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The Impact of General Laboratory Animal Health on Experimental Models in Antimicrobial Chemotherapy

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Introduction

Animal health is important for all types of animal experimentation. The elimination of infectious agents which act as undefined experimental factors has attracted much attention over the last decades. There is no difference between the use of animal models for antimicrobial research and other kinds of animal research. However, it should be obvious that if animals are to be used as reliable tools for experimental infection, spontaneous infections should not be allowed to interfere. Therefore, microbiologists should feel a special obligation to define the health of their laboratory animals. In this chapter some examples will be given of how poor health status may interfere with animal models for antimicrobial research, and precautions to avoid such interference will be described.

The impact of animal health on experiments

Concerns for the health of laboratory animals have mostly focused on spontaneous infections, although disease in laboratory animals may also be caused by genetic or environmental determinants. Examples of infectious agents which are of importance for antimicrobial animal models are listed in Tables 6.1–6.3. Some of these may cause disease in laboratory animals, and such disease might interfere with research. However, if animals are clinically ill, they are seldom used for experiments, and therefore research interference is more to be feared from those complications which are not clinically observable. Some microorganisms only have the ability to influence the animal temporarily, while others act through a long period of the animal's life, maybe lifelong.

Table 6.1 Common virus infections occurring spontaneously in laboratory animals

Genera	*Strain (species affected)*
Adenoviruses	Various rodent-specific strains, infectious canine hepatitis
Herpesviruses	Cytomegaloviruses (many), Aujeszky (pig), Rhinotracheitis (cat)
Parvoviruses	Minute virus of mice, mouse orphan parvovirus, Kilham rat virus, Toolan's H1 virus (rat), rat orphan parvovirus, canine parvovirus, panleukopenia (cat)
Arenaviruses	Lymphocytic choriomeningitus (mouse, hamster)
Caliciviruses	Rabbit haemorrhagic disease
Coronaviruses	Mouse hepatitis virus, rat coronavirus, sialodacryoadenitis (rat), guinea-pig coronavirus, rabbit coronavirus, haemagglutinating encephalomyelitis (pig), transmissible gastroenteritis (pig), feline infectious peritonitis
Paramyxoviruses	Sendaivirus (mouse, rat, hamster, guinea-pig), pneumoniavirus (mouse, rat, hamster, guinea-pig), simian virus 5-like (hamster, guinea-pig), parainfluenzavirus 3 (guinea-pig), distemper (dog), canine parainfluenzavirus
Orthomyxoviruses	H1N1, H3N2 (pig)
Poxviruses	Ectromelia (mouse), myxomavirus (rabbit), rabbit poxvirus, feline poxvirus
Picornaviruses	Theiler's murine encephalomyelitis virus (mouse, rat), guinea-pig poliovirus, stillbirth mummification embryonic death infection (pig)
Reoviruses	Type 3 (all mammals), rotaviruses (mouse, rat, rabbit, pig)
Retroviruses	Feline leukaemia, feline immunodeficiency virus
Togaviruses	Lactic dehydrogenase virus (mouse)

The impact on animal models in antimicrobial chemotherapy differs between different viruses. Clinically apparent viral disease is seldom seen in laboratory animals, while all viruses may have an impact on the immune system and may contaminate biological products sampled from animals. The strains listed are examples. Several viruses which may infect the species included are not given here as they are less common. A comprehensive list may be found in Hansen *et al.* (1994). The table does not include primates, as different primate species harbour a wide range of viruses. A comprehensive list referring to primates may be found in *Working Committee for SPF and Gnotobiotic Laboratory Animals* (1980).

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Table 6.2 Common bacterial infections occurring spontaneously in laboratory animals

