



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Medical Management and Diagnostic Approaches

Glen Otto and Craig L. Franklin

I. Introduction	548
II. Individual Animal Monitoring and Care	548
A. Observation and Examination	548
B. Signs of Illness and Distress	548
C. Treatment of Disease	548
III. Colony Health Management	550
A. Need for Monitoring	550
B. Health Status Terminology	550
C. Colony Health Management Considerations	550
D. Specific Components of Microbiological Monitoring	551
1. Random Testing of Resident Animals	551
2. Targeted Sentinel Programs	552
3. Vendor Screening	553
4. Quarantine	554
5. Screening of Imported Biological Materials	554
IV. Diagnostic Testing	555
A. Agents to be Monitored	555
B. Tests Used in Health Monitoring	555
1. General Test Performance Guidelines	556
2. Testing Methodologies	556
C. Testing Profiles	560
D. Test Interpretation and Retesting	561
V. Management of Colony Disease Outbreaks	562
A. Confirmation and Containment	562
B. Response Plan	562
C. Eradication Options	562
References	563

I. INTRODUCTION

This chapter reviews the basic principles of medical management of rat colonies and diagnostic approaches to detect infectious diseases of rats. As is the case with all other species, rats are susceptible to a variety of injuries and diseases that can cause distress, morbidity, or mortality. Any facility that houses rats must develop monitoring programs designed to rapidly identify health-related problems so they can be communicated to appropriate veterinary or animal care personnel to be resolved. These programs generally consist of multiple components, some of which are directed towards individual animals, and others that assess the health status of rat populations as a whole. Relevant aspects of medical management of rat colonies include individual animal monitoring and care; signs of illness and distress; colony health management; components of microbiological monitoring programs, including agents commonly targeted and sentinel programs; quarantine; biological material screening; diagnostic testing methodologies, including culture, serology, molecular diagnostic and histopathology; test profiles and interpretation; management of disease outbreaks; and treatment and prevention strategies for infectious agents.

II. INDIVIDUAL ANIMAL MONITORING AND CARE

A. Observation and Examination

Daily direct observation of rats for signs of abnormality is important, from a humane and ethical standpoint (Institute for Laboratory Animal Research-National Research Council, 1996a), and is vital in order to quickly identify problems caused by mechanical failure, trauma, pathogenic organisms, spontaneous disease, or research manipulations (Institute for Laboratory Animal Research-National Research Council, 1996b). The persons given the responsibility for this observation need to have had sufficient training or previous experience with rats in order to adequately detect abnormalities that might be present. Grossly visible trauma or lesions may not always be present in ill rats, but more subtle behavioral clues might be present. For this reason, it is important to be familiar with the normal range of behavioral patterns observed in healthy captive rats, which has been described (Saibaba et al., 1995).

Using appropriately sized equipment and methods similar to those used for larger species, it is possible to perform a relatively complete physical exam upon rats

when indicated (Sharp and LaRegina, 1998). The use of fabric or plastic restraint devices and/or protective gloves may be needed if the animal is fractious or the individual doing the examination is inexperienced, but most strains of rats are fairly amenable to gentle handling.

Careful observation and diagnostic evaluation is indicated for animals that show clinical signs when irradiated, exposed to corticosteroids or other immunosuppressive agents, or subjected to other types of significant stress, because latent infections might become symptomatic at these times (Small, 1984). Likewise, genetically immunodeficient animals (such as *rnu/rnu* rats) may manifest signs of disease from agents that are clinically silent in immunocompetent animals housed in the same area. Although the overall health status of most institutional rat colonies is monitored by routine screening of asymptomatic animals, it is important to realize that daily individual animal observation can sometimes identify an "index case" of a newly introduced disease that has not yet been revealed via routine scheduled testing.

B. Signs of Illness and Distress

Abnormal physical findings in rodents are not always useful in localizing an illness to a specific organ system. A very common constellation of findings indicative of pain or distress is piloerection, decreased activity, an ungroomed appearance, and often a hunched posture (Institute for Laboratory Animal Research-National Research Council, 1992). Weight loss is another nonspecific finding, but since weight determination is a simple, rapid, objective, and noninvasive technique, it is commonly used to assess the general health status of an animal placed under. It should be realized that stress is not always manifested as an absolute weight loss in a growing animal, so it may be necessary to take into account the normal weight gain of young rats in order to document a variation (Dymysz et al., 1963).

Table 16-1 describes signs of illness that can be seen in rats, along with possible diagnoses. This list is not meant to be an exhaustive summary, but it includes some of the more common clinical signs and suggests potential differential diagnoses.

C. Treatment of Disease

The majority of drugs administered to laboratory rats are provided prophylactically (for example, as part of perioperative care) or as a direct component of the research study. Because both the disease state and the use of xenobiotics (antibiotics) can affect the physiology of animals in a way that is difficult to control within the experimental design and could invalidate a study

TABLE 16-1
PHYSICAL FINDINGS

Abnormality	Potential diagnosis
Pale mucous membranes, extremities, or eyes	Anemia (if rat appears otherwise relatively normal) Excessive or too frequent blood collection Circulatory deficiency (if animal appears weak or depressed)
Alopecia with normal, intact skin	Physical abrasion from cage or feeder Self-grooming or barbering from cage mate
Alopecia with crusted, inflamed or ulcerated skin	Ectoparasites or dermatophytes Bacterial opportunists such as <i>Staphylococcus aureus</i> Pruritic syndromes
Dermal or subcutaneous masses	Tumors of skin or mammary origin Lymphadenopathy Abscess, granuloma, cyst
Nodular deformity of ears "Red" or "bloody" tears	Auricular chondritis Chromodacryorrhea caused by Harderian gland secretions (can also be seen on front paws and over the back from grooming) Frequent clinical sign of SDAV infection
Circumferential, annular constrictions on tail	Ringtail (generally seen in suckling animals under conditions of low humidity and a cool or poorly insulated environment)
Head tilt, circling or spinning when lifted by tail	Bacterial or mycoplasmal otitis interna/media Tumor or other space-occupying brain lesion
Hairless, swollen, or bleeding plantar lesions	"Sore hock" syndrome associated with large and/or aged rats kept on wire or mesh flooring
Salivation, weight loss, swollen oral tissues	Malocclusion
Fecal staining	Diarrhea
Dyspnea/rales/hyperventilation	<i>Mycoplasma pulmonis</i> , CAR bacillus, <i>C. kutschneri</i> , or <i>S. pneumoniae</i> infection Overheating
Facial swellings	Parotid and/or submandibular salivary gland swelling from coronavirus (RCV/SDAV) infection Abscess of lymph nodes (lymphadenitis) Zymbal gland tumor at the base of the ear
Abdominal distension (pot-bellied appearance)	Ascites Intestinal distension from toxicity (chloral hydrate) Enteritis (possibly megalooileitis associated with Tyzzer's disease) Obesity Abdominal mass (tumor, abscess)
Excessively wet hair coat and/or bedding	Pregnancy Diabetic polyuria Leaking bottle or automatic water system Behavioral water wastage from "playing" Overheating
Eye lesions	Blepharospasm, corneal opacities, keratitis due to coronaviral infection Cataracts (aging lesion)

RCV = rat corona virus; SDAV = Sialodacryoadenitis virus.

(Lipman and Perkins, 2002), ill rats are often euthanized rather than treated. However, the situation surrounding the incident should be carefully considered to determine whether it is prudent to gather appropriate antemortem diagnostic samples and to submit the carcass for necropsy evaluation even if the animal is terminated. Likewise, a process to monitor animal mortality records and to perform necropsy on animals whose death is suspicious is quite important, because in some cases such an evaluation can allow early detection of a problem that otherwise would reoccur and eventually affect a much larger group of animals.

In some situations, it certainly is useful to treat individual animals or larger groups if the animals are considered valuable to an ongoing study or are not being used to generate sensitive data. It is beyond the scope of this chapter to describe particular pharmaceutical dosages and treatment indications, but the reader can be directed elsewhere in this volume for disease-specific recommendations. Well-referenced and comprehensive formularies that include rat-specific drug dosages are also available, written for veterinarians in both the laboratory animal and "exotic" pet specialties (Carpenter et al., 1996; Hawk and Leary, 1999).

III. COLONY HEALTH MANAGEMENT

A. Need for Monitoring

Despite the fact that some infectious agents (for example, virulent strains of rat coronavirus) can induce readily identifiable abnormal physical signs, most infectious agents encountered in laboratory rat populations cause only subclinical disease, that is often signaled by interference with a research process. Such conditions can only be detected and identified via sensitive and comprehensive testing protocols (Institute for Laboratory Animal Research-National Research Council, 1991). Despite the lack of observable morbidity or mortality, these subclinical infections pose a significant risk to the research conducted with affected animals because they can alter the background physiology of experimental subjects or cause variation and alteration in specific experimental responses. These adverse effects have been summarized in a number of reviews (Institute for Laboratory Animal Research-National Research Council, 1991; Baker, 1998; Nicklas et al., 1999; Lipman and Perkins, 2002; Baker, 2003), and more recent studies continue to add to the list of potential adverse implications (Ball-Goodrich et al., 2002). Two of the physiologic processes that have specifically been shown to be altered by the presence of these infectious agents in rodents include immune function and neoplasia, which are quite relevant since immunogenetics, transplantation, and tumor biology are three of the disciplines that most heavily depend on the use of rats (Gill et al., 1989). As a result, one of the primary aims of a rodent colony health monitoring program is to document the presence or absence of particular infectious agents irrespective of any observable disease state. A term which is generally synonymous with colony health monitoring and is often used to convey this emphasis is *microbiologic monitoring* (Fujiwara and Wagner, 1994; Waggie et al., 1994).

