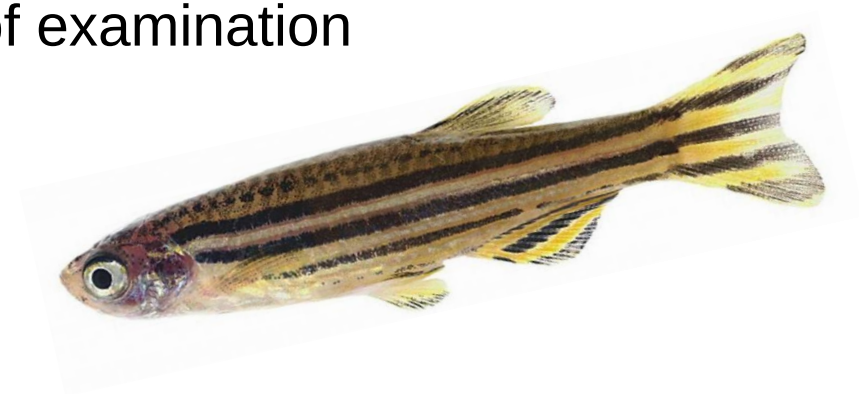
Lab Anim 2014 48: 178 originally published online 4 February 2014

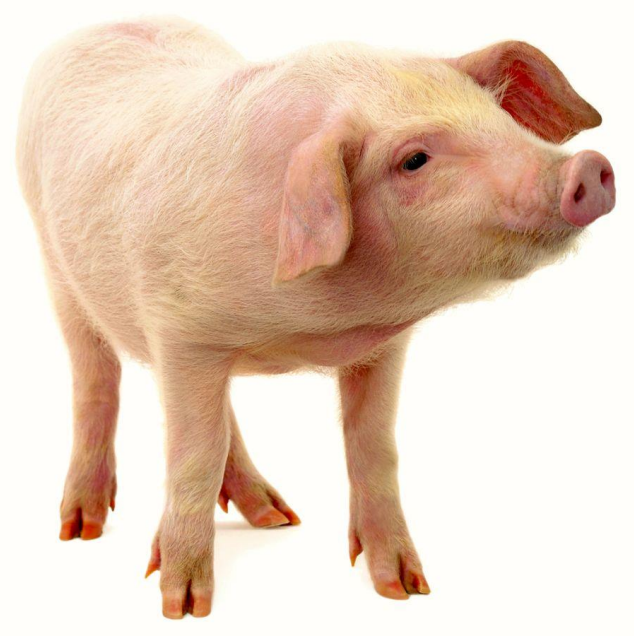
DOI: 10.1177/0023677213516312

The online version of this article can be found at:
http://lan.sagepub.com/content/48/3/178

In all vertebrate species in any type of examination

- behavior, including food intake
- activity and coordination
- Skin and hair condition.





In warm-blooded animals attention is also paid to

- skin turgor
- forced position
- vocalization

In the course of the examination, it is necessary to minimize animal stress by regular handling.

For most mammalian species used in laboratory practice, scales of facial expressions indicating pain and distress have been developed.

National Centre for the Replacement Refinement & Reduction of Animals in Research

The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye			
Nose bulge • Bulging on the bridge of the nose • Vertical wrinkles on the side of the nose			
Cheek bulge • Bulging of the cheeks			
Ear position • Ears rotate outwards and/or backwards, away from the face • Ears may fold to form a 'pointed' shape • Space between the ears increases			
Whisker change • Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' • Whiskers may clump together • Whiskers lose their natural 'downward' curve			

Read the original paper:
Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AMJM, Ferrari MD, Craig KD, Mogil JS. 2010. Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7(6): 447–449. doi:10.1038/nmeth.1455

For guidance on using the Mouse Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimace-scales.
To request copies of this poster, please email: enquiries@nc3rs.org.uk
The NC3Rs provides a range of 3Rs resources at: www.nc3rs.org.uk/resources

Images kindly provided by Dr Jeffrey Mogil, McGill University

National Centre for the Replacement Refinement & Reduction of Animals in Research

The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats.

