“实验动物国家标准论坛”专题

定义“SPF”级实验大小鼠和制定健康监测方案时需考虑的因素

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【摘要】 “无特定病原菌（SPF）”名词意味着排除了对宿主致病的特定的病原菌清单。许多机构在使用活体动物开展生物医学研究时，考虑到许多不同的因素，包括动物来源、设施布局、微生物学背景、工程技术标准、运行实践和试验需要，倾向于制定机构层面的生物排除清单。同样地，机构层面的健康监测方案的设计和实施也根据需要有所不同。相比之下，中国的实验动物生产者和使用者受制于国标，国标中根据动物健康分类建立了国家层面生物排除清单，同时也规定了特定的动物健康等级对应的详细的动物房工程设计标准。此外，本文还汇总了北美主要的啮齿类供应商的 SPF 清单，来提示实验动物使用者在评估自己的实验大小鼠的生物排除清单和制定机构层面的健康监测方案时应该考虑不同的因素，来确保动物种群的健康和科学的完整性。

【关键词】 无特定病原菌，生物排除，健康监测，考虑因素，大鼠，小鼠
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Factors to be considered when defining “SPF” and health monitoring programs in laboratory mice and rats

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【Abstract】 The term “specific pathogen free”（SPF）implies the bioexclusion of a defined list of organisms that can cause disease in a host. Due to many different factors including animal source, vivarium layout, microbiological history, engineering standards, operation practice, and experimental needs, the bioexclusion list tends to be specific for many institutions using live animals in biomedical research. As such, the design and implementation of institution-specific health monitoring program can also vary based on needs. By comparison, laboratory animal producers and users in China are subject to GB regulations which have established a national bioexclusion list based on animal health categories, as well as set detailed engineering standards for vivarium operations based on specific animal health profiles. In addition to summarizing the SPF list of major rodent vendors in North America, the purpose of this article is to bring attention to the different factors animal users should take into consideration when evaluating one’s own bioexclusion list and designing institution-specific health monitoring program for laboratory mice and rats, in order to assure animal colony health and scientific integrity.

【Key words】 Specific pathogen free; Bioexclusion; Health monitoring; Factors for considerations; Mice; Rats

Introduction

The term “specific pathogen free”（SPF）implies the exclusion of a defined list of organisms that can cause disease in a host. Within the field of laboratory animal science, this list generally includes viruses,

bacteria, fungi, and parasites with known potential to impact animal health, affect research outcome, or post as an occupational health hazard. For the discussion purpose, this article will cover bioexclusion list for laboratory mice and rats only. The current body of literature related to pathogens of concern is continuing to increase over the years. Technical refinements in microbial detection methods have allowed scientists to identify infective or carrier-state animals with more ease. They can study the epidemiology and pathogenesis of the agent, investigate changes in the physiological and immunological systems through controlled infections, and assess how animal model performance might be altered. Interesting and unique clinical cases published by human and veterinary clinicians, as well as periodic discussion amongst field experts during laboratory animal science forums, also help to direct public attention to any new or re-emerging microbial agent of rodent colony health concern. All these knowledge has allowed the general laboratory animal users, including researchers, laboratory animal specialists, and vivarium managers, to make informed decisions when faced with the decision: to test/exclude or not to test/exclude? For purpose of keeping the scope of this article narrow, limitations with regards to different diagnostic assays are not discussed, but readers are encouraged to review current literature and consult area specialists when deciding which specific assays should be used.

Consideration Factors for Setting Bioexclusion List

There can be differences in professional opinion on what microbial agents are of concern and therefore must be monitored and/or excluded from one's vivarium. All vivariums are structured and organized as microbiological units, with self-contained microbiological entity within a defined space. For example, a barrier facility within one or more rooms where personnel, equipment and animals move freely or where animals are kept in open cages; isolators or a group of microisolation cages where direct contact allows for horizontal transmission; single individually ventilated cage handled in a laminar flow cabinet following strict hygienic measures[1]. Once the unit structure is identified, a biosecurity program can be defined, with the understanding that biosecurity breach and introduction of pathogens could result from introduction of other animals (e.g. different species, different animal sources), fomites (e.g. bedding and experimental equipment), human caretakers, feed, and water. Biological materials such as cell lines, antibodies, conditioned media, and serum have also been implicated as potential source of contamination in a vivarium[2]. Therefore, for institutions designing their surveillance program and defining their own SPF or bioexclusion list, one must also take into consideration the effect of each microbial agent on animal health, impact on biomedical research, species specificity, zoonotic potential, prevalence, host factors (e.g. immune status, genetic background), as well as past and current microbiological status of the animal housing environment[1].

