Specific Pathogen-Free Macaques: Definition, History, and Current Production

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Abstract

Specific pathogen-free (SPF) macaque colonies are now requested frequently as a resource for research. Such colonies were originally conceived as a means to cull diseased animals from research-dedicated colonies, with the goal of eliminating debilitating or fatal infectious agents from the colony to improve the reproductive capacity of captive research animals. The initial pathogen of concern was Mycobacterium tuberculosis (M.tb.), recognized for many years as a pathogen of nonhuman primates as well as a human health target. More recently attention has focused on four viral pathogens as the basis for an SPF colony: simian type D retrovirus (SRV), simian immunodeficiency virus (SIV), simian T cell lymphotropic/leukemia virus (STLV), and Cercopithecine herpesvirus 1 (CHV-1). New technologies, breeding, and maintenance schemes have emerged to develop and provide SPF primates for research. In this review we focus on the nonhuman primates (NHPs) most common to North American NHP research facilities, Asian macaques, and the most common current research application of these animals, modeling of human AIDS.

Key Words: Cercopithecine herpesvirus 1; herpes B virus; Mycobacterium tuberculosis; nonhuman primate; simian immunodeficiency virus; simian T cell lymphotropic virus; specific pathogen-free; tuberculin testing

Introduction

Specific pathogen-free (SPF) nonhuman primates (NHPs) are now commonly requested both for research purposes and to maintain standardized health levels in established colonies. Technological advances in the past decade have led to more sensitive research outcomes that are recognizable by the presence of unwanted microorganisms and may thus require changes in decades-old practices. For example, for years the most economically efficient environments for both housing and breeding nonhuman primates in warmer climates have been outdoor social settings. But NHPs' exposure in such settings naturally occurring pathogens in rodents, birds, and other sources makes this a questionable practice for the creation and maintenance of SPF colonies. Future breeding and rearing conditions for SPF nonhuman primates will require a significantly more controlled environment. Furthermore, in light of an increased understanding of NHP social needs, provisions for enhanced psychological well-being and environmental enrichment programs will be necessary.

Thus the combination of increasingly sophisticated research needs and greater understanding of NHP needs will require that primates be raised under far more stringent conditions, and will significantly add to the cost of SPF nonhuman primates. In this review we briefly discuss various methods (also described in the literature; e.g., Sariol et al. 2005) for establishing and maintaining SPF colonies.

SPF-Targeted Pathogens

Perhaps the most commonly defined SPF primate is one that is free of Mycobacterium tuberculosis (M.tb.) as well as four specific viruses: simian immunodeficiency virus (SIV), simian type D retrovirus (SRV), herpes B virus or Cercopithecine herpesvirus 1 (CHV-1), and simian T cell lymphotropic/leukemia virus (STLV). Efforts to create colonies free of these four viruses have accelerated since the advent of acquired immunodeficiency syndrome (AIDS) and AIDS-related research in nonhuman primates using the SIV macaque model.

The now well-known lentivirus SIV is not naturally present in wild populations of macaques in Asia or India but can readily infect these animals, as is evident in the numerous controlled inoculations that occur in AIDS-modeling experiments (Haigwood 2004). The risk to humans who handle SIV-infected macaques or their blood or tissues is clear based on the AIDS-inducing disease it causes in macaques and the virus’s close phylogenetic relationship to HIV.

Although the disease caused by SIV is serious and deadly for many experimentally infected macaques, the vi-
rus is not present in macaque populations unless they have been experimentally exposed. Therefore the risk of disease caused by SIV is low if appropriate containment procedures are followed at institutions that conduct AIDS studies using macaques, and the risk is almost zero at institutions or centers without macaque AIDS models. If animals are infected with SIV the resulting immune suppression and dysregulation will be a confounding factor for most research studies. Thus, SPF colonies should be free of SIV-infected or exposed animals.

SRV, discovered at about the same time as SIV (Daniel et al. 1984; Marx et al. 1984), exists in most macaque research colonies in the United States and Europe. Although not the cause of a human immunodeficiency virus (HIV) AIDS-like disease in macaques, SRV is a major health concern and may confound research outcomes, particularly when the research involves immunocompromised primates.

From an occupational health viewpoint, the agent of greatest concern in Asian macaques is CHV-1. Although it has only minimal disease complications in the macaque (e.g., mucosal lesions in the mouth), human CHV-1 infections cause progressive and irreversible lower-extremity paralysis and death (Weigler 1992). With an increasing emphasis on occupational health-related issues and the occurrence of several deaths among research and veterinary personnel during the 1990s from CHV-1 infection, there have been increased efforts to create NHP colonies free of this pathogen.