Although there has been great progress in defining and improving the overall health status of laboratory rodent colonies in recent decades, many of the agents of concern remain endemic in institutional colonies (Institute for Laboratory Animal Research-National Research Council, 1991; Jacoby and Lindsey, 1998; Livingston and Riley, 2003), or may be introduced via human contacts or feral rodent contamination. Significant risks of one or more of these agents being introduced still exist in contemporary colonies. Due to the fact that a comprehensive colony health monitoring program is so vital in protecting the validity and reproducibility of experimental research data, it must be devoted an appropriate priority in terms of budget, personnel, and other resources. The policies and practices should be defined in written plans, and

agreement with the principles set forth should be secured by the scientific and administrative leadership of the institution, as well as by the veterinary and animal care group.

B. Health Status Terminology

The terms *axenic* and *gnotobiotic* refer to animals that harbor no cultivatable organisms or have a completely defined microbiological flora, respectively (see gnotobiology chapter); as a consequence, the health status of these animals regarding pathogenic or opportunistic agents is relatively easy to characterize. Terms that are less useful without detailed backup information include *specific pathogen-free* (SPF), and *conventional*. In general use, SPF refers to animals that are 1) considered to be free of major pathogens and some or all opportunists, 2) maintained under housing and use conditions designed to protect this high-quality status by excluding infectious agents, and 3) monitored closely to assure that there is no undetected introduction of excluded agents. Conventional animals are usually considered to be those that originate from uncontrolled colonies that are not subjected to routine health monitoring, or those in which some degree of monitoring occurs but there is no action taken if infectious agents are found. However, these terms are not really descriptive or representative enough to use when assigning risk to animals proposed for introduction to monitored, disease-free animal facilities. For example, animals from an institution that experienced an outbreak of rat coronavirus and decided not to undertake the steps needed to eliminate the agent from the facility can still be considered to be "SPF", since by definition this status is only defined by the particular list of agents of which the animals are specifically free. The term *conventional* also suffers from some ambiguity, since it can be used to refer to not only to animal health status but also to facility design. For example, a facility that allows direct staff entry into rat rooms without changing out of street clothes would be termed a conventional facility rather than a "barrier" in the purest sense, but if micro-isolators and high efficiency particulate air (HEPA)-filtered changing hoods have been used to successfully institute pathogen exclusion at the cage level, the animals themselves might possess a high-quality health status that is far from "conventional." In practice, it may be more effective to evaluate a specific panel of agents that animals have been tested for than to assign quality descriptions.

C. Colony Health Management Considerations

In contrast to a program designed to monitor individual animal health through the use of direct methods such as

close observation and physical examination, a program created to monitor the overall health status of a colony population will often utilize more indirect methods. Routine testing of selected representative animals (even in the absence of any signs of illness or disease) can provide valuable information regarding the viral, parasitic, and bacterial agents that such animals are either currently harboring or have been exposed to in the past.

Risk analysis should be done by any institution planning on holding rodents, a process which should involve a discussion of the relative costs and benefits of the various options available for routine health monitoring as well as quarantine isolation and testing. Although the available expertise of trained veterinarians, colony managers, and other professionals must be utilized, the discussions should not completely exclude the primary research directors and institutional officials who are needed to support the program both financially and administratively. It is also important to establish good communications with those individuals utilizing the animals for research so that they can report abnormal physiological responses or other experimental variation. It is not uncommon for research personnel to identify a problem with rodent-derived experimental data that ultimately is found to be due to microbiological contamination (Small, 1986; McKisic et al., 1993).

Risk-based sampling strategies will take into account 1) the frequency of introduction of new animals, 2) the quality and reliability of the source of introduced animals, 3) the mode of transport, 4) the pattern of personnel traffic into and out of the room, 5) the frequency of animal transport into and out of the room as part of the research project, 6) the potential for cross-contamination from other rooms inherent in the facility design, 7) the housing system, 8) the facility configuration (barrier design), 9) the proportion of the animals that are irreplaceable, 10) the proportion that are immunocompromised due to genetic factors, chemotherapy, or experimental stress, 11) the prevalence of infectious pathogens within the animal facility among laboratory rats in general, and 12) the potential for introduction of pathogens through the use of biological materials. The development of genetically engineered rats will add additional factors to consider when assessing risks of infectious disease.

It is important to realize that the window of detection varies for different types of diagnostic tests, and this must be taken into account whenever vendor screening or quarantine test results are interpreted. For example, when an antibody detection method (serology) is performed upon arrival, it is often considered representative of the vendor's colony, while subsequent seroconversion evident in serum drawn 2 to 4 weeks after delivery may be indication of exposure during transport or shortly after arrival at the user facility. However, tests that directly

identify components of the agent (such as an antigen detection assay or polymerase chain reaction, or PCR test) could theoretically be positive upon arrival due to either a pre-existing vendor problem or in-transit contamination.

D. Specific Components of Microbiological Monitoring

The primary goal of health monitoring is to detect the presence of an organism in at least one animal in the sample population, provided the organism is present. Equally as important, such testing is the means by which any of a panel of agents may be confirmed as not present. The components of most colony health monitoring programs include 1) periodic routine assessment of resident animals via random screening or targeted testing of dedicated sentinel animals, 2) the assessment of incoming animals through the use of vendor screening and/or quarantine testing, and 3) the assessment of biological materials destined for use in rat experiments. However, as stated earlier, the program for individual animal monitoring should include an attempt to identify index cases of diseases in the early stages of outbreaks that have not yet been detected by overall colony monitoring.

There is great variety in rodent health surveillance programs, and no two designs are usually identical (Institute for Laboratory Animal Research-National Research Council, 1991). However, some authorities advocate a certain degree of standardization (Jacoby and Homberger, 1999), and there are regional organizations that provide detailed specific guidelines for institutions that wish to participate (Yamamoto et al., 2001; Nicklas et al., 2002). Ultimately, health monitoring program design should cater to the needs of the institution. Consideration should also be given to needs of other institutions that could receive rats from the home institution (for example, the sharing of genetically engineered rats).

1. Random Testing of Resident Animals

Health monitoring is often performed on representative residents removed from the colony for specific testing. When selecting animals for screening, there are certain points to keep in mind. Animals to be sampled should be taken from rack and shelf locations spread throughout the room to maximize the possibility of detecting an isolated focus of contamination. If multiple stocks or strains are present, an attempt should be made to sample representative rats from each of these subcolonies. It is also desirable to test both young and old animals (avoiding geriatric animals) if they are available, since parasite burdens may be higher in the young (Institute for Laboratory Animal Research-National

Research Council, 1991), while the old would have had the best chance of seroconverting to agents that may have not yet affected younger animals. In a breeding colony, ideal choices might be retired breeders and surplus weanlings. Immunodeficient animals are a good choice for detecting parasites and bacterial contamination, since they may have a lowered resistance to such agents. However, it is important to remember that serology will be subject to false negative results if performed on an animal with a genetic or induced immunosuppression that may impair the antibody response. The purpose of health surveillance is generally not to accurately determine the specific *prevalence* of infection or disease in an area, but rather to accurately identify its *presence* by finding at least one positive animal in an endemic colony (Institute for Laboratory Animal Research-National Research Council, 1991). The minimum number of animals from a population that need to be tested in order to identify one positive animal can be viewed as a statistical exercise in random sampling. Probability theory can provide the equation necessary to determine the sampling size needed, based on the assumption that one is dealing with an ideal population (100 or more animals, where all animals have an equal opportunity for pathogen exposure) and calculated based on variables such as the prevalence of infection (often estimated at 30%) within the population and the degree of confidence required in the result (Clifford, 2001). These equations have been used to prepare charts that have been published to assist in the selection of sample size (Small, 1984; DiGiacomo and Koepsell, 1986; Institute for Laboratory Animal Research-National Research Council, 1991). For example, if an infectious agent affects 25% of the rats in a population, one would only need to test 15 randomly selected individuals in order to have a 99% probability of detecting the agent. These calculations have the most robust application when dealing with large populations of animals held under conditions that provide little or no barrier to cage-to-cage transmission (that is, an open shoe box or suspended caging with no filter tops) because, under those circumstances, agents are fairly uniformly distributed and would be expected to have a prevalence of 30% or higher. Under those circumstances, even if a room holds 1000 rats, it would still only be necessary to sample 8 of them to be 95% sure that an agent is not present. Many vendor quality assurance programs are based on this type of calculation.

2. Targeted Sentinel Programs

Alternatives to a random sampling approach are needed because a large percentage of rats in contemporary research colonies are housed under circumstances that do not result in a uniform distribution of transmissible agents, due to the popularity and utility of cubicles (segregating

fewer than 100 animals into functional groups) and/or systems that provide a barrier at the cage level, such as static microisolators or ventilated rack caging. These housing systems are beneficial in decreasing the likelihood of disease transmission, but they also make it harder to detect infectious agents based on random screening protocols, because the agent distribution is not uniform and the prevalence of infection may be far below 30%. Another problem with random screening techniques is the impracticality of selecting and testing animals from active research colonies without disrupting the ongoing research. For this reason, it is common to place *sentinel* animals into a colony for the sole purpose of health status testing. These animals are not assigned to any particular study, and under ideal circumstances they will be exposed to the same agents as the *principal* animals actually being used for research. Because they exist solely for the screening program, sentinels can be bled, sampled, or removed for nonsurvival testing at the discretion of the colony management, without interfering with ongoing experiments.