The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye			
Nose/cheek flattening • Flattening and elongation of the bridge of the nose • Flattening of the cheeks (potentially sunken look)			
Ear changes • Ears curl inwards and are angled forward to form a 'pointed' shape • Space between the ears increases			
Whisker change • Whiskers stiffen and angle along the face • Whiskers may 'clump' together • Whiskers lose their natural 'downward' curve			

Read the original paper:
Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JCS, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Molecular Pain 7: 55. doi:10.1186/1744-8069-7-55

For guidance on using the Rat Grimace Scale, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimace-scales.
To request copies of this poster, please email: enquiries@nc3rs.org.uk
The NC3Rs provides a range of 3Rs resources at: www.nc3rs.org.uk/resources

Images kindly provided by Dr Jeffrey Mogil, McGill University

National Centre for the Replacement Refinement & Reduction of Animals in Research

Newcastle University

The Rabbit Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rabbits.

The specific facial action units shown below comprise the Rabbit Grimace Scale. These action units increase in intensity in response to post-procedural pain and can form part of a clinical assessment alongside other validated indices of pain.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Action units		
	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye			
Cheek flattening • Flattening of the cheeks. When 'obviously present', cheeks have a sunken look. • The face becomes more angular and less rounded			
Nostril shape • Nostrils (nares) are drawn vertically forming a 'V' rather than 'U' shape • Nose tip is moved down towards the chin			
Whisker shape and position • Whiskers are pushed away from the face to 'stand on end' • Whiskers stiffen and lose their natural, downward curve • Whiskers increasingly point in the same direction. When 'obviously present', whiskers move downwards			
Ear shape and position • Ears become more tightly folded / curled (more cylindrical) in shape • Ears rotate from facing towards the source of sound to facing towards the hindquarters • Ears may be held closer to the back or sides of the body			

Read the original paper: Keating SCJ, Thomas AA, Flecknell PA, Leach MC (2012) Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. PLOS ONE 7(9): e44457. doi:10.1371/journal.pone.0044457

For guidance on using the Rabbit Grimace Scale, additional images of each action unit, research papers that underpin this technique, and for grimace scales in other species, visit: www.nc3rs.org.uk/grimacescales

To request copies of this poster, please email: enquiries@nc3rs.org.uk
The NC3Rs provides a range of 3Rs resources at www.nc3rs.org.uk/resources

Images kindly provided by Dr Matthew Leach, Newcastle University, and Dr Patricia Hedenqvist, Swedish University of Agricultural Sciences

The Rabbit Grimace Scale would not have been developed without the continuing work of the Pain and Animal Welfare Sciences Group (PAWS) at Newcastle University

Ear Position

Absent (0)

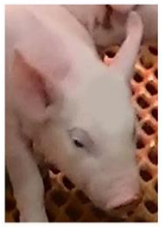
Moderately present (1)

Obviously present (2)

When the animal is in pain, the ears are drawn back from forward (baseline) position

Cheek Tightening/Nose Bulge

Absent (0)

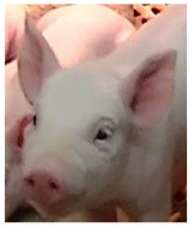


Moderately present (1)

Obviously present (2)

When the animal is in pain, a bulge of skin is apparent on the snout in response to cheek tightening

Orbital Tightening



Absent (0)

Present (1)

When the animal is in pain, the orbital area is narrowed as the eyelids are squeezed together (scored on a two-point scale)