STLV infection in macaques is not associated with a prevalent disease manifestation, but the more subtle effects of viral expression on host cells and the latent nature of infection make this virus a serious concern for human health and safety as well as a complicating factor in macaque studies. The pX portion of the HTLV and STLV genomes encodes the tax gene product and several other regulatory proteins that interact with cellular genes, including nuclear factor-kappaB, E2F, activator protein 1 (AP-1), and p53. A recent study has shown that a novel protein encoded on the antisense strand of HTLV, the basic leucine zipper protein HBZ, is important in cell transformation and is a likely tumor promoter (Satou et al. 2006). The tax protein has pleotropic effects on cells, from cell cycle promotion and gene activation to repression of some tumor suppressor proteins. These effects combine to yield T cell dysregulation that may be responsible for the T cell leukemia–inducing properties of HTLV (Yoshida 2001) and are most likely recapitulated by STLV in macaques and baboons. These well-characterized properties of HTLV make the presence of STLV in macaque colonies an unacceptable risk that should be reduced or eliminated.

History of Pathogen Elimination

With the escalation in the number of nonhuman primates used in research following efforts to develop and produce a polio vaccine during the 1950s, more time, money, and planning have been directed toward the elimination of M. tb. than any other pathogen. This goal is still foremost in any NHP colony health plan, either in the quarantine requirements for newly acquired primates or in established colonies. Thus when considering SPF requirements, it is necessary to look at some of the earliest persistent efforts to eliminate M. tb. from nonhuman primate colonies as a major health hazard.

M. tb. has been recognized as a serious health concern for nonhuman primates since the late 1800s (Ruch 1959) and has recently reemerged as a common pathogen in macaques imported from Asia. It is highly contagious and in some cases rapidly fatal among Asian macaques, particularly those kept in the close quarters of a research colony. By definition, then, all research colonies of Asian macaques should be M. tb. free. However, maintaining an M. tb.-free colony is a daunting, never-ending challenge for research support and occupational health staff, who must strive to eliminate or prevent infections in both the research animals and the researchers. Frequent mammalian tuberculin skin testing of all colony animals and human staff has become the norm at most NHP facilities.

Routine methods of intradermal skin testing using 0.1 cc of mammalian old tuberculin were established in the 1940s and 1950s and still form the cornerstone of most NHP M. tb. screening programs (Lerche et al. 2008; Roberts and Andrews 2008). Although purified protein derivative (PPD) is now used in cattle and humans for M. tb. and M. bovis screening, this product is ineffective in NHPs (Bennett et al. 1995).

However, two new assays have become commercially available in recent years to augment the tuberculin skin test. Whole blood in vitro interferon gamma assays, used alone or in parallel with traditional skin testing, appear to be reliable in identifying M. tb. in naturally and experimentally infected macaques (Garcia et al. 2004a; Vervenne et al. 2004). Similarly, a recent study has shown that a rapid lateral flow test consisting of a combination of M. tb.-specific proteins (ESAT-6 and MPB83) used in parallel with skin testing detected M. tb. infection in experimentally infected Old World monkeys more reliably than either test used alone (Lyashchenko et al. 2007).

Although questions remain as to whether any existing M. tb. screening test can identify latently infected animals, personnel maintaining SPF macaque colonies should not rely on tuberculin skin testing alone to ensure that their colonies are M. tb. free. The most effective testing regimen consists of routine tuberculin skin testing accompanied by one of the new assays as well as other, traditional diagnostics (e.g., thoracic radiography, gastric aspirate, or bronchoalveolar lavage culture).

The pathogens mentioned above are the foundation of an SPF colony, although specific research requirements may call for the inclusion of additional pathogens. Numerous species of nematodes, flukes, and amoebic, microfilarial, and malarial parasites commonly infect animals captured or maintained in their tropical habitats or in out-
door enclosures at domestic breeding facilities. Because such infections are often subclinical in nature, many facilities automatically treat for them during quarantine. Animals that present with bacterial or viral diarrheal agents are, when possible, isolated and treated or supported until symptoms subside.

**Viral Biology and Host Interactions Important for SPF Testing**

As discussed, the viruses of most concern for SPF macaque colonies are SRV, STLV, SIV, and CHV-1. Each possesses unique characteristics that are important to consider when devising a strategy for successful establishment of an SPF breeding colony of macaques designated for AIDS-related research. In addition, to conserve this valuable animal resource, comprehensive SPF programs should recognize alternate uses (non-AIDS- and AIDS-related alike) for animals infected by one or more of the four target viruses. Such animals should be considered for inclusion in less stringent SPF colonies.

Table 1 summarizes a scheme for SPF level designation where increasing levels represent animals with increasing numbers of excluded viruses. Animals that seroconvert to one or more of the excluded viruses may be reassigned to a lower level. The most common reassignment is from level 3 to level 2 due to newly detected CHV-1 infections, although animals at both these levels remain suitable for AIDS-related research. Other candidate viruses for exclusion (i.e., for an SPF-4 colony and greater) may include simian foamy virus (SFV) or additional simian herpesviruses.