Sentinels should be immunocompetent young adult rats (6 to 8 weeks of age) (Koszdin and DiGiacomo, 2002). The use of aged rats should be avoided if possible, as these animals may be more prone to false positive seroreactivity (Wagner et al., 1991). Selection of a particular stock or strain of rat to be used as sentinels will vary, and there is no single correct choice. Using the same stock and source as the principals that are being monitored may be ideal because it eliminates the additional risk of contamination that would occur if animals were imported from another source specifically to be used as sentinels (Institute for Laboratory Animal Research-National Research Council, 1991). For closed breeding colonies, this can be done by setting aside some of the animals bred locally to be used as sentinels, and if animals are commercially obtained, extra animals can be ordered along with the principal shipment. However, this approach is not always practical in non-closed colonies, and it is common for facilities to specifically order sentinel animals from a reliable commercial source to be used as sentinels. In this case, a readily available outbred line is often chosen for sentinel use, since they are inexpensive and will generally mount a robust antibody response. Inbred lines can also be used, but it is important to consider any strain-specific limitations of disease susceptibility or immune responses, since these may affect their utility as sentinels. Occasionally, sentinels will be chosen so that they have a coat color that differs from the principal animals they are associated with to minimize the possibility that they will be mistaken for experimental animals.

Sentinels should be placed in physical proximity to the principal animals they are associated with to ensure that they are exposed to equivalent environmental

contamination. It is desirable to place them in a consistent spot on each rack so that husbandry and research staff can anticipate their location. If a single cage is used, it is customary to place it on the bottom shelf, since it is assumed that the concentration of aerosolized agents and particulate fomites will be highest near the floor. There are no firm guidelines for the relative density of sentinels, but for logistical reasons at least one sentinel cage should be in place on each rack or in each cubicle. The placement of one sentinel cage on each standard 25-cage rack has historically worked well in most situations. Other approaches can be taken, such as allocating sentinel cages to each breeding or experimental subgroup, placing multiple cages on each rack to increase the theoretical sensitivity of the program, etc. Depending on the specific design of the program, each sentinel cage may contain either a single animal or a small group of rats. When multiple sentinels of the same age are kept in a cage, it is rarely useful to sample more than one at any time, since the microbiological status of cohabitating animals is generally uniform. The use of small groups offers an advantage: the other sentinels in the cohort can be used to confirm positive results found in the rat initially submitted for testing.

In housing situations where filter-topped cages are being monitored, it is a common practice to remove the lids from the cages used to hold sentinels, effectively keeping them in "open" cages. This is done to increase the exposure of the sentinels to environmental contamination that might be transmitted by either true aerosols or small particulate fomites that are generated and dispersed within the room as part of routine rodent care and use. However, it should be noted that, in this type of situation, the subpopulation of rats with the highest cumulative risk of becoming infected with an agent (for example, the sentinel cages receiving a constant flow of dirty bedding) are NOT being held with the same degree of cage-level containment as the principal animals, and if they do become infected, the amount of environmental contamination and subsequent cross-contamination to other cages in the home room or elsewhere may be increased. Since other open sentinel cages in the room would be at highest risk for secondary transmission, it also may become more difficult to determine the point of origin of an agent within a room if sentinels are becoming infected, not from their assigned principal cages, but from other sentinel cages, essentially giving a type of false positive result (Weisbroth et al., 1998). During the sentinel program planning process, the benefit of a potential increase in the sensitivity of open-caged sentinels to detect an agent needs to be balanced against these potential adverse effects.

The process of routinely transferring *soiled* bedding from principal rodent cages into sentinel cages will increase the sensitivity of a monitoring program and can decrease the duration of sentinel exposure needed to detect endemic

agents (Thigpen et al., 1989). The specific procedures utilized for the collection of soiled bedding and the transfer to sentinel cages vary widely as a result of the different types of cage/rack/hood configurations that are used and because they must integrate with the specific procedural methods being used for overall cage changing. However, to ensure that the transfer of bedding is having a net positive impact on colony health (by aiding in the detection of excluded agents) rather than a net negative impact (by increasing the cage-to-cage transmission between principal cages) this practice should be standardized and incorporated into both written procedural descriptions and employee training programs. It should be realized that dirty bedding transfer may not reliably transmit all agents of concern in a rat colony (Dillehay et al., 1990; Artwohl et al., 1994; Cundiff et al., 1995).

The optimum time interval between the placement of sentinels and their screening is another factor that has not been definitively determined. The time it takes a sentinel to be exposed to endemic infection would be expected to vary depending on specifics such as 1) the relative density of sentinels, 2) the frequency of cage changing and soiled bedding transfer, 3) the percentage of principal cages that have bedding sampled at each change, 4) the caging system in place, 5) the prevalence and transmissibility of the infectious agent present, and 6) possibly the macro-environmental characteristics of the room, such as relative humidity and ventilation. Once a sentinel is exposed, there will be an additional delay until the development of an immune response ascends to levels that can be detected by serologic means. Experimentally, it has been shown that sensitive antibody determination tests can identify seroconversion in a period as short as 1 week post-exposure for rats infected with agents such as the rat coronavirus/sialodacryoadenitis virus (RCVSDAV) (Smith, 1983) and the RV parvovirus (Ball-Goodrich et al., 2002). However, a more "average" timeframe is within the range of 2 to 3 weeks, and it is felt that the utility of testing results will be greatest if a period of 21 to 28 days is allowed for seroconversion. For this reason, sentinels should generally not be sampled before they have had at least 1 month of exposure. It cannot be assumed that an agent will make its way into a sentinel cage during the first week or two, so many programs allow for an exposure period longer than 1 month (for example, utilizing 2 to 3 months of exposure as part of a quarterly monitoring schedule).

3. Vendor Screening

Facilities wishing to verify the reports obtained from commercial colonies may establish formal vendor screening programs whereby a small group of rats are obtained specifically for diagnostic testing without the vendor's knowledge. Sampling of animals that are euthanized

immediately upon arrival can provide confirmation of the health status of the animals as maintained by the vendor, although it should be recognized that serology would generally not be expected to consistently identify animals infected less than a week previously. If the intent is to fully evaluate the status of all animals delivered, this testing must be repeated for each breeding unit of animals accepted from the vendor, and it should also account for the fact that vendors may produce the same strain in multiple, physically distinct breeding or holding areas (Small, 1984). Such a program might be feasible for facilities with a very limited list of vendors and a small number of strains in use, but is often impractical for facilities that serve large, multidisciplinary institutions. In this situation, a more limited and targeted vendor surveillance program might be useful (for example, surveying animals when a new vendor is under consideration, or getting more information if there are specific concerns about the status of animals from a particular vendor for some reason). Occasionally, the status of the vendor's production colony is not in question, but possible contamination during transport and delivery is suspected. If that is the case, incoming vendor animals for testing should not be killed upon arrival, but should be placed in a quarantine facility that provides for not only containment but also exclusion of infectious agents (to eliminate confounding cross-contamination within the facility). They can then be given time to fully colonize with and/or seroconvert to agents they were exposed to in transport, and tested on a schedule similar to other animals subjected to quarantine.

4. Quarantine

In many cases, the relative risk to the existing colony from newly-acquired animals that are shipped from a high-quality vendor and arrive in intact, filtered shipping containers is small enough to allow direct introduction into the room (Small, 1986; Institute for Laboratory Animal Research-National Research Council, 1996b). The documented procedures for rodent receipt under these circumstances should include a careful inspection of the containers upon arrival, the rejection of those that are damaged, and careful handling and disinfection of the external surfaces to minimize the risks from superficial contamination of the crate.

In contrast, animals proposed for introduction from noncommercial sources are often bred, packed, and shipped under less stringent conditions, and the establishment of a quarantine program for this type of transfer is very important.

The type of health monitoring documentation available when animals are obtained from a university, research institute, or biotechnology/pharmaceutical company may

be quite variable, and should be carefully interpreted as plans are made to receive and quarantine rats. Terms such as *SPF* or *conventional* are useful in relaying the general status of a colony, or to contrast the differing characteristics of animals from different rooms/buildings/facilities (much the same as the terms *clean* and *dirty*) but much more specific information should be obtained from the sending institution. From a health monitoring perspective, the status of each cohort of imported animals must be defined individually, based on the recent and historical findings of specific health monitoring tests. When introducing animals into a disease-free facility and making decisions about the relative risk, all animals should be considered suspect until there is data to suggest otherwise.

It is vitally important to achieve functional segregation and isolation of animals during a quarantine period, not only to protect the health status of other rodents in the facility, but also to ensure the ability to accurately determine the actual source of any contamination identified during quarantine. Room-level isolation would be ideal, but often there are space constraints when dealing with small shipments of rodents, and the common procedure is to utilize flexible-film isolators, cubicles, or ventilated cabinets of some type to partition a quarantine room (Small, 1984). In contemporary colonies, the introduction of a novel, noncommercial rat strain is a much less frequent occurrence than the transfer of a mutant mouse line. However, if this activity increases in the future (as many in the field feel that it will) it may be necessary to consider programs similar to those described for mouse quarantine that group multiple shipments into a single cohort for batch testing (Rehg and Toth, 1998). The availability of microisolator-type caging, either as static units or within ventilated racks, has also allowed programs to be designed that are not all-in-all-out but still allow functional isolation and segregation of multiple shipments within the same room (Institute for Laboratory Animal Research-National Research Council, 1996b; Otto and Tolwani, 2002). Although this option will provide more flexibility and may reduce the space requirements for quarantine, proper operational procedures are extremely important, since the whole system is reliant upon proper technique.

5. Screening of Imported Biological Materials

All tissue cultures and tumors should be tested and approved as free of infective contaminants prior to use in rats (Sharp and LaRegina, 1998). Recent experiences have shown that even cell-free biologicals have the potential to introduce agents to rodent colonies when imported (Lipman et al., 2000). Similar to the procedures used for mouse tissues, a rat antibody production (RAP) bioassay can be performed, whereby naïve animals held in

quarantine are inoculated with a representative aliquot of the suspect material and tested 4 to 6 weeks later for seroconversion to excluded agents (Small, 1984; Johnson, 1986). Alternatively, newer technology makes it possible to utilize various types of polymerase chain reaction (PCR) assays on the materials themselves to more directly assess them for the presence of infectious agents (Besselsen et al., 2002; Bootz and Sieber, 2002; Blank et al., 2004).