Danio Rerio health status external monitoring

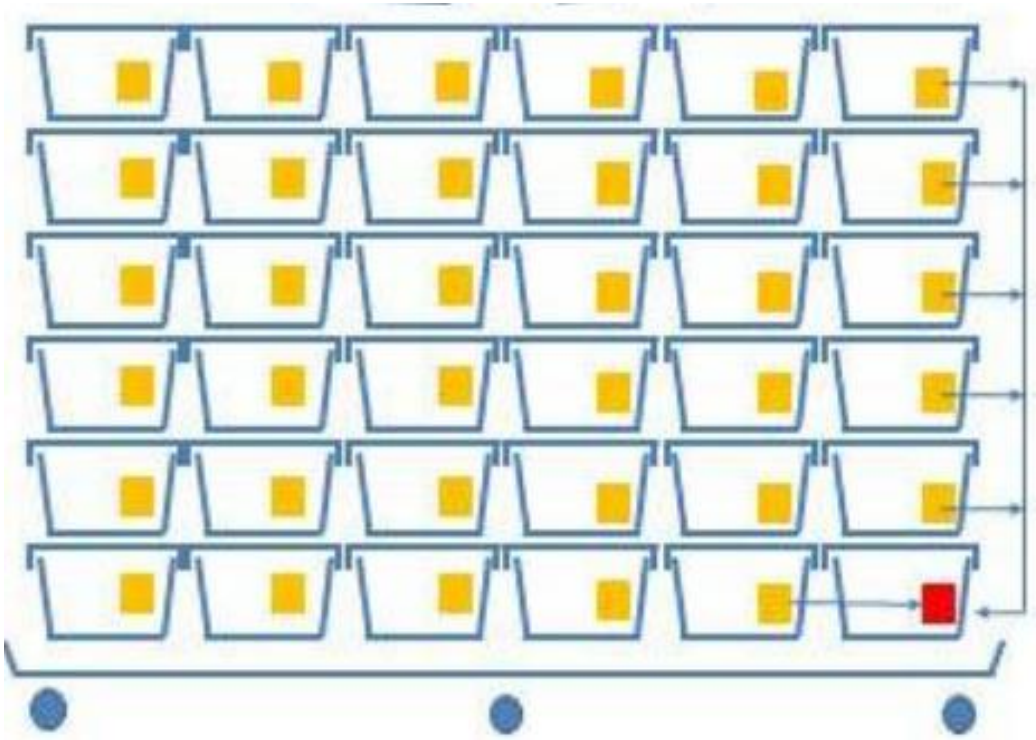
Frozen or alcohol-fixed euthanized fishes are used for PCR tests.

Basic list of pathogens to control	**Extended list of pathogens to control** **In addition to basic one,** **actual for SPF Danio Rerio**
Mycobacterium abscessus	Ichthyophthirius multifiliis
Mycobacterium chelonae	Flavobacterium columnare
Mycobacterium fortuitum	Infectious spleen & kidney necrosis virus (ISKNV)
Mycobacterium haemophilum	Zebrafish picornavirus (ZfPV-1)
Mycobacterium marinum	Mycobacterium gordonae
Mycobacterium peregrinum	Mycobacterium saopaulense
Edwardsiella ictaluri	Myxidium streisingeri
Pseudocapillaria tomentosa	Piscinoodinium pillulare
Pseudoloma neurophilia	Pleistophora hyphessobryconis

Standard practice of quarantine Danio Rerio: not the fishes themselves can move from quarantine content to the main one, but only processed embryos. Under less stringent conditions, for non-biomedical studies, it is allowed to quarantine adult fishes for 14-21 days.

Laboratory rodents health status external monitoring

Soiled bedding transfer methodology is used to prepare sentinel animals for IVC housing system in rodents.



Usually, the preparation of sentinel animals takes 2-3 months, but the period may vary depending on the requirements of the experiment. During this period, sentinel animals should receive contaminated materials from all controlled cells. NB: one month of the incubation period is needed.

Evaluation of presence of bacterial and viral agents in laboratory animals in feces and mouth swabs is carried out by quantitative PCR method. Blood serum is examined for specific antibodies by the ELISA method.

NB: immunocompetent sentinels are used to monitor the health of immunodeficient animals.

To change laboratory rodents colony status from conventional to SPF, special methods of rederivation are used (foster mother, embryotransfer etc.).

Laboratory rodents health status external monitoring

INTERCEPTOR – IVC Microbiological Monitoring

- Reduce the number of sentinel animals – a positive step forward for the 3Rs
- Closed system to avoid sample contamination (patent pending)
- Greater sensitivity than traditional bedding methodology
- Quick to identify pathogenic microorganisms, even with low infection prevalence
- Eeasier and cheaper than bedding sentinels

Recommended infectious agents to monitor and frequencies of monitoring for laboratory mice (*Mus musculus*).