	Bacteria (species affected)
Gram-positive cocci	*Staphylococcus aureus* (many), *S. hyicus* (pig)
	Streptococci
	β-haemolytic type A/B/D/G (many), *Streptococcus zooepidemicus* (guinea-pig, pig), *S. pneumoniae* (rodents, rabbit), *S. suis* (pig)
Gram-positive rods	*Corynebacterium kutscheri* (mouse, rat)
	Erysipelothrix rhusiopathiae (pig)
	Eubacterium suis (pig)
	Clostridium piliforme (rodents, rabbit)
	Clostridium difficile (hamster, guinea-pig)
	Clostridium perfringens (pig)
Enterobacteriaceae	*Citrobacter freundii* Type 4280 (mouse)
	Klebsiella pneumoniae (many)
	Salmonellae, subgenus I (all mammals)
	Yersinia spp.
	Yersinia pseudotuberculosis (guinea-pig), *Y. enterocolitica* (pig, dog, cat)
Pasteurellaceae	*Actinobacillus pleuropneumoniae* (pig)
	Pasteurella pneumotropica
	Haemophilus parasuis (pig)
Spiral bacteria	*Treponema cuniculi* (rabbit)
	Campylobacter coli/jejuni (many)
	Helicobacter spp.
	H. hepaticus/bilis (mouse), *H. suis* (pig), *H. felis* (cat)
Other Gram-negative bacteria	*Bordetella bronchiseptica* (mouse, guinea-pig, pig, dog, cat)
	Cilia-associated respiratory (CAR) bacillus (rat, rabbit)
Mycoplasma	*Mycoplasma pulmonis* (mouse, rat), *M. hyopneumoniae* (pig)

The bacteria listed are examples and under certain circumstances may all cause disease. A few bacteria, such as streptococci and salmonellae, may have an impact on the immune system. Most spontaneous bacterial infections possess the potential for competing with spontaneous infections and become activated by immunosuppression. *Mycoplasma* are common contaminants of biological products. Also bacteria from the normal flora, such as *Escherichia coli* and *Pseudomonas aeruginosa*, may act as opportunistic pathogens.The table does not include primates, as different primate species harbour a wide range of bacteria. A comprehensive list for primates may be found in *Working Committee for SPF and Gnotobiotic Laboratory Animals* (1980) and a more detailed list for the other species may be found in Hansen *et al.* (1994).

This division is mainly connected with the ability of the agent to persist in the organism. However, that it is not always so can be illustrated by the very simple example given by those infections which introduce resistance against reinfection. It cannot be overemphasized that even good experimental designs cannot eliminate all kinds of microbial interference because infections may inhibit the induction of a certain animal model, may make it difficult to interpret the final results, may show a dose-related response or, last, but not least, increase variations within the experiment.

The impact of spontaneous infections on the animal model

Pathological changes, clinical disease and mortality

Clinically apparent disease due to infections with specific pathogens is rare in laboratory animals, as the most pathogenic agents, such as *Ectromelia* virus in mice, have been eliminated from most colonies. However, the presence of various less pathogenic microorganisms may cause changes in the organs, resulting in difficulties in the interpretation of the pathological diagnosis included in the evaluation of many microbiological models—a phenomenon often referred to as background noise. Furthermore, the pathology of experimental infections may be changed by spontaneous infections.

Example. Rodents used as models for acute pneumonia may be naturally infected with agents altering the pathology of other infectious agents. For example, rats infected with either *Mycoplasma pulmonis* alone, Sendai virus alone or both together show three different pictures of respiratory pathology (Schoeb *et al.*, 1985).

Immunomodulation

The immune system may be modulated by spontaneous infections in the absence of clinical disease. This effect may be either *suppressive* or *activating* or both at the same time, but on different parts of the immune system. As a general rule of thumb all viruses should be regarded as immunosuppressive. One of the reasons for this is the viraemic phase in the pathogenesis of many virus infections: during this phase cells of the immune system may be infected.

Table 6.3 Common parasite infestations occurring spontaneously in laboratory animals

Genera	*Parasite species*	*Animal species commonly infected*
Pinworms	*Syphacia obvelata/muris*	Rodents
	Passalurus ambiguus	Rabbit
	Ascaris suum	Pig
	Toxocara canis/cati	Dog, cat
	Toxascaris leonina	Dog
Cestodes	*Dipylidium caninum*	Dog, cat
Flagellates	*Giardia muris*	Rodents
	Spironucleus muris	Rodents
	Tritrichomonas spp.	Rodents
Microspora	*Encephalitozoon cuniculi*	Rodents, rabbit
Coccidia	*Eimeria* spp.	Rabbit, pig
	Isospora spp.	Pig
	Toxoplasma gondii	Cat
Hair follicle mites	*Demodex* spp.	Dog
Body mange mites	*Notoedres cuniculi (cati)*	Rabbit, cat
	Sarcoptes scabiei	Pig
Ear mange mites	*Otodectes cynotis*	Cat, dog
	Notoedres cati	Cat
Fur mites	*Chirodiscoides caviae*	Guinea-pig
	Cheyletiella parasitovorax	Dog, cat
Ticks	*Ctenocephalides* spp.	Dog, cat