For obvious reasons, zoonotic agents are excluded from a vivarium unless research needs dictates otherwise, and that sufficient engineering controls with structural and resource support for containment are present. These pathogens are rarely found in purpose-bred rodent colonies, and most institutions check against these pathogens infrequently unless if working with wild-caught rodents or with biological materials. Institutions with researchers using immunodeficient and humanized rodent models for studying the immune system, xenotransplantation, and infectious disease models are encouraged to pay attention to potential introduction and amplification of microorganisms that are of rodent and human origin through biological materials.[3] For example, *Mycoplasma* spp. and viral contamination have been identified in tumor cell lines,[4,5] embryonic stem cells can be susceptible to persistent infection with mouse hepatitis virus and may produce viruses[6,7], monoclonal antibody have been found to be contaminated with lactate dehydrogenase elevating virus[8], murine germplasms can harbor mouse parvovirus,[9] and outbreaks of ectromelia linked to contaminated serum[10].

In general, animal users are more concerned with

prevalent infectious agents that can overtly impact rodent colony health, cause inapparent infections that lead to changes of animal model phenotype or alters research data, and to some extent, opportunistic and emerging (or re-emerging) pathogens. Detailed descriptions of infectious diseases in mice and rats have been discussed at length in books and review papers, including clinical signs and impact on research[11-18]. Through regular review of current literature, laboratory animal veterinarians and vivarium managers gather pertinent information to help them allocate and concentrate health monitoring resources on higher risk, prevalent infectious agents, and monitor less frequently for the remaining, lower-risk agents. As example, Charles River's Research Animal Diagnostic Services presented retrospective data on the prevalence of pathogens identified in rodent samples submitted from pharmaceutical, biotechnology, academic, and government institutions to diagnostic labs in North America and Europe. In mice, commonly detected infectious agents include mouse norovirus, the parvoviruses, mouse hepatitis virus, rotavirus, Theiler's murine encephalitis virus, *Helicobacter* spp. *Pasteurella pneumotropica*, and pinworms. In rats, commonly detected infectious agents include *Pneumocystis carinii* (previously termed "rat respiratory virus"), parvoviruses (rat minute virus, Kilham's rat virus, rat parvovirus, and Toolan's H-1 virus), rat theilovirus, *Helicobacter* spp., *P. pneumotropica*, and pinworms. Although *Staphylococcus aureus* was also prevalent in samples from both rodent species, this bacteria is typically considered opportunistic and are of minimal health concern in immunocompetent colonies, while continued monitoring against *Corynebacterium bovis* by PCR assays and culture suggest this bacterium continues to be found in immunodeficient mouse colonies[19].

In order to provide researchers with consistent, high quality animal models, laboratory rodent vendors will disclose their health monitoring program and define specific pathogens to be excluded. Influenced by production environment, vendors will classify the animals under different categories. For example, barrier reared animals tend to be free from a specified list of pathogens, but otherwise have undefined microflora. On the other hand, gnotobiotics including axenic and defined flora animals have defined microbial status and maintained using aseptic techniques. Table 1 is a list of microbes typically screened by well known rodent vendors in North America[20-22]. Samples may be collected from colony animals and from environment, while sampling frequency ranged from monthly to annually depending on disease prevalence and biosecurity risk. Some microbial agents, if identified, require immediate recycling of the production unit. Some are considered opportunistic and are only of concern to specific types of animal models; therefore are excluded from gnotobiotic or isolator reared colonies only. Some are non-pathogenic and commensal organisms and are therefore monitored but not excluded. Tables 2 and 3 summarizes the bioexclusion list from three different global vendors of laboratory mice and rats with comparison with the most current Chinese GB requirements[20-24].