	Every 3 months	Annually
Viruses		
Mouse hepatitis virus	x	
Mouse rotavirus	x	
Murine norovirus	x	
Parvoviruses:		
Minute virus of mice	x	
Mouse parvovirus	x	
Theiler's murine encephalomyelitis virus	x	
Lymphocytic choriomeningitis virus		x
Mouse adenovirus type 1 (FL)		x
Mouse adenovirus type 2 (K87)		x
Mousepox (ectromelia) virus		x
Pneumonia virus of mice		x
Reovirus type 3		x
Sendai virus		x
Bacteria		
Helicobacter spp.	x	
If positive, speciation for *H. hepaticus*, *H. bilis* and *H. typhlonius* is recommended		
Pasteurella pneumotropica	x	
Streptococci β-haemolytic (not group D)	x	
Streptococcus pneumoniae	x	
Citrobacter rodentium		x
Clostridium piliforme		x
Corynebacterium kutscheri		x
Mycoplasma pulmonis		x
Salmonella spp.		x
Streptobacillus moniliformis		x
Parasites		
Endo- and ectoparasites (reported to the genus level)	x	
Additional agents*		
Viruses:		
Hantaviruses		
Herpesviruses (mouse cytomegalovirus, mouse thymic virus)		
Lactate-dehydrogenase elevating virus		
Polyomaviruses (mouse polyomavirus, K virus)		
Bacteria and fungi:		
Cilia-associated respiratory bacillus		
Klebsiella oxytoca, Klebsiella pneumoniae		
Other *Pasteurellaceae*†		
Pneumocystis murina		
Pseudomonas aeruginosa		
Staphylococcus aureus		
Others as necessary		

Recommended infectious agents to monitor and frequencies of monitoring for rats (*Rattus norvegicus*).

	Every 3 months	Annually
Viruses		
Parvoviruses:		
Kilham rat virus	x	
Rat minute virus	x	
Rat parvovirus	x	
Toolan's H-1 virus	x	
Pneumonia virus of mice	x	
Rat coronavirus/Sialodacryoadenitis virus	x	
Rat theilovirus	x	
Hantaviruses		x
Mouse adenovirus type 1 (FL)		x
Mouse adenovirus type 2 (K87)		x
Reovirus type 3		x
Sendai virus		x
Bacteria and fungi		
Clostridium piliforme	x	
Helicobacter spp.	x	
If positive, speciation for *H. bilis* is recommended		
Mycoplasma pulmonis	x	
Pasteurella pneumotropica	x	
Streptococci β-haemolytic (not group D)	x	
Streptococcus pneumoniae	x	
Cilia-associated respiratory bacillus		x
Pneumocystis spp.		x
Salmonella spp.		x
Streptobacillus moniliformis		x
Parasites		
Endo- and ectoparasites (reported to the genus level)	x	
Additional agents*		
Bacteria and fungi:		
Bordetella bronchiseptica		
Corynebacterium kutscheri		
Encephalitozoon cuniculi		
Klebsiella oxytoca, Klebsiella pneumoniae		
Other *Pasteurellaceae*†		
Pseudomonas aeruginosa		
Staphylococcus aureus		
Others as necessary		

Recommended infectious agents to monitor and frequencies of monitoring for guinea pigs (*Cavia porcellus*).

	Every 3 months	Annually
Viruses		
Guinea pig adenovirus	x	
Guinea pig parainfluenza virus 3/Caviid parainfluenza virus 3	x	
Sendai virus	x	
Guinea pig cytomegalovirus		x
Bacteria and fungi		
Bordetella bronchiseptica	x	
Corynebacterium kutscheri	x	
Streptococci β-haemolytic (not group D)	x	
Streptococcus pneumoniae	x	
Clostridium piliforme		x
Encephalitozoon cuniculi		x
Salmonella spp.		x
Streptobacillus moniliformis		x
Parasites		
Endo- and ectoparasites (reported to the genus level)	x	
Additional agents*		
Bacteria and fungi:		
Chlamydophila caviae		
Cilia-associated respiratory bacillus		
Dermatophytes		
Pasteurellaceae		
Pseudomonas aeruginosa		
Staphylococcus aureus		
Yersinia pseudotuberculosis		
Others as necessary		

Recommended infectious agents to monitor and frequencies of monitoring for hamsters (*Mesocricetus auratus*).