The parasites listed under each species are examples. Several other infestations — although less common — may occur. Except for coccidia and the mange mites, most parasites only cause mild disease or no disease at all, but they have an impact on the immune system and may affect the absorption of compounds from the gastrointestinal tract. Other parasitic infestations than those listed in this table may occur. A comprehensive list may be found in Hansen *et al.* (1994) or *Working Committee for SPF and Gnotobiotic Laboratory Animals* (1980).

Examples. Infection with lactic dehydrogenase virus in mice, which clinically is totally inapparent, seems to influence the function of the macrophages (Stevenson *et al.*, 1980), e.g. an impaired antigen presentation has been described (Isakov *et al.*, 1982). This may lead to an increase in the severity of the symptoms shown by experimental infections (Bonventre *et al.*, 1980). Also non-viral microorganisms are known to influence the immune system, e.g. *Mycoplasma pulmonis* infection in mice and rats may change the symptoms observed after experimental infection (Howard *et al.*, 1978; Cassell *et al.*, 1986). Another mycoplasma, *M. arthritidis*, has been reported to increase susceptibility to experimental pyelonephritis (Thomsen and Rosendal, 1974).

Physiological modulation

Some microorganisms have a specific effect on enzymatic, haematological and other parameters which are monitored in the animal during an experiment. Organic function disturbances may change the outcome of the experiment without the knowledge of the scientist. For example, the altered function of the liver caused by some spontaneous hepatic infections may influence the pharmacokinetics of antimicrobial drugs.

Example. Acute infection with *Clostridium piliforme* in mice prolongs the half-life of trimethoprim (Friis and Ladefoged, 1979).

Competition between microorganisms within the animal

Spontaneous infections in an experimental infection model may compete with the experimental infection, which in worst cases may fail.

Example. Helicobacter pylori mouse models have been difficult to develop. Lately, it has been reported that the "cleaner" the mouse, the more successful the colonization rate of *H. pylori* in mice (Fox and Lee, 1997). This is equivalent to the fact that gnotobiotic or microbiologically defined (see below) pigs may be experimentally infected with *H. pylori*, while conventional pigs may not (Krakowka *et al.*, 1987). Such differences between different microbiological categories of animals may be explained by natural infection with related agents, and today species-specific *Helicobacter* spp. have been isolated from both mice (Fox *et al.*, 1994, 1995) and pigs (Queiroz *et al.*, 1990). Infections with species other than *Helicobacter* may play a role as well.

Contamination of biological products

Microorganisms present in the animal may contaminate samples and tissue specimens, such as cells and sera. This may complicate the *in vitro* maintenance of cell lines, and may interfere with experiments performed on cell cultures or isolated organs. Further, the reintroduction of such products into animal laboratories will impose a risk to the animals kept in that laboratory. Nicklas *et al.* (1993) found

that 3% of a high number of cell lines and monoclonal antibodies were contaminated with murine viruses, the most important ones being lactic dehydrogenase virus, reovirus type 3 and minute virus of mice.

Example. An unidentified virus culture received at an American virus centre from a Romanian institute was inoculated on mice. Before the virus was identified as the poxvirus, mouse ectromelia, the virus had spread to the sarcoma cells used for the induction of ascites. Mice inoculated with the sarcoma cells now died earlier than previously. Afterwards, 28% of the institute's stored ascitic fluids were found to be contaminated with ectromelia, and so were 15 virus strains, which the institute had deposited at the American Tissue Culture Collection. The virus centre had to stop all experimental work for half a year, be totally depopulated of mice, disinfected and restocked with caesarian-derived mice (Shope, 1986).