With exception of axenic animals, all rodents carry with them a unique set of microflora from the environment they were born into. There will always be health risks, great or small, when exposing new animals to a different set of microflora within a new microbiological unit. Microorganisms that do not usually cause clinical signs in immunocompetent animals may cause disease in immunodeficient animals, or in animals whose resistance is lowered (e.g. by other diseases, experimental procedures, drugs). Genetically modified rodents may have unanticipated phenotypes including overt or subtle immunomodulation which result in disease induced by organisms thought to be commensal or previously unknown in that species.[1] Any organism has the potential to be an opportunist, provided that it finds a suitable host or favorable circumstances.

Lastly, when defining an institution-specific bioexclusion list, one must consider the past and current microbiological status and the intended use within a microbiological unit. Table 4 lists a few high and low risk scenarios for introducing unwanted agents

into a microbiological unit[1]. With exception of breeding and maintaining axenic animals where the use of specialized equipment (e. g. isolators) and labor intensive procedures are necessary to accomplish this goal, once the primary housing space (e. g. cage level) has been occupied by live animals, it is unrealistic to expect a completely sterile environment. Furthermore, in the secondary space (e. g. room level), with continuous movement of personnel and equipments, it is not surprising to expect the presence of some level of environmental microbes. On the other hand, what specific environmental microbes are actually present, and whether they are detrimental to animal health or science, should be assessed based on scientific evidence and operation risk. For example, organisms such as *Pseudomonas aeruginosa* is currently listed as a pathogen to be excluded in SPF rodents following GB requirement. This agent is widespread in nature and abundant in soil and water, but rarely causes disease in animals and humans. Althought it is not part of the indigenous microbiota of mice, it is commonly isolated from oropharynx and feces, especially in conventionally housed animals[12,15]. It may cause clinical disease in immunocompromised hosts, but has low significance in immunocompetent animals; therefore *P. aeruginosa* is often tolerated within commercial barrier facilities. Another example is *Staphylococcus* species, which commonly colonize the skin, mammary glands, mucous membranes, and gastrointestinal tract of man and animals. Surveys of staphylococcal carriage revealed cutaneous colonization of about 90% of healthy people and approximately 75% of conventional laboratory mice, with *S. epidermidis* being most predominant isolate from man, *S. xylosus* and *S. sciuri* from mice, and fewer than 10% carried *S. aureus*[25]. Several host factors, including age, physiologic state, and genotype, appear to increase the susceptibility of mice to staphylococcal infections, where *S. xylosus*, *S. epidermidis*, and *S. aureus* have all been isolated from dermal wounds. In general, staphylococci is considered as an opportunistic pathogen, as it harmlessly colonizes host tissue but is capable of proliferating and releasing virulence factors once the epithelial barrier is breached and bacteria contaminate the wounded tissues[15]. If working with animal models with compromised immune system, then it may be desirable to reduce the presence of pseudomonas and staphylococcus from the animal colony. One should make sure that: 1) newly arrived animals are demonstrably negative; 2) the agents are not enzootically present within the receiving microbiological unit; 3) the agents are included in the health monitoring program for the microbiological unit; and 4) there is an action plan developed in case of identification of one or more agents on the bioexclusion list.

For rodent vendors in North America, viruses and pathogenic ecto- and endo-parasites are generally screened and excluded, while monitoring of other microbes does not necessarily equate with automatic exclusion.

Consideration Factors when Designing a Health Monitoring Program

The goal of health monitoring is to detect a pre-determined list of excluded agents and see if the animal population being monitored meets the expected health specifications. There are multiple ways to design one's surveillance program; however, like all assays, none are perfect. The results from health testing provide insight into the microbial status of animals tested at a particular time. Take into context basic animal information such as clinical observations, husbandry practice, and experimental goals, it is the cumulative data from periodic testing of animals housed in a defined microbiologic unit over a longer period of time that proves more informative about the health profile of a population. Harmonization of health monitoring programs internationally have been discussed at international forums[1, 26-27], but some researchers argued that such guidelines or recommendations are not feasible as no animal facilities are identical, different monitoring programs (e. g. agents to be monitored, frequency of testing) may be different between microbiological units within the same facility, and different approaches may be necessary when monitoring immunocompetent vs. immunodeficient animals.[27]

Never the less the guidelines and recommendations serve as good starting points when developing institution-specific health surveillance programs.

Tab. 1 Compilation of microbes typically monitored by rodent vendors in North America as part of rodent health quality assurance program[20-22].