IV. DIAGNOSTIC TESTING

A. Agents to be Monitored

There should be a very specific justification for each agent tested, based on the potential for an adverse effect on animal health or research studies (Institute for Laboratory Animal Research-National Research Council, 1991). There is no firm agreement on exactly which agents should be eliminated from high-quality rat populations, but there is a general consensus on the organisms which have the most potential detrimental impact and thus are almost universally monitored for and excluded (Waggie et al., 1994; Institute for Laboratory Animal Research-National Research Council, 1996b; Nicklas et al., 2002). These agents are listed in Table 16-2.

There are a number of agents not on this list that also have the potential for significant impact and merit monitoring in colonies being carefully maintained. *Clostridium piliforme* (the agent of Tyzzer's disease) and the cilia-associated respiratory (CAR) bacillus have proven difficult to detect as part of routine screening, but innovative diagnostic tests are making this more easily done (Boivin et al., 1994; Franklin et al., 1999).

Members of the *Pasteurella pneumotropica* complex often colonize the murine respiratory tract and can cause opportunistic infections in rats as well as mice (Kohn and Clifford, 2002). Emerging pathogens such as newly-discovered parvoviruses and members of the bacterial genus *Helicobacter* have been studied and characterized primarily in mice, but since they can colonize rats, facilities should begin to develop plans for monitoring these agents and taking action should they be found (Riley et al., 1996; Ball-Goodrich et al., 1998; Haines et al., 1998; Goto et al., 2000; Wan et al., 2002). Although it would be rarely encountered in rat colonies maintained at a high health status, many facilities opt to monitor for the bacterial agent *Streptobacillus moniliformis*, because it can cause a zoonotic disease (rat bite fever) and also could be a marker for wild rat contamination. Other potential pathogens include the Theiler murine encephalomyelitis virus-like agent, reovirus-3, mouse adenovirus-1, and *Pneumocystis carinii*. The latter is likely carried by most rats, but its potential to cause clinical or histologic disease is primarily in immunodeficient rats.

Agent-specific frequencies of testing can be determined based on the perceived risks of infection, transmissibility of the agent, potential impact of the agent to the population and associated research, immunocompetence of the colony being screened, ubiquity of agent, and the requirements of the biomedical research community, but it must be recognized that economic considerations also will play a role (Institute for Laboratory Animal Research-National Research Council, 1991). Tests for agents that are considered to pose a similar risk can be grouped together. For example, commonly encountered agents such as coronavirus, parvovirus, *Mycoplasma pulmonis*, and pinworms might be tested for on a quarterly basis, while more infrequently-detected agents such as Hantaan and Sendai virus could be surveyed on an annual or semi-annual basis.

TABLE 16-2
CORE AGENTS FOR SCREENING

Type of organism	Specific agent
Viruses	Sendai virus
	Rat corona virus (RCV or SDAV)
	Rat virus (RV or KRV)
	H-1 parvovirus
	Rat parvovirus (RPV)
	Pneumonia virus of mice (PVM)
Bacteria	Hantaan virus
	<i>Streptococcus pneumoniae</i>
	<i>Mycoplasma pulmonis</i>
	<i>Corynebacterium kutscheri</i>
Parasites	<i>Salmonella</i> spp.
	<i>Syphacia muris</i>
	<i>Radfordia ensifera</i>

SDAV = Sialodacryoadenitis virus.

B. Tests Used in Health Monitoring

The monitoring of laboratory rats for infectious disease utilizes a variety of tests. These include those in the general categories of gross necropsy, examination of serum for antibodies to infectious agents (serology), culture of bacterial pathogens, molecular tools designed to amplify infectious agent genomes, microscopic examination for parasites, and histologic examination of tissues. For routine health monitoring, these tests are often packaged, depending on the institution's needs, into profiles that include one or more testing modalities. For additional reading, a number of excellent reviews are available (Weisbroth et al., 1998; Compton and Riley, 2001; Feldman, 2001; Livingston and Riley, 2003).

1. General Test Performance Guidelines

Determining which test or which battery of tests to use in detecting infectious disease requires some knowledge about test performance. An ideal test is one that in all cases clearly distinguishes between exposed and unaffected animals (Weisbroth et al., 1998). Diagnostic tests can be assessed via several parameters (Table 16-3; from Bellamy and Olexson, 2000). In general, diagnostic sensitivity and specificity are of greatest importance when designing a health monitoring program. Tests with high sensitivity will generate a very low percentage of false negative results, whereas tests with high specificity will generate a low percentage of false positive results. Tests with high (>90%) sensitivity and specificity (for example, serology) should be used when available. For those tests that lack sensitivity or specificity (for example, histology), results must be interpreted accordingly. Other parameters, such as positive and negative predictive values, may also be of value in interpreting results, however these parameters can be affected by agent prevalence. For example, when agent prevalence is low, the calculated positive predictive value may also be misleadingly low (Lipman and Homberger, 2003). While highly sensitive and specific tests are available, it should be realized that no test is 100% sensitive or 100% specific. To this end, all unexpected results should be confirmed either through the use of corroborative testing platforms, the testing of additional

animals, or both. In no case, should a decision about colony status be made based on a single positive result.

As discussed earlier, daily observation is a critical component to any health monitoring program. Recognition of clinical signs is especially important in the early detection of outbreaks of disease and documenting emerging diseases. However, because most agents that infect rats cause subclinical disease, observation is a very insensitive means of screening for infectious disease. As a result, sentinel and colony monitoring programs have been developed. For health monitoring, animals may either be euthanized and a necropsy examination performed, or samples for testing may be collected from live animals such as blood for serology, feces for molecular diagnostics, or perianal tape test samples for pinworms.

2. Testing Methodologies

a. **PRE-NECROPSY EXAMINATION.** A pre-necropsy examination should include collection of important circumstantial and historical data that may be important to test interpretation. Historical data include, but are not limited to, housing and husbandry conditions, rat strains housed, the genetic and immune status of the rats to be screened, number of animals in the colony, and individual identification, including sex, approximate age, and pelage color (Weisbroth et al., 1998). Prior to necropsy, animals should

TABLE 16-3

PARAMETERS FOR ASSESSING DIAGNOSTIC TEST PERFORMANCE.

Test characteristics	Formula
Diagnostic Sensitivity—likelihood that an animal will be positive for a particular test given that the animal is truly infected with the agent	$TP / (TP + FN) \times 100\%$
Diagnostic Specificity—likelihood that an animal will be negative for a particular test given that the animal is truly free of that agent	$TN / (FP + TN) \times 100\%$
Positive Predictive Value—estimate of the likelihood that an animal with a positive test has an infection; provides an estimate of the percentage of animals that are likely to have an infection given that they are positive for a particular test	$TP / (TP + FP) \times 100\%$
Negative Predictive Value—estimate of the likelihood that an animal with a negative test is free of the infection; provides an estimate of the percentage of animals that are likely to be free of an infection given that they are negative for a particular test	$TN / (TN + FN) \times 100\%$
Diagnostic Accuracy—provides a measure of all results (positive and negative) that correctly classify infectious disease status	$(TP + TN) / (TP + FP + TN + FN) \times 100\%$
Prevalence—an estimate of the frequency of an infection in a population at a point in time	$(TP + FN) / (TP + FP + TN + FN) \times 100\%$

FN = false negative results; FP = false positive results; TN = total negative results; TP = total positive results.

be examined for normal activity, ambulation, posture, hair coat appearance, and the presence or absence of discharges (Weisbroth et al., 1998).

b. **NECROPSY EXAMINATION.** A gross necropsy examination of the rat is critical in situations where rats exhibit clinical signs or when increased mortality is noted (see Euthanasia and Necropsy chapter). For example, if multiple rats develop cervical swellings and/or excessive periocular and perinasal porphyrin accumulation, a gross necropsy may reveal swollen salivary glands that support a tentative diagnosis of SDAV coronavirus infection. As a result, colony management decisions can be implemented while confirmatory tests are being pursued. Gross necropsy examinations are also often a component of health monitoring screens. Such necropsy examinations usually include a thorough examination of major organ systems with sample collection dependent on gross findings. Gross necropsy may reveal a multitude of lesions including abscesses, pneumonia, developmental defects, urolithiasis, neoplasia, trauma, and malocclusion. Unfortunately, there are few gross lesions that are pathognomonic for specific infectious diseases. Moreover, in many infectious diseases, gross lesions are not evident and the gross necropsy serves to enable sample collection for other more sensitive assays.

c. **SEROLOGY.** Examination for antibodies produced during an infection is the most economical and efficient means of screening rats for infectious disease. Serology offers several advantages: 1) testing requires serum, which can be obtained from an either euthanized or anesthetized rat; 2) multiple tests can be performed on a single serum sample; 3) antibodies (IgM followed by IgG) are detectable 1 to 2 weeks following exposure to the infectious agent; 4) serum antibody is long lasting (months), so the organism does not need to persist in the host for the infection to be detected; and 5) the antigens used in serologic assays can be highly purified, rendering these tests very sensitive and specific (Livingston and Riley, 2003).

A variety of serologic assays have been developed; the enzyme-linked immunosorbent assay (ELISA) and the immunofluorescence assay (IFA) have emerged as the two most popular platforms. A new multiplex fluorescent immunoassay (MFI), that utilizes antigen coated beads, has recently been developed and may supplant ELISA in high-throughput diagnostic laboratories. Other methods such as hemagglutination inhibition, complement fixation, and serum neutralization are time consuming and not as sensitive as ELISA or IFA and are thus no longer routinely used in rat infectious disease diagnosis. Additional methods such as Western blot analysis are valuable adjunct tests for ELISA and IFA but are not commonly used as primary tests.