The impact of the environment, genetics and the experiment on the health of the animals

Susceptibility to the development of spontaneous infectious diseases is under the control of genetics, sex, sexual cycle, age, and other characteristics of the host. It is obvious that immune-deficient rodents such as the nude mouse, the severe combined immunodeficiency (SCID) mouse, and the nude rat are more susceptible, e.g. in nude mice abscesses caused by bacterial species such as *Staphylococcus aureus*, *Pasteurella pneumotropica*, *Morganella morganii*, *Citrobacter freundii* and *Streptococci* (Rygaard, 1973; Custer *et al.*, 1973; Fortmeyer, 1981) are common. Variation in susceptibility to the development of infectious disease between inbred strains of rats and mice is often connected with the histocompatibility type of the animal (Brownstein, 1983; Hansen *et al.*, 1990). Infectious disease symptoms are most common in young animals (Fujiwara *et al.*, 1973; Onodera and Fujiwara, 1973; Lai *et al.*, 1976; Zurcher *et al.*, 1977), but if for some reason disease is developed at a later age, there is a tendency for the disease to be worse than in younger animals (Jersey *et al.*, 1973). Differences between sexes in susceptibility to the development of infectious disease may be observed, e.g. colitis and rectal prolapse caused by *C. freundii* in mice are more common in males (Fortmeyer, 1981).

Animals should be transported in specifically designed containers being sufficiently supplied with food and water. Road transport in purpose-equipped vehicles by trained staff directly from vendor to user is preferable. A British set of guidelines may be followed (LABA and LASA, 1993). Any kind of transportation will stress the animals, and therefore a period of acclimatization in the experimental facilities is a must. One of the most common errors in transportation is the use of vehicles without proper ventilation, cooling or heating, which may be the cause of various grades of respiratory infection, often with opportunistic bacteria, such as *Staphylococcus* spp. and *Pseudomonas* spp., involved. Primates are often transported long-distance by air, during which journey they are handled by untrained staff. This may result in wounds and scratches, which if necessary should be treated immediately upon arrival. In all cases of such improper transportation any trauma should be reported to the transporting agent as well as to the vendor in order to improve future transportations.

Principles for housing have been dealt with in another chapter. Improper housing may change different aspects of an animal model. For example, mycoplasmosis is more severe in rats exposed to ammonia than in unexposed rats (Pinson *et al.*, 1986)—a situation which may be caused by infrequent cage-changing and bad ventilation.

Subclinical respiratory disease caused by *Mycoplasma*, respiratory viruses and some bacteria may be responsible for increased mortality during anaesthesia.

Post-surgical infections are more common in large animals than in rodents. Infections are mostly caused by non-specific members of the normal flora, such as *Staphylococcus aureus, Bacillus fragilis, Pseudomonas aeruginosa* and *Escherichia coli*. Rats seem to be more resistant than mice and hamsters (Donelly and Stark, 1985).

Immunosuppression of laboratory animals, a common tool in infection research, may activate latent infections. Some examples from mice are given in Table 6.4.

Long-term treatment of laboratory animals with antibiotics may change the normal flora of the animals from Gram-positive to Gram-negative dominated flora. Some of the propagated bacteria, e.g. *Klebsiella pneumoniae*, may be opportunistic pathogens, and may, as with humans, be multiresistant to a wide range of antibiotics (Hansen, 1995). Application of certain antibiotics, e.g. all β-lactams (Young *et al.*, 1987), in hamsters and guinea-pigs may often lead to fatal *Clostridium difficile* enterotoxaemia. The effect may also be observed with other antibiotics, e.g. tetracyclines and macrolides. In rabbits, the enterotoxaemia is mostly observed after oral dosing (Olfert, 1981), and in general, rabbits should not be given antibiotics *per os*, if disturbance in the intestinal flora is to be avoided.

Zoonoses caused by laboratory animals

Zoonoses are infections which may spread from one animal species to another. In this connection a zoonosis will be regarded as an infection with the potential of spreading from animals to humans. Selected zoonoses are listed in Table 6.5.