	Microbial Agents Monitored	
	Mice	Rats
Viruses	Sendai virus (SEND) 仙台病毒 Pneumonia virus of mice (PVM) 小鼠肺炎病毒 Mouse hepatitis virus (MHV) 小鼠肝炎病毒 Minute virus ofmMice (MVM) 小鼠微小病毒 Mouse parvovirus (MPV) 小鼠细小病毒 Murine norovirus (MNV) 鼠诺瓦克病毒 Theiler's mouse encephalomyelitis virus (GDVII) 小鼠脑脊髓炎病毒 Reovirus 3 (REO3) 呼肠孤病毒Ⅲ型 Mouse rotavirus (EDIM) 小鼠轮状病毒 Lymphocytic choriomeningitis virus (LCMV) 淋巴细胞脉络丛脑膜炎病毒 Ectromelia virus (Mouse Pox) 鼠痘病毒 Mouse adenovirus 1 and 2 (MAV) 小鼠腺病毒 Mouse cytomegalovirus (MCMV) 小鼠巨细胞病毒 Mouse pneumonitis virus (K) K 病毒 Polyoma virus (POLY) 多瘤病毒 Hantaan virus (HANT) 汉坦病毒 Mouse thymic virus (MTLV) 小鼠胸腺病毒 Lactate dehydrogenase elevating virus (LDV) 乳酸脱氢酶增高病毒	Sendai virus (SEND) 仙台病毒 Pneumonia virus of mice (PVM) 小鼠肺炎病毒 Sialodacryoadenitis/rat coronavirus (SDAV/RCV) 大鼠涎泪腺炎病毒/大鼠冠状病毒 Kilham rat virus (KRV) 大鼠细小病毒 RV 株 Toolan's H-1 virus (H1) 大鼠细小病毒 H1 株 Rat parvovirus (RPV) 大鼠细小病毒 Rat minute virus (RMV) 大鼠微小病毒 Reovirus 3 (REO3) 呼肠孤病毒Ⅲ型 Rat theilovirus (RTV) 泰勒病毒 Lymphocytic choriomeningitis virus (LCVM) 淋巴细胞脉络丛脑膜炎病毒 Hantaan virus (HANT) 汉坦病毒 Mouse adenovirus (MAV) 小鼠腺病毒
Bacteria, Mycoplasma, Fungi	*Beta hemolytic streptococcus* spp. 乙型溶血性链球菌 *Bordetella bronchiseptica* 支气管鲍特杆菌 Cillia-associated respiratory bacillus (CAR bacillus) 呼吸道纤毛杆菌 *Corynebacterium bovis* * 牛棒杆菌 *Corynebacterium kutscheri* 鼠棒状杆菌 *Clostridium piliforme* 泰泽病原体 *Citrobacter rodentium* 枸橼酸杆菌 *Helicobacter bilis* 胆型螺旋杆菌 *Helicobacter hepaticus* 肝型螺杆菌 Other *Helicobacter* sp. 其他螺杆菌 *Klebsiella oxytoca* 产酸克雷伯氏杆菌 *Klebsiella pneumonia* 肺炎克雷伯氏杆菌 *Mycoplasma pulmonis* 支原体 *Pasteurella multocida* 多杀巴斯德杆菌 *Pasteurella pneumotropica* 嗜肺巴斯德杆菌 *Pseudomonas aeruginosa* 绿脓杆菌 *Proteus mirabilis* * 奇异变形杆菌 *Salmonella* spp. 沙门菌 *Staphylococcus aureus* 金黄色葡萄球菌 *Streptobacillus moniliformis* 念珠状链杆菌 *Streptococcus pneumoniae* 肺炎链球菌 *Pneumocystis* spp. * 肺孢子菌 Dermatophytes 皮肤病原真菌	*Beta hemolytic Streptococcus* spp. 乙型溶血性链球菌 *Bordetella bronchiseptica* 支气管鲍特杆菌 Cillia-associated respiratory bacillus (CAR bacillus) 呼吸道纤毛杆菌 *Corynebacterium bovis* * 牛棒杆菌 *Corynebacterium kutscheri* 鼠棒状杆菌 *Clostridium piliforme* 泰泽病原体 *Helicobacter bilis* 胆型螺旋杆菌 *Helicobacter hepaticus* 肝型螺杆菌 Other *Helicobacter* sp. 其他螺杆菌 *Klebsiella oxytoca* 产酸克雷伯氏杆菌 *Klebsiella pneumonia* 肺炎克雷伯氏杆菌 *Mycoplasma pulmonis* 支原体 *Pasteurella multocida* 多杀巴斯德杆菌 *Pasteurella pneumotropica* 嗜肺巴斯德杆菌 *Pseudomonas aeruginosa* 绿脓杆菌 *Proteu mirabilis* * 奇异变形杆菌 *Salmonella* spp. 沙门菌 *Staphylococcus aureus* 金黄色葡萄球菌 *Streptobacillus moniliformis* 念珠状链杆菌 *Streptococcus pneumonia* 肺炎链球菌 *Pneumocystis carinii* ("RRV") 卡氏肺囊虫 Dermatophytes 皮肤病原真菌
Parasitology	Ectoparasites[a] 体外寄生虫 Endoparasites[b] 体内寄生虫 Protozoa[c] 原生动物 *Encephalitozoon cuniculi* (ECTRO) 兔脑原虫	Ectoparasites[a] 体外寄生虫 Endoparasites[b] 体内寄生虫 Protozoa[c] 单细胞生物 *Encephalitozoon cuniculi* (ECTRO) 兔脑原虫
Pathology	Necropsy and histopathology	Necropsy and histopathology