ELISAs are highly sensitive and can be highly specific depending on the choice of antigen. Because ELISA is

adaptable to automation, a large number of samples can be rapidly screened, and this testing platform is relatively inexpensive. Indirect ELISA methodology utilizes antigen bound to a solid phase (96 well plates or beads; Kendall et al., 1999). Serum is added and if antibody to the antigen is present, it will bind in a specific manner. Antibodies not specific for the antigen are removed in subsequent washing steps. Following washing, enzyme-labeled antibodies that are specific for rat antibody (enzyme conjugated anti-immunoglobulin) are added. These bind to rat antibodies that were bound in the first step. The last step involves the addition of a substrate for the enzyme label. If specific antibody is present, the secondary enzyme labeled antibody will have bound and the enzyme will cleave the substrate resulting in color change; the latter can be measured spectrophotometrically to give a semiquantitative assessment of serum antibody to the specific antigen (usually measured in absorbance units).

Serum quality is critical in ELISA testing. Non-specific absorbance may also occur in serum from aged rodents (over 6 months old; Wagner et al., 1991), strains subject to autoimmunity, animals whose immune systems are non-specifically stimulated because of injury, neoplasm, other noninfectious disease processes, or other types of antigenic stimulation (Wagner et al., 1991; Weisbroth et al., 1998). Additionally, a variety of experimental manipulations of rodents may result in non-specific absorbance.

Antigens employed in ELISA testing vary in complexity from crude extracts containing multiple antigens and impurities to select recombinant proteins generated in viral vectors. The use of highly purified subunit antigens may increase specificity as cross-reactive impurities are not present. However, the use of these subunit antigens may negatively impact sensitivity (Compton and Riley, 2001). This happens because the host response is polyclonal, with many antibodies being produced to different epitopes on the infectious agent. Highly purified subunit antigens may lack the immunodominant epitopes to which antibodies have been produced and result in a test with decreased sensitivity when compared to one that utilizes crude protein preparations. Moreover, agents may express different epitopes during different stages of disease. Therefore, an ELISA that uses an antigen that is only expressed at certain stages may miss some infections. In practice, a balance is sought so that purified preparations of multiple antigens are used, resulting in very sensitive and specific assays.

Variations in ELISA methodology also exist. One such variation, often referred to as antigen capture ELISA, allows for the detection of antigen rather than antibody. These assays are particularly useful in detecting agents in feces or secretions and may ultimately serve as adjuncts or alternatives for more expensive molecular-based techniques.

IFA methodology is similar in principle to ELISA (Kendall et al., 1999). In contrast to ELISA, the antigen is supplied in the form of a virus or bacteria growing in culture. Virus-infected cells and uninfected cells (internal controls) are fixed to wells of a glass slide. Test serum is added to wells, and if antibody is present it binds to the antigen. The secondary antibody is labeled with a fluorescent molecule rather than an enzyme (fluorescent dye-conjugated anti-immunoglobulin). Sensitivity is similar to ELISA. Specificity is equal to or better than ELISA because patterns or location of fluorescence may provide additional information (granular or nuclear fluorescence may be consistent with certain viral infections as opposed to diffuse fluorescence, which may indicate a non-specific reaction). IFAs are relatively inexpensive, but more expensive than ELISA. The main disadvantages of IFA are that it is labor intensive, and interpretation is subjective and dependent on the expertise of the observer. An additional requirement is a specialized epifluorescence microscope. The choice between ELISA and IFA is based on personal preference of the laboratory. These tests are often used in combination, with the ELISA serving as the primary test and the IFA serving as a confirmatory test.

As described earlier, serology is ideal because detectable antibodies persist for months, allowing for a large window of opportunity to detect infections. This is very advantageous in health monitoring programs. Because of this large window of opportunity, serologic testing at a single time point cannot distinguish active from prior infections (for example, the infectious potential of a rat tested). Serology also allows for multiple tests to be run on a single serum sample. Although serology has few limitations, it is unreliable in the diagnosis of infections in immunodeficient rodents (Compton and Riley, 2001; Livingston and Riley, 2003) and, as mentioned earlier, cannot distinguish exposure from active infection. Both ELISA and IFA are subject to non-specific reactivity, which can lead to false positive results. This is especially true in bacterial ELISAs due to the complexity and abundance of potentially cross-reactive bacterial antigens. With improvements in antigen production and reagents, false positive results are uncommon. However, because of this possibility, a single positive should always be confirmed with additional testing.

d. **CULTURE.** Culture of bacteria, using a variety of media may be incorporated into health monitoring programs. Culture is especially useful when evidence of infection such as abscess formation or pneumonia is present. Culture is most effective during the height of infection, and prior to administration of antibiotics or the development of an immune response (Compton and Riley, 2001). Culture may also be used as a screening tool for pathogens or agents capable of causing opportunistic infections. In the latter scenario, mucosal sites of the intestinal tract (for example, the

cecum) and respiratory tract (for example, the nasopharynx) are cultured on broad spectrum or selective media. Bacterial speciation is based on colony morphology, Gram staining characteristics, organism morphology, biochemical tests, and growth on selective media or in selective conditions (Feldman, 2001; Livingston and Riley, 2003). Culture has the advantage of determining whether a live agent is present, as opposed to potentially nonviable DNA remnants or anti-bacterial antibodies from a past infection, in the animal. Culture and subsequent biochemical analyses are also very specific for most agents and can be supplemented with molecular techniques where speciation is desired. Culture does have some drawbacks. For example, agents colonizing the mucosal surface may be present in low numbers or sequestered in areas not accessed by routine procedures (for example, the deep recesses of the nasal turbinates). Moreover, fastidious organisms may not grow well unless conditions are optimized, or their growth may be hindered by the growth of more vigorous bacteria. Some agents may take several days to grow into identifiable colonies, while some agents such as *CAR* bacillus and *Clostridium piliforme* have yet to be cultivated on cell-free media. Collection of samples for submission to diagnostic laboratories may also be problematic in that some bacteria, notably the *Pasteurellaceae*, do not survive well in transfer media.

Culture of viruses is also possible using cell culture systems or embryonated eggs; however, procedures are time consuming, expensive, and require considerable expertise. Viral culture is important in the characterization of novel viral infections. However, because other means of detecting viral infection are readily available, viral culture is rarely used in rat health monitoring programs.

e. **MOLECULAR DIAGNOSTICS.** Molecular diagnostic techniques, primarily those based on the PCR technique are rapidly replacing traditional culture techniques (Compton and Riley, 2001). PCR utilizes specific oligonucleotide primers to exponentially amplify small amounts of target deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) from a particular organism that is present in a clinical specimen. PCR offers exquisite sensitivity and specificity, detecting as few as 1 to 10 viral virions and 3 to 10 bacteria (Compton and Riley, 2001), and is readily adapted to the detection of bacterial, viral, parasite, and fungal agents.

Details of PCR technique can be found in a number of technique manuals. Briefly, PCR consists of repetitive cycles of a 3-step amplification procedure. Double-stranded sample DNA is denatured into two single strands. Oligonucleotide primers specific for the agent (complementary to the specific microorganisms genome and typically situated a few hundred base pairs apart) are added and allowed to anneal to target sequences in the sample DNA. A polymerase (for example, Taq polymerase), an enzyme

that functions in DNA synthesis, is added along with nucleotide bases and new DNA strands of a specific size are created. After “n” cycles of this 3-step process of denaturation, annealing, and synthesis, the target sequence is amplified 2^{nth} times (30 cycles = $2^{30} = 1,073, 741, 824$ copies of DNA). The product is then subjected to gel electrophoresis, and if a targeted sequence is amplified, it will migrate to a specific location based on its molecular weight. RNA (RNA viral genomes) may also be detected by PCR. In this case, reverse transcriptase PCR (RT-PCR) is utilized. With RT-PCR, RNA is converted to complementary DNA (cDNA) using the enzyme, reverse transcriptase. This cDNA becomes the template for PCR, as described earlier. Because RNA is very susceptible to degradation, additional protective steps in sample preparation and storage should be incorporated.

PCR offers superior sensitivity and specificity, and results can be obtained in a single working day. The main disadvantages of PCR are directly related to its advantages. The exquisite sensitivity renders contamination especially problematic, and false positives may occur if strict laboratory technique protocols are not in place or followed. The test is also relatively expensive due to the need for expensive equipment and its labor-intensiveness. This expense can be partially overcome by pooling of samples. In addition, costs will likely be lowered as technology allows for more automation of PCR. The expense of PCR also relates to the need for multiple samples from which multiple tests must be performed. This problem is being addressed by the development of high-throughput multiplexed assays where multiple agents can be tested in a single reaction. Lastly, PCR is often performed on biological samples, many of which contain inhibitors of components of the PCR reaction such as heme and plant products that contaminate feces (Panaccio and Lew, 1991; Al-Soud and Radstrom, 2001; Compton and Riley, 2001; Feldman, 2001). This possibility must be considered when testing these samples; however, the use of highly purified DNA can eliminate or sufficiently dilute inhibitors, so that accurate results are obtained.

Sampling for PCR requires knowledge of the pathogenesis of the agent, including tissue tropism and duration of infection (Compton and Riley, 2001). PCR is an ideal primary test for the detection of active or persistent infections (for example, infections by parvoviruses, LCMV, RCMV, *Mycoplasma pulmonis*, *Helicobacter* spp.) or those agents for which other diagnostic tests are of poor sensitivity (for example, the culture of *Helicobacter* spp.). In contrast, detection of infections where colonization is transient (many viral infections) is possible for only brief periods of time. In the latter case, PCR may serve as an adjunct test. In this scenario, infections may be detected by a primary test such as serology. To confirm infection, additional rats of appropriate target age (an age at which

colonization or shedding is expected) are selected and target tissues are tested by PCR. This two-methodology approach provides very convincing evidence of infection. Moreover, although PCR cannot distinguish between live and dead organisms, results from PCR testing can provide valuable information about the current status (actively shedding, free of colonization) among individual animals or groups of animals.