Because SIV-infected Asian macaques (with rare exception) progress to a terminal disease state, such animals must be culled immediately from all SPF colonies. Furthermore, although it is rare, nonexperimental horizontal transmission of SIV has occurred between macaques and therefore continuous SIV monitoring of SPF colonies associated with AIDS research centers is warranted.

**Table 1 SPF level designations**

<table>
<thead>
<tr>
<th>SPF level</th>
<th>SRV</th>
<th>STLV</th>
<th>CHV-1</th>
<th>Other</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td></td>
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<td>4</td>
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<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

*CHV-1, *Cercopithecine herpesvirus* 1; SIV, simian immunodeficiency virus; SPF, specific pathogen-free; SRV, simian type D retrovirus; STLV, simian T cell lymphotropic/leukemia virus.

**Simian Type D Retrovirus (SRV)**

Simian type D retroviruses (betaretroviruses) are one of the most pleotropic members of the retrovirus family and can replicate in many tissues of the body (Bryant et al. 1986; Moazed and Thouless 1993). Their pleotropic nature leads to very productive acute infections with high levels of virus in bodily fluids, making SRV one of the most problematic viruses for SPF colonies due to horizontal and vertical transmission. However, all SRV infections are not equal in terms of transmissibility: some host animals effectively neutralize the virus with strong antibody responses against the envelope glycoproteins, while a small percentage of animals are continuous viral shedders (Wilkinson et al. 2003). In this section we describe how these SRV characteristics are important to the viral testing assays used for routine screening and how the timing of testing and retesting should be designed in consideration of these characteristics.

Surveys of captive macaque colonies and wild troops with a high prevalence of SRV infection show that more than 80% of the animals raise an effective antibody response, with the result that very little virus is detectable by polymerase chain reaction (PCR) or viral culture techniques (Grant et al., in preparation; Lerche et al. 1987).

When the antibody response is targeted at the outer membrane glycoproteins (gp70), the infected animals rarely express infectious virus and therefore pose little immediate threat of transmission (Benveniste et al. 1993; Brody et al. 1992). But a high anti-gp70 response should not be construed as “sterilizing” because in some animals the SRV suppression is only transient and transmission can occur again later, especially perinatally (i.e., through maternal-fetal or maternal-infant contact) (Tsai et al. 1990).

Most healthy animals exposed to an SRV viremic animal mount a measurable immune response within 6 weeks of exposure, so it is important to test animals 6 to 8 weeks after possible exposure or after a change in housing environment where SRV-positive animals are present. Antibodies to the SRV envelope glycoproteins are generally protective and result in little or no virus in the periphery, but antibody-positive animals can still produce infectious virus under situations of stress or immune suppression and should be considered a threat to any SPF colony.

**SRV Serotypes and Cross Reactivity**

The occurrence of multiple SRV serotypes is another important consideration when implementing screening protocols for SPF colonies. Five different serotypes of SRV (SRV-1 through SRV-5) have been identified to date; SRV-1, -3, and -5 are most common in Asian and Indian M. mulatta (Li et al. 2000; Maul et al. 1986), SRV-2 is most common in Southeast Asian M. fascicularis and M. nemestrina (Grant et al. 1995a), and SRV-4 is less common and less well defined. SRV types based on PCR DNA sequencing from langur monkeys appear to be variants of endogenous langur viruses (Nandi et al. 2000) and do not
represent a concern for macaques in SPF colonies, but their significance in wild settings is unknown at this time. The presence of many different serotypes of SRV and their wide geographic distribution are a cause for concern for primate colonies worldwide.

Cross reactivity of antibodies between different SRV serotypes occurs and is directed predominantly at the major capsid protein, p27, and transmembrane glycoprotein gp20-gp22, as these are the most highly conserved regions of the type D retrovirus genomes. Ligand-binding assays have been developed that use these conserved portions of the virus to give the strongest reactions (Khan et al. 2006; Kuller et al. 2005). Minor capsid proteins and the outer membrane glycoprotein, gp70, are less conserved among the different serotypes and show little or no cross reactivity between serotypes. Low cross reactivity is due to low ligand-antibody binding and leads to low signals in ligand-binding assays such as enzyme-linked immunosorbent assay (ELISA), immunoblot, and bead-based assays. Knowledge of the serotypes present or likely to be present in the population to be tested is important in order to achieve maximum reactivity of antibodies in common serological assays (for a thorough discussion of assay development and use, see Simmons 2008). Robust ligand-binding reactivity is the key to successful SRV diagnosis and the elimination of infected or exposed animals from SPF colonies.