	Every 3 months	Annually
Viruses		
Lymphocytic choriomeningitis virus	x	
Sendai virus	x	
Bacteria		
Pasteurella pneumotropica	x	
Clostridium piliforme		x
Corynebacterium kutscheri		x
Helicobacter spp.		x
Salmonella spp.		x
Parasites		
Endo- and ectoparasites (reported to the genus level)	x	
Additional agents*		
Viruses:		
Hamster polyomavirus		
Pneumonia virus of mice		
Bacteria and fungi:		
Encephalitozoon cuniculi		
Lawsonia intracellularis		
Other *Pasteurellaceae*†		
Others as necessary		

Recommended infectious agents to monitor and frequencies of monitoring for rabbits (*Oryctolagus cuniculus*).

	Every 3 months	Annually
Viruses		
Rabbit haemorrhagic disease virus (RHDV)*	x	
Rabbit rotavirus	x	
Bacteria and fungi		
Bordetella bronchiseptica	x	
Clostridium piliforme	x	
Encephalitozoon cuniculi	x	
Pasteurella multocida	x	
Cilia-associated respiratory bacillus		x
Salmonella spp.		x
Parasites		
Endo- and ectoparasites (reported to the genus level)	x	
Additional agents†		
Viruses:		
Adenovirus		
Coronavirus		
Myxomatosis virus		
Bacteria and fungi:		
Clostridium spp.		
Dermatophytes		
Escherichia coli (enteropathogenic strains)		
Other *Pasteurellaceae*		
Pneumocystis oryctolagi		
Staphylococcus aureus		
Treponema paraluiscuniculi		
Others as necessary		

Laboratory pigs health status external monitoring

Control of pathogens in pigs is based primarily on local veterinary regulations.

In course of quarantine, animal samples are examined for

- Salmonellosis
- Helminthoses
- Tuberculosis
- Brucellosis
- Classical swine fever

In prolonged keeping conditions the control scheme is similar plus african swine fever study is applied. Helminthoses screening should be performed twice a year

In SPF-status mini-pigs, according to the WHO program, are also excluded

- Enzootic pneumonia
- Actinobacillosis
- Atrophic rhinitis of pigs
- Porcine dysentery
- Aueschi's disease
- Leptospirosis

Pigs are surgically delivered by hysterectomy or cesarean section, followed by maintenance in a sterile zone and six-week quarantine before administration to the SPF herd.

SPF indicator syndromes in swine

Turbinate Atrophy and Snout Distortion	Bacterial agents • Bordetella bronchiseptica • Pasteurella multocida	A disease of young growing pigs, which results in atrophy and distortion of the bones of the nasal cavity. Commonly called Atrophic Rhinitis
Pneumonia	Numerous viral and bacterial agents • Swine influenza virus • Porcine reproductive and respiratory syndrome virus • Actinobacillus pleuropneumonia • Pasteurella multocida • Mycoplasma hypopneumoniae • Haemophilus parasuis • Bordetella bronchiseptica	Signs can range from clinically inapparent to severe respiratory difficulty. Severe infections are characterized by head down posture and forced breathing called “thumping”
Swine Dysentery	Bacterial agent • Serpulina hyodysenteriae	A muco-hemorrhagic colitis causing varying degrees of diarrhea
Lice	Sucking Louse • Haematopinus suis	Infestation with this louse can cause severe skin irritation and pruritus. Agent can spread blood borne diseases between pigs
Mange	Burrowing mite • Sarcoptes scabiei var. suis	Causes severe pruritus and loss of condition
Pseudorabies	Viral agent • Herpesvirus suis	Usually inapparent in chronically infected herds. In naive young pigs may see nervous and respiratory signs. Like other herpesviruses can cause death in aberrant hosts such as dogs, cats, and cattle.
Brucellosis	Bacterial agent • Brucella suis	Usually a reproductive disease of swine, leading to decreased herd reproductive performance.

Joe Safron, Janet C. Gonder, The SPF Pig in Research, *ILAR Journal*, Volume 38, Issue 1, 1997, Pages 28–31, https://doi.org/10.1093/ilar.38.1.28

In conclusion

The microbiological status of animals is of fundamental importance in preclinical trials, since even in an asymptomatic flow, infectious diseases can affect the study outcome. Controlled microbiota is one of the most important components of laboratory animals quality. The level of control should be determined by the specific purposes of the test center.