* Tested in gnotobiotic or defined flora colonies

[a] Ectoparasites screened can include *Myobia musculi*, *Myocoptes musculinus*, *Radfordia affinis*, etc.

[b] Endoparasites screened can include helminthes such as *Aspiculuris tetraptera* and *Syphacia* spp.

[c] Protozoa screened can include *Giardia muris* and *Spironucleus muris*, as well as non-pathogenic or commensal organisms such as *Entamoeba*, *Chilomastix*, *Hexamastix*, trichomonads, etc.

Tab. 2 Summary of some vendor bioexclusion lists for laboratory mice based on health profile as compared with the GB testing requirements[20-24].

Bioexclusion List	Vendor 1		Vendor 2			Vendor 3[@]			GB 14922.2-2011 14922.1-2001	
	Barrier reared (VAF/Plus®)	Isolator reared (VAF/Elite®) & immuno-deficient	Murine pathogen free™	Restricted flora™	Excluded flora & defined flora	Bio-exclusion level	Barrier reared	Isolator reared	Clean	SPF
SEND	X	X	X	X	X	1	X	X	X	X
PVM	X	X	X	X	X	1	X	X	—	X
MHV	X	X	X	X	X	1	X	X	X	X
MMV	X	X	X	X	X	1	X	X	—	X
MPV	X	X	X	X	X	1	X	X	—	—
TMEV (GDVII)	X	X	X	X	X	1	X	X	—	O
REO3	X	X	X	X	X	1	X	X	—	X
EDIM	X	X	X	X	X	1	X	X	—	—
MAV	X	X	X	X	X	1	X	X	—	O
POLY	X	X	X	X	X	1	X	X	—	O
K	X	X	X	X	X	1	X	X	—	—
MCMV	X	X	X	X	X	1	X	X	—	—
MTLV	X	X	X	X	X	1	X	X	—	—
LCMV	X	X	X	X	X	1	X	X	O	O
HANT	X	X	X	X	X	1	X	X	O	O
ECTRO	X	X	X	X	X	1	X	X	X	X
LDV	X	X	X	X	X	1	X	X	—	—
MNV	X	X	X	X	X	1	X	X	—	—
B. bronchiseptica		X	X	X	X	3	X	X	—	—
C. bovis		X*		X	X	1	—	X	—	O
C. kutscheri	X	X	X	X	X	1	X	X	X	—
C. rodentium	X	X	X	X	X	1	X	X	—	—
H. bilis		X	X	X	X	1	X	X	—	—
H. hepaticus	X	X	X	X	X	1	X	X	—	—
Helicobacter sp.		X	X	X	X	1	X	X	—	—
K. oxytoca		X		X	X	3	X	X	—	—
K. pneumoniae		X		X	X	3	X	X	—	X
P. multocida		X			X^	1	X	X		—
P. pneumotropica		X	X	X	X	1	X	X	—	X
P. mirabilis		X			X	3	—	X	—	—
P. aeruginosa		X		X	X	2	X	X	—	X
Salmonella spp.	X	X	X	X	X	1	X	X	X	—
Staph. aureus		X		X	X	2	X	X	—	X
S. moniliformis	X	X	X	X	X	1	X	X	O	—
Strep. pneumoniae		X	X	X	X	1	X	X	—	O
Beta hemolytic Strep. spp.		X		X	X	3	X	X	—	O
Pneumocystis spp.		X	X	X	X	1	X	X	O	O
C. piliforme	X	X	X	X	X	1	X	X	X	X
CAR bacillus	X	X	X	X	X	1	X	X	—	—
M. pulmonis	X	X	X	X	X	1	X	X	X	X
Ectoparasites	X	X		X	X	1	X	X	X	X
Helminths	X	X	X	X	X	1	X	X	X	X
Other endoparasites			X	X	X	1 / 2	X[#]	X[#]	—	X[a]
Pathogenic protozoa	X	X	X	X	X	1	X	X	—	—
E. cuniculi	X	X	X	X	X	1	X	X	O	O
Dermatophytes						1	X	X	O	O
Non-spore forming rod bacteria					X^		N/A	N/A	—	—
Cocci bacteria					X^		N/A	N/A	—	—
Yersinia pseudotuberculosis	—	—	—	—	—	N/A	—	—	O	O
Yersinia enterocolitica	—	—	—	—	—	N/A	—	—	O	O
Escherichia coli	—	—	—	—	—	N/A	—	—	O	O
Toxoplasma gondii	—	—	—	—	—	N/A	—	—	X	X