Zoonoses from rodents, rabbits, dogs and cats used for experimental purposes are rare. Several agents with a potential of infecting both humans and animals exist, but nowadays all rodents for research can and should be purchased from vendors who efficiently keep their animals free of zoonoses. The most important zoonosis from rodents is lymphocytic choriomeningitis virus, an arenavirus, which

Table 6.4 Examples of latent infections in mice which may be activated by immunosuppression

Protection level needed to avoid infection	*Agent*	*Reference*
Barrier	*Citrobacter rodentium*	Juhr (1988)
	Clostridium piliforme	Fujiwara *et al.* (1964)
	Corynebacterium kutscheri	Takagaki *et al.* (1967)
	Streptobacillus moniliformis	Juhr (1988)
	Cytomegaloviruses	Sekizawa and Openshaw (1984)
	Mouse hepatitis virus	Dupuy *et al.* (1975)
	Sendai virus	Anderson *et al.* (1980)
Isolators	*Enterobacter cloacae*	Matsumoto (1980)
	Klebsiella pneumoniae	Matsumoto (1982)
	Pseudomonas aeruginosa	Taffs (1974)
	Staphylococcus spp.	Detmer *et al.* (1990)

Those infections which can be kept absent by barrier protection will normally be absent in mice from commercial breeders, as all of these keep their colonies behind barriers. However, if problems are to be expected from those infections which can only be avoided in isolators, one will have to specify the need for gnotobiotic animals (see Table 6.6).

may occasionally be found as a spontaneous infection in hamsters, and on rare occasions also in mice and gerbils. The portals of entry are probably the mucous membranes and broken skin. Most reported cases of infection in humans derive from hamsters (Baum *et al.*, 1966). Human symptoms are mostly influenza-like, but meningitis, abortions and, in rare cases, fatalities may occur (Shek, 1994). The microspore, *Encephalitozoon cuniculi*, is widespread in laboratory rabbits and guinea-pigs. Therefore, it should be noted that it has recently emerged as an opportunistic parasite in human immunodeficiency virus (HIV)-infected humans (Desplazes *et al.*, 1996). Dermatophycoses, i.e. skin infections with *Trichophyton* and *Microsporum* spp., may occur in a wide range of laboratory animals. Cats, in which the infection is often asymptomatic, impose the highest risk for humans. The coccidium *Toxoplasma gondii* may also occur in a wide range of species, but its natural reservoir is in the cat, and care should be taken only to purchase cats free of this infection. In pigs the major risk seems to be development of erysipeloid after contact with pigs infected with *Erysipelothrix rhusiopathiae*, a Gram-positive rod found in most agricultural pig herds (Takahashi *et al.*, 1987).

Non-human primates share a range of infections with humans. Although most research primates of the western world today are purpose-bred, they are in general not produced under hygienic regimes as strict as those which are applied to rodents. Therefore, primates should be regarded as a risk for human health. The most serious problem is herpesvirus type B, which in macaques produces symptoms equivalent to herpes simplex in humans, while in humans it may cause fatal encephalitis (Weigler, 1992). The virus is present in several commercial breeding colonies. Humans bitten by seropositive monkeys should as a routine be treated with acyclovir. Hepatitis viruses of various types, especially type A, are of greatest concern, when working with chimpanzees (Friedmann *et al.*, 1971), although it should be noted that antibodies may be found in other primate species as well. It is, however, not clear whether these are due to viruses capable of infecting humans or just equivalent monkey strains without such a potential. Fatal filoviruses, i.e. Ebola and Marburg, are not present in commercial colonies and can simply be avoided by not purchasing wild-caught monkeys.

When dealing with primates the opposite transmission of infections, i.e. from humans to monkeys, must be regarded as a serious risk for ruining projects. For example, infection with measles virus can easily eradicate a colony of the common marmoset, *Callithrix jacchus* (Working Committee for SPF and Gnotobiotic Laboratory Animals, 1980). Also herpes simplex may produce devastating disease in marmosets (Hendrickson, 1972). Therefore, people with symptoms of these or other kinds of viral disease should not be allowed to handle monkeys. For the same reasons, staff from primate facilites are commonly screened for tuberculosis in those parts of the world where this disease is prevalent in the human population.