Newer modifications of PCR, such as fluorogenic 5' nuclease PCR are also being developed (Feldman, 2001; Besselsen et al., 2002; Drazenovich et al., 2002; Besselsen et al., 2003; Uchiyama and Besselsen, 2003). These assays offer improved sensitivity and specificity in some cases, require no post-PCR processing, and can be used to quantify infectious agents. Moreover, other molecular techniques such as microarrays, which allow for the screening of thousands of agents simultaneously, will surely add to the arsenal of molecular techniques available to the diagnostician in the near future. Molecular techniques are also applicable in many other areas of rat medicine and biology, including the detection of contaminants in tissue culture material and monitoring of genetic purity of inbred strains or genetically engineered rats.

f. PARASITE SCREENING. Screening for parasites is usually accomplished by a subgross or microscopic examination of parasite niches. The three general classes of parasites that infect rats include ectoparasites (mites and lice), endoparasitic helminths (pinworms, other nematodes and cestodes), and endoparasitic protozoa. For ectoparasites, the pelage can be collected and examined for mite or louse infestation. Most protocols suggest allowing the sample to cool to encourage mites to venture to the tips of the hair shaft in search of a warmer host. Alternatively, scotch tape tests may be effective for detecting mites or mite eggs attached to hair shafts. The latter can also be utilized in the live animal.

Endoparasitic helminths may be detected by direct examination of the intestinal tract for adult worms. *Syphacia muris* pinworms usually inhabit the cecum and *Rodentolepis (Hymenolepis)* spp. tapeworms the small intestine. Detection of helminths in gross specimens may be enhanced by the use of a dissecting microscope. Incubation of a section of intestine in saline for a short period of time may also facilitate detection by allowing worms to migrate out of the dark fecal matter into the more transparent saline. Pinworms of the genera *S. muris* also deposit ova on the perineum and can thus be detected by perineal tape testing. For this test, a piece of clear cellophane tape is applied to the perineal skin, placed on a microscope slide, and examined for typical banana-shaped ova. This test offers the advantage of being usable in live animals. As an alternative, fecal flotation may be used to detect pinworm or cestode ova.

Endoparasitic protozoa are usually commensal organisms of questionable pathogenicity. These agents are generally detected by wet mount preparations of intestinal contents. Protozoa are readily identified based on motility, morphology, and intestinal locale. For example, *Spironucleus muris* is most often found in the small intestine and is characterized by its small tear drop shape with darting motility. *Giardia* sp. are also found in the small intestine but are larger, have a cup-shaped morphology with an "owl face" appearance, and a "falling leaf" motility. Other protozoa include trichomonads (lemon-shaped with undulating membrane and rolling motility), *Chilomastix* sp. (oval to bar-shaped with spiraling motility), and *Entamoeba* sp. (amoeboid shape with slow motility by pseudopod formation).

In general, patent infections by parasites are more readily detectable in young animals (Wagner et al., 1991; Weisbroth et al., 1998). Microscopic and gross examinations for parasites are advantageous in that they are relatively simple, straightforward techniques; some (tape tests, fecal flotation) can be performed on live animals; and they are relatively specific. The disadvantage of these tests is that they lack sensitivity, and ultimately the development of more sensitive techniques, such as PCRs, may be warranted.

g. SCREENING OF TISSUE BY HISTOLOGY FOR LESIONS OF INFECTIOUS DISEASE. A variety of tissues may be screened for lesions indicative of infectious disease. While there are very few pathognomonic lesions of rodent infections, screening of tissues may provide presumptive diagnoses that can be confirmed by other means. The disadvantages of histology as a screening tool include the narrow window of opportunity to detect certain transient infections and the fact that many opportunistic pathogens do not cause histologic disease. Screening of tissues may be useful in several situations: 1) screening of target tissues for known lesions of infectious disease; 2) screening immunodeficient rats in which tests such as serology are not appropriate; 3) detecting disease early in its time course prior to the development of detectable antibody; 4) detecting bacterial agents that are difficult to cultivate (such as CAR bacillus and *Clostridium piliforme*); and 5) detecting emerging or previously unrecognized infectious diseases. The latter is exemplified by the recent discovery of rat respiratory virus. This agent was discovered after the recognition that chronic idiopathic interstitial pneumonia became prevalent in several colonies of rats (Simmons and Riley, 2002). In addition, it is only by histopathology that noninfectious degenerative conditions, such as renal and cardiac calcinosis, may be recognized.

The use of tissue screening for infectious disease relies upon the selection of certain target tissues. It is unrealistic

to screen all tissues for signs of disease and many tissues are not common sites of infection. Most commonly, systems exposed to the external environment (for example, respiratory and enteric systems) are screened. Other tissues often screened are based on known disease pathogenesis. These include the Harderian and salivary glands, which are screened for lesions of rat coronavirus (RCV/SDAV), and the urinary bladder, which is screened for *Trichosomoides crassicauda* infections.

h. OTHER TESTING STRATEGIES. Historically, other testing platforms were employed, including stress testing for *C. piliforme* (Fries and Ladefoged, 1979) or *P. carinii* (Armstrong et al., 1991). These tests may still be used as a diagnostic tool or for the characterization of a novel pathogen, but they are rarely if ever used in routine health monitoring. Moreover, certain strains of rats, such as gnotobiotic or axenic rats, may require additional tests, such as flora confirmation, that can be coupled with health monitoring.

Table 16-4, adapted from Livingston and Riley (Livingston and Riley, 2003), lists agents commonly tested for in rat health monitoring programs and methodologies used to test these agents.

C. Testing Profiles

Tests used in the monitoring of rats for infectious disease are often packaged, depending on the institution's needs, into profiles that include one or more testing modalities. These profiles almost invariably include serologic examination for antibodies to viral and bacterial agents and may include gross necropsy examination, parasite examination, examination for enteric or respiratory pathogens using culture or molecular techniques, and histologic examination of target tissues. The design of these testing profiles requires consideration of several factors as outlined in earlier sections of this chapter. Tiered testing strategies are very economical and are becoming commonplace (Laber-Laird and Proctor, 1993). With these strategies, the most prevalent agents are tested for on a frequent basis via inexpensive high-throughput tests, while testing for agents of low prevalence or screening of animals for indication of emerging diseases occurs on a less frequent basis. For example, rats may be screened for pinworms, fur mites, *M. pulmonis*, RCV, SDAV, pneumonia virus of mice (PVM), and parvoviruses on a quarterly basis while less prevalent agents such as *Corynebacterium kutscheri*, *Streptococcus pneumoniae*, Sendai, REO3, LCM, MAD1, CAR bacillus and *Clostridium piliforme* are tested for once a year (Nicklas et al., 2002).

TABLE 16-4
COMMONLY USED TESTING METHODOLOGIES FOR RAT PATHOGENS

Agent (species)	Primary testing methodology (sample tested)	Confirmatory testing methodology
Viruses		
Hantaan (HTN)	Serology (serum)	PCR (kidney)
Lymphocytic choriomeningitis virus (LCMV)	Serology (serum)	PCR (kidney)
Mouse adenovirus 1 (MAD1)	Serology (serum)	PCR (lung)
Pneumonia virus of mice (PVM)	Serology (serum)	PCR (trachea, lung)
Rat coronavirus (RCV/SDAV)	Serology (serum)	PCR, Histology (salivary and Harderian glands)
Rat parvoviruses	Serology (serum)	PCR (mesenteric lymph node)
Reovirus type 3 (REO 3)	Serology (serum)	PCR (liver, lung feces)
Sendai virus (Sendai)	Serology (serum)	PCR (trachea, lung)
Theiler murine encephalomyelitis virus (TMEV)*	Serology (serum)	PCR (feces, intestine)
Rat respiratory virus	Histology (lung)	
Bacteria		
Cilia-associated respiratory (CAR) bacillus	Serology (serum), PCR (trachea)	Histology (nasopharynx, trachea, lung)
<i>Corynebacterium kutscheri</i>	Culture (NP)	
<i>Helicobacter</i> spp.	PCR (feces)	Culture (feces)
<i>Mycoplasma pulmonis</i>	Serology (serum)/PCR (NP)	Culture (nasopharynx)
<i>Pasteurella pneumotropica</i>	Culture (NP)	PCR (nasopharynx)
<i>Salmonella</i> spp.	Culture (cecal contents, feces)	PCR (cecal contents, feces)
<i>Streptococcus pneumoniae</i>	Culture (NP)	PCR (nasopharynx)
<i>Clostridium piliforme</i>	ELISA (serum)	PCR, Histology (intestine, liver)
Parasites		
<i>Radfordia ensifera</i>	Direct exam (pelage)	
<i>Rodentolepis (Hymenolepis) spp.</i>	Direct exam (small intestine)	
<i>Syphacia muris</i>	Direct exam (cecal contents)	
	Direct exam (perianal tape test)	
Fungus		
<i>Pneumocystis carinii</i> **	PCR (lung)	Histology (lung)

ELISA = enzyme-linked immunosorbent assay; NP = nasopharynx; PCR = polymerase chain reaction.

*TMEV-like agent (see Virology chapter)

**Monitored only in immunodeficient rats.

D. Test Interpretation and Retesting

In many cases, interpretation of health monitoring results is straightforward. For example, when several rats with cervical swellings are found to be seropositive for RCV/SDAV, it is reasonable to determine that an outbreak of this infection is occurring. In other cases, the diagnosis is not so clear-cut, and test results require careful interpretation and follow-up testing. For example, in cases where a single animal is found to be seropositive for PVM, this may indicate either an early outbreak or a false positive result.