Notes. "X"- excluded; "—"- not tested; "*"- exclusion in immunodeficient animals or gnotobiotic/defined flora colonies only; "^"- allowed in excluded flora colonies; [@] Vendor houses microbiologically defined rodent colonies within maximum security production barriers and flexible-film isolators; Bioexclusion levels "1" = excluded from all animals; "2" = excluded from immunodeficient animals, but not immunocompetent animals; "3" = excluded based on customer demand; "#"- endoparasites excluded in immunodeficient animals include *Chilomastix* sp. flagellates, *Entamoeba muris*, and trichomonads; "[a]"- flagellates and ciliates; "O"- when testing is required, colony must be negative; "N/A"- not applicable

Tab. 3 Summary of some vendor bioexclusion lists for laboratory rats based on health profile as compared with the GB testing requirements[20-24].

Bioexclusion List	Vendor 1			Vendor 2		Vendor 3[@]			GB 14922.2-2011 14922.1-2001	
	Barrier reared (VAF/Plus®)	Isolator reared (VAF/Elite®) & immuno-deficient	Murine pathogen free™	Restricted flora™	Excluded flora & defined flora	Bio-exclusion level	Barrier reared	Isolator reared	Clean	SPF
SEND	X	X	X	X	X	1	X	X	X	X
PVM	X	X	X	X	X	1	X	X	—	X
SDAV/RCV	X	X	X	X	X	1	X	X	—	X
KRV	X	X	X	X	X	1	X	X	—	X
H1	X	X	X	X	X	1	X	X	—	X
RPV	X	X	X	X	X	1	X	X	—	—
RMV	X	X	X	X	X	1	X	X	—	—
REO3	X	X	X	X	X	1	X	X	—	X
RTV	X	X	X	X	X	1	X	X	—	—
LCMV	X	X	X	X	X	1	X	X	—	—
HANT	X	X	X	X	X	1	X	X	X	X
MAV	X	X	X	X	X	1	X	X	—	—
B. bronchiseptica			X	X	X	3	X	X	X	X
C. bovis		X	—	X	X	1	—	—	—	—
C. kutscheri	X	X	X	X	X	1	X	X	X	X
H. bilis			X	X	X	1	X	X	—	—
H. hepaticus	X	X	X	X	X	1	X	X	—	—
Helicobacter sp.			X	X	X	1	X	X	—	—
K. oxytoca		X		X	X	3	X	X	—	—
K. pneumoniae		X		X	X	3	X	X	—	X
P. multocida					X^	1	X	X	—	—
P. pneumotropica		X	X	X	X	1	X	X	—	X
P. mirabilis		X			X	3	—	X	—	—
P. aeruginosa		X		X	X	2	X	X	—	X
Salmonella spp.	X	X	X	X	X	1	X	X	X	X
Staph. aureus		X		X	X	2	X	X	—	X
S. moniliformis	X	X	X	X	X	1	X	X	O	O
Strep. pneumoniae		X	X	X	X	1	X	X	—	O
Beta hemolytic Strep. spp.		X		X	X	3	X	X	—	O
Pneumocystis carinii ("RRV")		X	X	X	X	1	X	X	O	O
C. piliforme	X	X	X	X	X	1	X	X	X	X
CAR bacillus	X	X	X	X	X	1	X	X	—	—
M. pulmonis	X	X	X	X	X	1	X	X	X	X
Ectoparasites		X	X	X	X	1	X	X	X	X
Helminths		X	X	X	X	1	X	X	X	X
Other endoparasites		X	X	X	X	1 / 2	X[#]	X[#]	—	X[a]
Pathogenic protozoa	X	X	X	X	X	1	X	X	—	—
E. cuniculi	X	X	X	X	X	1	X	X	O	O
Dermatophytes						1	X	X	O	O
Non-spore forming rod bacteria					X^		N/A	N/A	—	—
Cocci bacteria					X^		N/A	N/A	—	—
Yersinia pseudotuberculosis	—	—	—	—	—	—	—	—	O	O
Yersinia enterocolitica	—	—	—	—	—	—	—	—	O	O
Toxoplasma gondii	—	—	—	—	—	—	—	—	X	X