Prevention of infections in laboratory animals

Production of animals free of research-interfering infections

To avoid disease, microbial interference and zoonoses laboratory animals are produced according to a three-step principle: *rederivation, barrier protection* and *health monitoring.*

Rederivation means the production of animals to initiate breeding colonies by either caesarian section (Foster, 1959) or embryo transfer (Bur, 1995). Such animals are *germ-free* at delivery, except in rare cases for transplacental infections. For both procedures foster mothers are used: these are

Table 6.5 Important zoonoses in laboratory animals

Disease or disease agent	*High-risk animal species*	*Transmission*	*Reference*
Primate zoonoses			
Filoviruses			
Marburg	African green monkeys	Contact with contaminated excretions	Held *et al.* (1968)
Ebola (African strains)	Macaques	Contact with contaminated excretions	Pattyn (1978)
Hepatitis viruses, types A and E	Mostly chimpanzees (type A)	Enterically	Gust and Feinstone (1988); Ticehirst *et al.* (1992)
Herpesvirus type B	Macaques	Invasive or mucosal contact	Hendrickson (1972)
Monkeypox	Various	Probably by contact	Ivker (1997)
Mycobacterium tuberculosis	All old-world monkeys	Aerogenously	Goss (1970)
Salmonella spp.	All species	Enterically	Williamson *et al.* (1963)
Shigella spp.	All species	Enterically	Williamson *et al.* (1963)
Zoonoses of other animal species			
Lymphocytic choriomeningitis virus	Hamster, mice, gerbils	Invasive or mucosal contact	Baum *et al.* (1966)
Hantaviruses	Rats	Invasive or mucosal contact	LeDuc (1987)
Erysipelothrix rhusiopathiae	Pigs	Skin contact	Takahashi *et al.* (1987)
Yersinia spp.	Guinea-pigs, pigs	Ingestion of contaminated faeces	Aleksic and Bockemuhl (1990)
Leptospira	Rats	Invasive or mucosal contact	Kiktenko (1985)
Pasteurella multocida	Cats	Bites	Griego *et al.* (1990)
Salmonella	All species	Enterically	Morris (1996)
Campylobacter	All species	Enterically	Morris (1996)
Rat bite fever			
Streptobacillus moniliformis	Rats, mice	Invasive or mucosal contact	Wullenweber (1995)
Spirillum minus	Rats, mice	Invasive or mucosal contact	Bhatt and Mirza (1992)
Ringworm			
Trichophyton	Guinea-pigs, rabbits	Skin contact	Pier *et al.* (1994)
Microsporum spp.	Cats	Skin contact	Pier *et al.* (1994)
Encephalitozoon cuniculi	Guinea-pigs, rabbits	Enterically	Desplazes *et al.* (1996)
Toxoplasma gondii	Cats	Ingestion of spores	Dubey (1996)
Hymeonolepis spp.	Rats, mice	Ingestion of eggs/insects	Schantz (1996)
Visceral larva migrans (*Toxocara* spp.)	Dogs, cats	Ingestion of eggs	Fenoy *et al.* (1997)

either germ-free or *gnotobiotic*, i.e. they have only a well-defined microflora. The foster mothers and the rederived offspring are housed in isolators (Figure 6.1). Approximately 8 weeks after birth or section, the foster mothers and some of the offspring are sampled for a microbiological screening.

Animals kept in isolators can be kept totally germ-free or gnotobiotic. Larger-scale production in isolators is, however, expensive, and therefore most laboratory animals are bred in a *barrier unit*, i.e. a unit where materials are decontaminated before introduction and the staff showers on the way in (Figure 6.1). Barrier-bred animals do not have a fully known flora, but they can be kept free of some specified agents. Before being moved from isolators into the barrier unit, the animals are often given a starter flora consisting of, for example, lactobacilli and some anaerobic rods. Furthermore, they catch microorganisms of human origin from the caretakers. All this becomes "the normal flora" (Hansen, 1992). Members of this flora can also interfere with some kinds of research, e.g. in immunosuppressed animals (Table 6.4).

To secure the absence of specific microorganisms in breeding and preferably also experimental colonies of laboratory animals, a number of animals from the colony are