There are several approaches to test interpretation. First, results should be interpreted in the context of the entire colony and the health monitoring program. Decisions about rodent health should rarely if ever be made on a single positive result and the latter should be assumed to be

a false positive until verified (Laber-Laird and Proctor, 1993; Compton and Riley, 2001; Livingston and Riley, 2003). Verification may include testing a sample (serum) using an alternative test platform, testing a second sample from the affected animals using an alternative test platform (for example, through PCR), or testing cohort animals (Weisbroth et al., 1998; Livingston and Riley, 2003). As discussed earlier, there are two primary serologic testing platforms: ELISA and IFA. These platforms can also be used as adjuncts for each other. In most diagnostic laboratories, the ELISA serves as the primary test, and borderline or solitary positive results are confirmed by IFA. Confirmatory testing may also involve the use of different testing platforms. For example, if a rat is found to be seropositive for CAR bacillus, the lungs may be examined with a silver stain to detect the presence of

the organism. Often, only serum is collected for health monitoring, so samples for confirmatory testing by other platforms may not be possible. In these cases, testing of cohort animals may be warranted and a diagnostic plan to test with different testing platforms should be designed. For example, if a rat is found to be seropositive for a rat parvovirus, additional animals from that colony may be tested by serology and their mesenteric lymph nodes may concurrently be tested by PCR for rat parvovirus. Testing of cohort animals is also warranted in the case where very few animals are seropositive. This scenario may indicate an early outbreak or a false positive result. If an early outbreak is occurring, cohort animals will have additional time to seroconvert and the percentage of positives should increase.

V. MANAGEMENT OF COLONY DISEASE OUTBREAKS

A. Confirmation and Containment

As discussed earlier, when laboratory testing suggests a change in colony status for a particular agent, it is important to verify the information. Once confidence in the lab result is obtained, the positive sample should be tracked back to confirm its origin by comparing the date of testing, or cage or animal identification numbers, etc., to the monitoring schedule and sampling documentation. Based on this information, if an excluded agent appears to be present, the room should be quarantined to prevent further spread throughout the facility. Scheduled incoming shipments should be diverted to other areas, and transfers out of the room should be canceled. Changes to standard practices that have the potential to affect cross-contamination should be considered, such as the room entry order, the handling and transport of soiled cages, the protective clothing and disinfectants used in the room, and the amount of personnel traffic allowed. A follow-up plan should be implemented to establish whether the agent truly does exist within the room by testing remaining sentinels or principal animals. It may be useful to draft a generic initial response plan for suspected contamination events in advance, so that these initial steps can be instituted promptly and efficiently.

B. Response Plan

When contamination has been confirmed, a plan of action should be developed by the veterinary and animal facility management groups in concert with others that are affected, such as the research groups holding animals in the

area. It is also prudent to include key individuals from the administration or upper management (since there may be a significant fiscal impact) and the institutional animal care and use committee. In some cases there will be no question what the follow-up response to contamination will be (for example, eradication of the agent), but in others the potential costs and benefits of the available options may need to be considered. Regardless of the decisions made, the plan must be documented and distributed so that the goal is very clear to all involved and the sequence of events and projected timeline is evident.

C. Eradication Options

There are a variety of methods that can be used to eliminate an infectious agent from an area, and careful professional judgment is needed to determine the most appropriate course of action. If the animals are replaceable and the primary consideration is to return the room to normal use, complete depopulation followed by environmental decontamination can be performed. Attempting partial depopulation by removing positive animals (via test and cull) is not a very productive approach for most rodent colony disease outbreaks due to the large numbers of animals often involved, the delay between exposure and seroconversion, and the possibility that the disease will be further spread during the handling and sampling procedures needed to test the entire population. As an alternative approach, if the agent does not establish persistent infection, it may be useful to test and remove the *negative* animals. Fully immune populations should pose little risk of shedding to naïve animals after infection by agents such as coronavirus or Sendai virus. By retaining only previously exposed seropositive animals to re-establish a breeding program, it is possible to produce seronegative offspring (Brammer et al., 1993). A related approach to break the chain of transmission and repopulate an area without determining the serologic status of each individual rat allowed to remain is the cessation of breeding method, also known as *burnout* or *stop-breeding* schemes. By eliminating the introduction of naïve animals from outside and eliminating all internal breeding for a period of time (6 to 8 weeks is recommended) coronavirus can be eliminated from a population (Bhatt and Jacoby, 1985; Jacoby and Gaertner, 1994). Reciprocal transfer of soiled bedding between all cages during the early weeks of a burnout period can help assure that all animals have had equivalent exposure to the agent. Of note, these techniques may not work in immunodeficient rats and should be used with caution in genetically engineered rats.

If rats having a valuable or irreplaceable genotype are involved in an outbreak, there are methods for rederiving the strain. Detailed description of techniques used for rat

cesarean section rederivation with or without superovulation have been published (Rouleau et al., 1993; Sharp and LaRegina, 1998) and are described elsewhere in the Assisted Reproductive Technologies chapter of this text. Although the techniques may not be as well established or efficient in rats, superovulation and embryo transfer methods similar to those used in mice can be successful in rats (Robl and Heideman, 1994).

REFERENCES

- Al-Soud, W.A., and Radstrom, P. (2001). Purification and characterization of PCR-inhibitory components in blood cells. *J. Clin. Microbiol.* **39**, 485–493.
- Armstrong, M.Y., Smith, A.L., and Richards, F.F. (1991). Pneumocystis carinii pneumonia in the rat model. *J. Protozool.* **38**, 136S–138S.
- Artwohl, J.E., Cera, L.M., Wright, M.F., Medina, L.V., and Kim, L.J. (1994). The efficacy of a dirty bedding sentinel system for detecting Sendai virus infection in mice: a comparison of clinical signs and seroconversion. *Lab. Anim. Sci.* **44**, 73–75.
- Baker, D.G. (1998). Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clin. Microbiol. Rev.* **11**, 231–66.
- Baker, D.G. (2003). "Natural Pathogens of Laboratory Animals Their Effects on Research." ASM Press, Washington.
- Ball-Goodrich, L.J., Leland, S.E., Johnson, E.A., Paturzo, F.X., and Jacoby, R.O. (1998). Rat parvovirus type 1: the prototype for a new rodent parvovirus serogroup. *J. Virol.* **72**, 3289–3299.
- Ball-Goodrich, L.J., Paturzo, F.X., Johnson, E.A., Steger, K., and Jacoby, R.O. (2002). Immune responses to the major capsid protein during parvovirus infection of rats. *J. Virol.* **76**, 10044–10049.
- Bellamy, J.E.C., and Olexson, D.W. (2000). Evaluating Laboratory Procedures. In: "Quality Assurance Handbook for Veterinary Laboratories." pp. 61–77, Iowa State University Press, Ames, IA.
- Besselsen, D.G., Wagner, A.M., and Loganbill, J.K. (2002). Detection of rodent coronaviruses by use of fluorogenic reverse transcriptase-polymerase chain reaction analysis. *Comp. Med.* **52**, 111–116.
- Besselsen, D.G., Wagner, A.M., and Loganbill, J.K. (2003). Detection of lymphocytic choriomeningitis virus by use of fluorogenic nucleic acid reverse transcriptase-polymerase chain reaction analysis. *Comp. Med.* **53**, 65–69.
- Bhatt, P.N., and Jacoby, R.O. (1985). Epizootiological observations of natural and experimental infection with sialodacryoadenitis virus in rats. *Lab. Anim. Sci.* **35**, 129–134.
- Blank, W.A., Henderson, K.S., and White, L.A. (2004). Virus PCR assay panels: an alternative to the mouse antibody production test. *Lab. Anim.* **32**, 26–32.
- Boivin, G.P., Hook, R.R., Jr., and Riley, L.K. (1994). Development of a monoclonal antibody-based competitive inhibition enzyme-linked immunosorbent assay for detection of Bacillus piliformis isolate-specific antibodies in laboratory animals. *Lab. Anim. Sci.* **44**, 153–158.
- Bootz, F., and Sieber, I. (2002). Replacement of mouse and rat antibody production test; comparison of sensitivity between the in vitro and in vivo methods. [German] *ALTEX* **19 Suppl 1**, 76–86.
- Brammer, D.W., Dysko, R.C., Spilman, S.C., and Oskar, P.A. (1993). Elimination of sialodacryoadenitis virus from a rat production colony by using seropositive breeding animals. *Lab. Anim. Sci.* **43**, 633–634.
- Carpenter, J., Mashima, T., and Rupiper, D. (1996). "Exotic Animal Formulary." Greystone Publications, Manhattan, KS.
- Clifford, C.B. (2001). Samples, sample selection, and statistics: living with uncertainty. *Lab. Anim.* **30**, 26–31.
- Compton, S.R., and Riley, L.K. (2001). Detection of infectious agents in laboratory rodents: traditional and molecular techniques. *Comp. Med.* **51**, 113–119.
- Cundiff, D.D., Riley, L.K., Franklin, C.L., Hook, R.R., Jr., and Besch-Williford, C. (1995). Failure of a soiled bedding sentinel system to detect cilia-associated respiratory bacillus infection in rats. *Lab. Anim. Sci.* **45**, 219–221.
- DiGiacomo, R.F., and Koepsell, T.D. (1986). Sampling for detection of infection or disease in animal populations. *J. Am. Vet. Med. Assoc.* **189**, 22–23.
- Dillehay, D.L., Lehner, N.D., and Huerkamp, M.J. (1990). The effectiveness of a microisolator cage system and sentinel mice for controlling and detecting MHV and Sendai virus infections. *Lab. Anim. Sci.* **40**, 367–370.
- Drazenovich, N.L., Franklin, C.L., Livingston, R.S., and Besselsen, D.G. (2002). Detection of rodent Helicobacter spp. by use of fluorogenic nucleic acid polymerase chain reaction assays. *Comp. Med.* **52**, 347–353.
- Dymsha, J., Miller, S., Maloney, J., and Foster, H. (1963). Equilibration of the laboratory rat following exposure to shipping stresses. *Lab. Anim. Care* **13**, 60–65.
- Feldman, S.H. (2001). Diagnostic molecular microbiology in laboratory animal health monitoring and surveillance programs. *Lab. Anim.* **30**, 34–42.
- Franklin, C.L., Pletz, J.D., Riley, L.K., Livingston, B.A., Hook, R.R., Jr., and Besch-Williford, C.L. (1999). Detection of cilia-associated respiratory (CAR) bacillus in nasal-swab specimens from infected rats by use of polymerase chain reaction. *Lab. Anim. Sci.* **49**, 114–117.
- Fries, A.S., and Ladefoged, O. (1979). The influence of Bacillus piliformis (Tyzzer) infections on the reliability of pharmacokinetic experiments in mice. *Lab. Anim.* **13**, 257–261.
- Fujiwara, K., and Wagner, J. (1994). Sendai virus. In "Manual of Microbiologic Monitoring of Laboratory Animals." (T. Nomura, ed.), pp. 107–109, NIH Publication No. 94–2498.
- Gill, T.J., 3rd, Smith, G.J., Wissler, R.W., and Kunz, H.W. (1989). The rat as an experimental animal. *Science* **245**, 269–276.
- Goto, K., Ohashi, H., Takakura, A., and Itoh, T. (2000). Current status of Helicobacter contamination of laboratory mice, rats, gerbils, and house musk shrews in Japan. *Curr. Microbiol.* **41**, 161–166.
- Haines, D.C., Gorelick, P.L., Battles, J.K., Pike K.M., Anderson, R.J., Fox, J.G., Taylor, N.S., Shen, Z., Dewhirst, F.E., Anver, M.R., and Ward, J.M. (1998). Inflammatory large bowel disease in immunodeficient rats naturally and experimentally infected with Helicobacter bilis. *Vet. Pathol.* **35**, 202–208.
- Hawk, C., and Leary, S. (1999). "Formulary for Laboratory Animals." Iowa State University Press, Ames, IA.
- Institute for Laboratory Animal Research-National Research Council (1991). "Infectious Diseases of Mice and Rats." National Academy Press, Washington, DC.
- Institute for Laboratory Animal Research-National Research Council (1992). "Recognition and Alleviation of Pain and Distress in Laboratory Animals." National Academy Press, Washington, DC.
- Institute for Laboratory Animal Research-National Research Council (1996a). "Guide for the Care and Use of Laboratory Animals." National Academy Press, Washington, DC.
- Institute for Laboratory Animal Research-National Research Council (1996b). "Laboratory Animal Management: Rodents." National Academy Press, Washington, DC.
- Jacoby, R., and Gaertner, D. (1994). Rat coronavirus/sialodacryoadenitis virus. In "Manual of Microbiologic Monitoring of Laboratory Animals." (T. Nomura, ed.), pp. 87–91, NIH Publication No. 94–2498.
- Jacoby, R.O., and Homberger, F.R. (1999). International standards for rodent quality. *Lab. Anim. Sci.* **49**, 230.