Notes. "X"- excluded ; "—"- not tested; "^"- allowed in excluded flora colonies; [@] Vendor houses microbiologically defined rodent colonies within maximum security production barriers and flexible-film isolators; Bioexclusion levels "1" = excluded from all animals; "2" = excluded from immunodeficient animals, but not immunocompetent animals; "3" = excluded based on customer demand; "[#]"- endoparasites excluded in immunodeficient animals include *Chilomastix* sp. flagellates, *Entamoeba muris*, and trichomonads; "[a]"- flagellates and ciliates; "O"- when testing is required, colony must be negative; "N/A"- not applicable

Tab. 4 High and low risk scenarios for introducing unwanted agents into a microbiological unit. [1]

High risk scenarios:
· Frequent introduction of animals into the biological unit
· Units of varying microbiologic status with close proximity
· Combining of animals from different breeding colonies or vendors
· Movement of animals out of the unit for manipulation and subsequent return
· Inadequate pest control program resulting in insects or wild rodents in animal rooms or feed/bedding storage area
· Frequent introduction of biological materials originating from the same animal species housed in the unit
· Multipurpose facilities with different types of experimental manipulation
· Frequent entry of research personnel into the unit in addition to animal care staff
· Frequent turnover of animal care personnel working in the unit
Shared equipment that cannot be disinfected easily
Low risk scenarios:
· Closed breeding colonies
· All-in-all-out system for the microbiological unit
· Occasional introduction of new animals
One or few types of experimental manipulation

Once a bioexclusion list has been developed, institutions could employ one or more approaches for sampling, including the use of sentinel animals with direct or indirect (e. g. via soiled bedding) exposure to colony animals, direct evaluation of feces/fur from colony animals, and direct evaluation of environment where pathogens will likely concentrate (e. g. filters, hoods, etc.)[28-30] Direct contact with the principal animals is the most efficient and reliable way to transmit infection to sentinels. If soiled-bedding sentinels are to be used, then several events need to occur in order for successful detection of infectious agents. In order for sentinels to become positive for an agent when tested, the colony animals must first be infected and be shedding an adequate amount (e. g. infectious dose) to be collected in bedding samples. The agent must remain infectious in soiled bedding, and sentinels must be susceptible. Sentinels must then self-inoculate with the infectious dose. Viruses must find the appropriate receptors on susceptible cells to replicate, and bacteria must compete with pre-existing flora. If the method of testing is by serology, then sufficient time must be waited for seroconversion to occur. Finally, conditions under which samples are collected, shipped, and tested, must be proper to avoid false positive or negative results[28]. For parasitology, traditional method of visual inspection can lack sensitivity, but could be compensated with increased sample size. On the other hand, contemporary PCR assays such as the high-density array of real-time PCR have adequate sensitivity to detect diverse pathogens in heavily pooled specimens collected noninvasively from colony animals, including viruses, bacteria, parasites, and protozoa. PCR has gained popularity in recent years as a complementary tool to the traditional health monitoring program that employs serology, culture, and visual assessment of samples and/or animals[29].