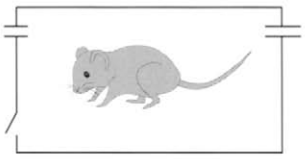
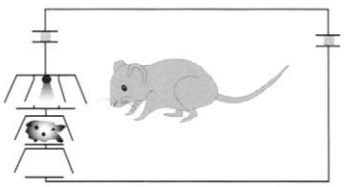
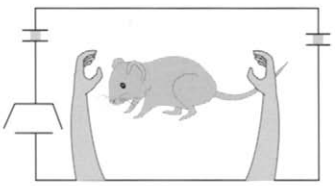
Protection level	*Designation of animal units*	*Designation of animals*
Ventilation No filters *Staff* Unrestricted entrance No quarantine *Materials* No decontamination *Protection obtained* Microbiological status is insecure	 Conventional	Conventional
Ventilation Filters in inlet and outlet *Staff* Entrance through a three-room shower Quarantine after contact with other animals *Materials* Autoclave Chemical disinfection lock *Protection obtained* Animals can be kept free of certain agents, but typically harbour microorganisms of human origin	 Barrier unit	*Preferred term* Microbiologically defined *Other terms* Specific pathogen-free (SPF) Virus Antibody-Free (VAF) Caesarian-Originated Barrier-Sustained (COBS)
Ventilation Filters in inlet and oulet *Staff* Animals are only handled from the outside through gloves *Materials* Disinfected, e.g. by irradiation and passed through a chemical disinfection lock *Protection obtained* Animals can be kept gnotobiotic (axenic), i.e. totally germ-free or with a fully defined microflora	 Isolator	*Preferred terms* Germ-free Gnotobiotic Axenic

Figure 6.1 Different hygienic levels for housing laboratory animals. Transition forms between the different levels exist, e.g. animal units in which materials are decontaminated but the staff do not shower on their way in. The so-called ventilated rack, in which each animal cage can be single-ventilated, is someway between a barrier unit and an isolator.

Table 6.6 An example of a health-monitoring report from a breeding unit for laboratory mice

FELASA-approved health monitoring report

Name and address of the breeder: Dept. Exp. Med., University of Copenhagen, Panum Institute
Date of issue: 5 May 1997 Unit no: 10.2 Latest test date: April 14 1997 Rederivation: 1987
Species: Mice Strains: Pan:NMRI, DBA1/J/Pan

	Historical results	*Latest test results*	*Laboratory*	*Method*
Viral infections				
Minute virus of mice	Negative	0/8	Panum	ELISA
Mouse hepatitis virus	Negative	0/8	Panum	ELISA
Pneumonia virus of mice	Negative	0/8	Panum	ELISA
Reovirus type 3	Negative	0/8	Panum	ELISA
Sendai virus	Negative	0/8	Panum	ELISA
Theilers encephalomyelitis virus	Negative	0/8	Panum	ELISA
Ectromelia virus	Negative	NT	Panum	ELISA
Hantaviruses	Negative	NT	Panum	IFA
Lymphocytic choriomeningitis virus	Negative	0/8	Panum	ELISA
Lactic dehydrogenase virus	Negative	NT	Panum	Enzymatic
Bacterial and fungal infections				
Bordetella bronchiseptica	Negative	0/10	Panum	Culture
Citrobacter freundii (4280)	Negative	0/10	Panum	Culture
Clostridium piliforme	Negative	0/8	Panum	ELISA
Corynebacterium kutscheri	Negative	0/10	Panum	Culture
Leptospira spp.	Negative	NT	Panum	Mic. agg.
Mycoplasma spp.	Negative	0/8	Panum	ELISA
Pasteurella spp.				
Pasteurella pneumotropica	Positive	3/10	Panum	Culture
Other *Pasteurella* spp.	Negative	0/10	Panum	Culture
Salmonella	Negative	0/10	Panum	Culture
Streptobacillus moniliformis	Negative	0/10	Panum	Culture
β-Haemolytic streptococci				
Group G	Positive	0/10	Panum	Culture
Other	Negative	0/10	Panum	Culture
Streptococcus pneumoniae	Negative	0/10	Panum	Culture
Other species associated with lesions: None				
Parasitological infections				
Arthropods	Negative	0/10	Panum	Inspection
Helminths	Negative	0/10	Panum	Flotation
Eimeria spp.	Negative	0/10	Panum	Flotation
Giardia spp.	Negative	0/10	Panum	Microscopy
Spironucleus spp.	Negative	0/10	Panum	Microscopy
Other flagellates	Negative	0/10	Panum	Microscopy
Klossiella spp.	NT	NT		
Encephalitozoon cuniculi	NT	NT		
Toxoplasma gondii	NT	NT		
Pathological lesions observed				
Stock: Pan:NMRI Lesions: None				