- Jacoby, R.O., and Lindsey, J.R. (1998). Risks of infection among laboratory rats and mice at major biomedical research institutions. *ILAR J* **39**, 266–271.
- Johnson, K. (1986). Hemorrhagic fever-Hantaan virus. In "Viral and Mycoplasma Infections of Laboratory Rodents: Effects on Biomedical Research." (E. New, ed.), pp. 193–216, Academic Press, Orlando, FL.
- Kendall, L.V., Steffen, E.K., and Riley, L.K. (1999). Indirect fluorescent antibody (IFA) assay. *Contemp. Top. Lab. Anim. Sci.* **38**, 68.
- Kohn, D., and Clifford, C. (2002). Biology and diseases of rats. In "Laboratory Animal Medicine." (F. Quimby, ed.), pp. 121–165. Academic Press, San Diego.
- Koszdin, K.L., and DiGiacomo, R.F. (2002). Outbreak: detection and investigation. *Contemp. Top. Lab. Anim. Sci.* **41**, 18–27.
- Laber-Laird, K., and Proctor, M. (1993). An example of a rodent health monitoring program. *Lab. Anim.* **22**, 24–32.
- Lipman, N.S., and Homberger, F.R. (2003). Rodent quality assurance testing: use of sentinel animal systems. *Lab. Anim.* **32**, 36–43.
- Lipman, N.S., and Perkins, S. (2002). Factors that may influence animal research. In "Laboratory Animal Medicine." (F. Quimby, ed.), pp. 1143–1184, Academic Press, San Diego.
- Lipman, N.S., Perkins, S., Nguyen, H., Pfeiffer, M., and Meyer, H. (2000). Mousepox resulting from use of ectromelia virus-contaminated, imported mouse serum. *Comp. Med.* **50**, 426–435.
- Livingston, R.S., and Riley, L.K. (2003). Diagnostic testing of mouse and rat colonies for infectious agents. *Lab. Anim.* **32**, 44–51.
- McKisic, M.D., Lancki, D.W., Otto, G., Padrid, P., Snook, S., Cronin, D.C., 2nd, Lohmar, P.D., Wong, T., and Fitch, F.W. (1993). Identification and propagation of a putative immunosuppressive orphan parvovirus in cloned T cells. *J. Immunol.* **150**, 419–428.
- Nicklas, W., Baneux, P., Boot, R., Decelle, T., Deeny, A.A., Fumanelli, M., and Illgen-Wilcke, B. (2002). Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Lab. Anim.* **36**, 20–42.
- Nicklas, W., Homberger, F.R., Illgen-Wilcke, B., Jacobi, K., Kraft, V., Kunst, I., Mahler, M., Meyer, H., and Pohlmeyer-Esch, G. (1999). Implications of infectious agents on results of animal experiments. Report of the Working Group on Hygiene of the Gesellschaft für Versuchstierkunde—Society for Laboratory Animal Science (GV-SOLAS). *Lab. Anim.* **33** Suppl 1, S39–87.
- Otto, G., and Tolwani, R.J. (2002). Use of microisolator caging in a risk-based mouse import and quarantine program: a retrospective study. *Contemp. Top. Lab. Anim. Sci.* **41**, 20–27.
- Panaccio, M., and Lew, A. (1991). PCR based diagnosis in the presence of 8% (v/v) blood. *Nucleic Acids Res.* **19**, 1151.
- Rehg, J.E., and Toth, L.A. (1998). Rodent quarantine programs: purpose, principles, and practice. *Lab. Anim. Sci.* **48**, 438–447.
- Riley, L.K., Franklin, C.L., Hook, R.R., Jr., and Besch-Williford, C. (1996). Identification of murine helicobacters by PCR and restriction enzyme analyses. *J. Clin. Microbiol.* **34**, 942–946.
- Robl, J., and Heideman, J. (1994). Production of transgenic rats and rabbits. In "Transgenic Animal Technology." (C. Pinkert, ed.), pp. 265–277. Academic Press, San Diego.
- Rouleau, A.M., Kovacs, P.R., Kunz, H.W., and Armstrong, D.T. (1993). Decontamination of rat embryos and transfer to specific pathogen-free recipients for the production of a breeding colony. *Lab. Anim. Sci.* **43**, 611–615.
- Saibaba, P., Sales, G., Stodulski, G., and Hau, J. (1995). Behaviour of rats in their home cages: daytime variations and effects of routine husbandry procedures analyzed by time sampling techniques. *Lab. Anim.* **30**, 13–21.
- Sharp, P., and LaRegina, M. (1998). "The Laboratory Rat." CRC Press, Boca Raton, FL.
- Simmons, J.H., and Riley, L.K. (2002). Hantaviruses: an overview. *Comp. Med.* **52**, 97–110.
- Small, J. (1986). Decision making, detection, prevention and control. In "Viral and Mycoplasma Infections of Laboratory Rodents: Effects on Biomedical Research." (E. New, ed.), pp. 777–799, Academic Press, Orlando, FL.
- Small, J.D. (1984). Rodent and lagomorph health surveillance: quality assurance. In "Laboratory Animal Medicine." (F. Loew, ed.), pp. 709–723, Academic Press, Orlando, FL.
- Smith, A.L. (1983). An immunofluorescence test for detection of serum antibody to rodent coronaviruses. *Lab. Anim. Sci.* **33**, 157–160.
- Thigpen, J.E., Lebetkin, E.H., Dawes, M.L., Amyx, H.L., Caviness, G.F., Sawyer, B.A., and Blackmore, D.E. (1989). The use of dirty bedding for detection of murine pathogens in sentinel mice. *Lab. Anim. Sci.* **39**, 324–327.
- Uchiyama, A., and Besselsen, D.G. (2003). Detection of reovirus type 3 by use of fluorogenic nuclease reverse transcriptase polymerase chain reaction. *Lab. Anim.* **37**, 352–359.
- Waggie, K., Kagiya, N., Allen, A., and Nomura, T. (1994). "Manual of Microbiologic Monitoring of Laboratory Animals." NIH Publication No. 94–2498.
- Wagner, J.E., Besch-Williford, C.L., and Steffen, E.K. (1991). Health surveillance of laboratory rodents. *Lab. Anim.* **20**, 40–45.
- Wan, C.H., Soderlund-Venermo, M., Pintel, D.J., and Riley, L.K. (2002). Molecular characterization of three newly recognized rat parvoviruses. *J. Gen. Virol.* **83**, 2075–2083.
- Weisbroth, S.H., Peters, R., Riley, L.K., and Shek, W. (1998). Microbiological assessment of laboratory rats and mice. *ILAR J* **39**, 272–290.
- Yamamoto, H., Sato, H., Yagami, K., Arikawa, J., Furuya, M., Kurosawa, T., Mannen, K., Matsubayashi, K., Nishimune, Y., Shibahara, T., Ueda, T., and Itoh, T. (2001). Microbiological contamination in genetically modified animals and proposals for a microbiological test standard for national universities in Japan. *Exp. Anim.* **50**, 397–407.