Sample size decision should be based on expected prevalence of infected (e. g. assay-positive) animals, not simply by the population size[31]. The binomial sampling formula suggested to ILAR Committee in 1976 assumes that the users have an idea of expected disease prevalence and animal population is >100[1, 28, 31-32]. Detection of disease with a low tendency to spread, such as mouse parvovirus, requires a considerable number of animals, whereas highly infectious agents such as mouse hepatitis virus or Sendai virus require only a few animals[27]. Negative test results does not prove that the population is free of the agent; instead, they simply give the users a level of confidence that the prevalence of assay-positive animals in the population is below the assumed minimum. As prevalence of positive animals decreases, the sample size required to achieve that same level of confidence increases. As compared to open-top caging systems, it is important to note that housing systems and husbandry practice play an important role in disease transmission. As such, popular use of microisolation cages (e. g. individually ventilated cages) can actually make detection of a low prevalent pathogen more challenging by serving as its own biocontainment zone[30, 31].

Frequency of sampling and testing should be driven by the historical rate of contaminations and the animal housing system. As examples, surveillance of commercial barrier rooms where viruses are the most frequent cause of adventitious infections, while contaminations with pathogenic bacteria and parasites are extremely rare, serology is performed more often than bacteriology and parasitology. On the other hand, gnotobiotic and immunodeficient colonies are typically maintained in isolators or in microisolation units to achieve high level of protection against opportunists, contamination with extraneous bacteria and fungi is much more common than viral and parasitic infestations, therefore bacteriology through culture and environmental swabs is done more frequently than serology and parasitology[31]. Generally institutions will conduct periodic testing (e. g. monthly, quarterly, annual) based on biosecurity risk assessment. Test parameters may be different at each time point, so long as the cumulative data is deemed adequate for providing vital information as to the animal colony health profile within the microbiological unit.

Finally, the choice of animals used for testing can be important for accurate health monitoring results. In general, immunocompetent animals are used for serologic monitoring, but institutions may choose to add immunodeficient mice to enhance the sensitivity of surveillance assays designed for the direct detection of infectious agents, such as PCR, bacteriology, or parasitology. Animal's age, sex, and genetics (e. g. different strains) may influence the susceptibility of infection and subsequent detection by different testing assays[1,33-36]. Outbred stocks are commonly used as sentinels because they are generally good serologic responders and are relatively inexpensive. Some inbred strains show partial or complete resistance to certain agents and should be avoided (e. g. C57BL/6 strains to MPV, SJL to respiratory strains of MHV)[35]; therefore, their appropriateness as sentinels should be carefully evaluated. Before placement of new sentinels into a health monitoring program, users should make sure that the animals are free of all infectious agents that would be of concern in the area that they are being chosen to monitor. The easiest option is to use animals raised in isolators of defined health profile, sourced from reputable vendors with strict quality control programs. In addition to dedicated sentinels, testing clinically ill animals will often provide useful information about the overall health profile within the microbiological unit.

Summary

Having a clearly defined and transparent health monitoring program will facilitate institutions in making informed decisions when introducing new animals into an established microbiological unit. It is not difficult for animal users to come up with a list of institution-specific microbial agents to exclude, including viruses, bacteria, fungi, and parasites that have known potential to impact animal and human health or confound research outcome. Many people look to major rodent vendors' microbial testing program and international guidelines for a list of organisms to monitor, but the need for monitoring opportunistic and commensal microbes will depend on several host and environmental factors. Similarly, detection assays of the agents can differ in sensitivity and specificity, which will influence the interpretation of the test results. Where appropriate, submission of biological samples for testing should be considered for a well-rounded health monitoring program. In the end, detection of an organism does not necessarily mean that it has to be eliminated, while a negative test result does not necessarily mean that the colony is free of the agent. Anyone charged with the responsibility of designing and implementing rodent health monitoring programs should have a sound understanding of rodent pathogens, take all of the aforementioned factors into consideration, and regularly review and adjust the health monitoring scheme as required, in order to ensure animal colony health and scientific integrity within the institution.

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