Abbreviations for laboratories

Panum	Dept. Exp. Med., Panum Institute, Univ. Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N		
Positive	Positive results previously observed	0/10	No positives out of 10 samples
Negative	Positive results never observed	NT	Not tested

The report form has been standardized according to guidelines issued by the Federation of European Laboratory Animal Science Associations (FELASA; Kraft *et al.*, 1994).

currently sampled and subjected to a range of bacteriological, parasitological and serological investigations. This procedure is referred to as *health monitoring*. All commercial vendors issue reports on the health monitoring performed in their colonies (Table 6.6), and animals should never be purchased without first having studied such a report. Typical intervals between screening of colonies are 6–12 weeks for rodents and 6 months for larger animals such as rabbits, pigs, dogs and cats. Results of health monitoring are historical, i.e. animals may become infected in the period between two samplings, and therefore infected animals may have been used for research, before the infection has been discovered. Although the number of animals to sample for health monitoring can be judged from statistical principles (Hansen, 1993), such statistical principles are not commonly applied in health monitoring, and therefore infections listed as "not found" in health-monitoring reports may be present but not found due to the use of too few animals. The typical sample size ranges from 6 to 10 animals. If this sample size is used to screen for *Clostridium piliforme* in rat colonies by the means of serology it would be statistically valid, while it would not be if pathology is used due to the lower sensitivity of the histopathological methods (Hansen *et al.*, 1994). To minimize such concerns about the microbial status of laboratory animal guidelines relating to a number of species have been published in Europe (Hem *et al.*, 1994; Kraft *et al.*, 1994). These guidelines list what to test for, how often to do it and how many animals to sample. In general, it is a sound principle to ask commercial vendors for health reports issued according to these guidelines, but it should be noted that the guidelines do not ensure statistical validity (Hansen, 1996).

Animals bred behind a barrier and being currently health-monitored are sold under different terms, among which *microbiologically defined* seems to be the most precise (Figure 6.1). Rodents, rabbits, pigs, cats and dogs can be purchased as microbiologically defined at several commercial vendors all over the world.

Also, animal units housing animals in long-term studies should run a health-monitoring system. For larger animals, such as dogs, cats and rabbits, this may be done by sampling directly from the animals. When using rodents in long-term studies the experimental animals will normally be supplemented by some equivalent animals — so-called *sentinels*, i.e. animals kept in the facilities only for health-monitoring purposes. The sentinels should be left in the unit for at least 30 days before examination. It is normal practice to add some bedding from the other animals in the unit to the fresh bedding of the sentinels (Hansen and Skovgaard-Jensen, 1995).

Quarantine housing

All introductions of new animals into an animal unit run the risk of introducing infections. Therefore, animals may be housed in a quarantine facility before introduction. In general, all animals not coming from suppliers with a reliable health-monitoring system should as a minimum be housed in a quarantine facility for at least 2 weeks and thereafter screened for those agents from which they must be free. For quarantine housing a *ventilated rack* may be used. This is a cage system where each cage can be single-ventilated. In this system different deliveries may be isolated from one another; this is not possible in a common quarantine facility. Alternatively, animals may be quarantine-housed in isolators. All primates, no matter what their origin, should be quarantined for at least 30 days before entering a running primate facility. During this period the animals should be inspected for signs of clinical disease and screened for unwanted infectious agents, especially those with a zoonotic potential, such as herpesvirus type B, *Salmonella* spp. and *Shigella* spp. During quarantine experiments should not be performed on the animals.

Screening biological materials for the absence of infections

To avoid biological materials of animal origin — sera, cells, tissue preparations — carrying infectious agents when used in an animal facility, such materials should either have their origin in an animal department with a reliable health-monitoring system or be screened directly for the presence of infectious agents. This has traditionally been done by the mouse antibody production (MAP) test (Collins and Parker, 1972): Mice are injected with the material intraperitoneally and nasally, and kept in isolators for 4 weeks, whereafter serum is sampled and screened for the infectious agents by serology. In recent years polymerase chain reaction has been used as a supplement to the MAP test (Yagami *et al.*, 1995). If biological materials of uncertain microbial status are to be used, inoculation and maintenance of the inoculated animals should only be performed in facilities efficiently separating these animals from all other animals, e.g. in negative-pressure isolators.

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