**SRV Antibodies and Endogenous Sequences**

A characteristic of SRV infections that is potentially problematic in SPF colonies is the incidence of viremia accompanied by very low or undetectable antibody response, a condition that has a low prevalence but can go unnoticed in colonies that use only serology for screening. Animals that express high levels of virus and proviral DNA are often those with the lowest levels of antibody (Wilkinson et al. 2003), and although they appear to be serologically negative they can shed virus in almost all bodily fluids for several years. This condition may be related to immune tolerance because such chronic virus shedders are almost always infants and juveniles born to antibody-positive dams and they appear to be incapable of mounting a robust immune response against SRV (Tsai et al. 1990). Because serology screening alone could miss animals of the highest threat to the colony, it is important for any SRV screening program to incorporate both serology and direct virus testing to detect low-antibody/high-virus animals.

A contrary finding should also be noted about PCR-positive animals: some animals that express high levels of viral DNA in circulating peripheral blood mononuclear cells (PBMCs) do not appear to transmit the virus to other animals. However, animals with high levels of virions in the blood and the accompanying high RNA levels transmit the virus more easily (Wilkinson et al. 2003). This may indicate that animals with high DNA levels effectively neutralize the virus through cellular and humoral responses but that DNA is still present in infected cells, yielding little or no viral transmission. In any case, PCR-positive animals need to be considered a threat to any SPF colony.

Another cautionary note about PCR and SRV concerns the hazard of detecting closely related endogenous sequences. Hundreds of copies of endogenous retroviruses related to type D viruses are present in every cell of Cercopithecine monkeys, and even PCR primers with imperfect matches to the endogenous viruses will successfully amplify the unwanted sequences (Grant et al. 1995). In regions of the gp20 and core genes of SRV, homology to endogenous sequences can be as high as 95%. It is therefore important to validate PCR amplification targets with DNA sequencing to confirm that all positive reactions are specific for exogenous SRV and not a product of endogenous sequences. Direct virus detection by PCR should be done after carefully considering the target sequence and its relationship to SRV and endogenous viruses.

**Simian T Lymphotropic/Leukemia Virus**

Simian T lymphotropic/leukemia virus (STLV) is a deltaretrovirus present in all New and Old World primate species. Closely related to human T lymphotropic/leukemia virus (HTLV), STLV has proteins and a genome organization very similar to those of its human counterpart (Van Dooren et al. 2007). The seroprevalence of STLV in most natural simian populations is 5-40%, but it can be much higher in captive colonies (Richards et al. 1998; Schillaci et al. 2005). The lower natural prevalence makes it possible to effectively eliminate STLV from breeding animals with moderate effort and low expense, thus it is one pathogen that all breeding colonies should target for removal.

The characteristic of STLV that makes it most amenable to elimination from SPF colonies is the cell-associated nature of virus particles in this family of retroviruses (Parrish et al. 2004; Tochikura et al. 1985): most mature STLV virions are contained within cells, not outside the cells as free virions (Gabet et al. 2003). The obvious benefit of a cell-associated virus is its low frequency of transmission due to low viral levels in bodily fluids. These low levels result in lower transmission rates among group-housed animals and almost zero transmission among individually housed animals (Parrish et al. 2004).

Effective methods for diagnosing STLV infection are antibody testing with commercially available HTLV reagents and PCR or other nucleic acid methods that use HTLV sequences (Mertens et al. 2001). The close relationship of these two primate retroviruses is evident in the high level of cross reactivity in ligand-binding assays and in the DNA sequence homology. In fact, HTLV ligand-binding assays and Western blots are routinely used for macaque testing (Khan et al. 2006; Kuller et al. 2005) as STLV purified reagents and controls are not commercially available. STLV-specific PCR uses primers in the tax region (Vandamme et al. 1997). Similarities between HTLV and STLV proteins and nucleic acid sequences have facilitated
the use of specific and cross-reactive assays for detection of both viruses, and these well-characterized reagents are important to the screening of SPF macaque colonies.

Simian Immunodeficiency Virus (SIV)

Simian immunodeficiency virus (SIV) is one of the SPF agents for elimination in macaque colonies because of its prevalence in US domestic primate centers with active AIDS research programs. There are no reports of SIV-positive macaques other than those experimentally infected or accidentally housed with infected animals. Viral characteristics of SIV have been extensively described in previous reviews (Geretti and Osterhaus 2001; Nathanson et al. 1999) and a wide range of reagents have been developed for multiple SIV variants.

The most important SIV screen for a general SPF colony is a widely reactive test that can identify animals infected with any possible SIV variant. One way to achieve this broad SIV test is to use whole virus preparations for immunoassays that contain conserved regions of the genome such as the core p27 gene region.

Specific Rationale for Assay Method Designs

Testing for the four primary pathogens that affect macaques has been established through published and unpublished work in diagnostic laboratories at primate centers, and most of the information on methods for viral testing is the result of screening domestic macaque colonies (Lerche and Osborn 2003). In this section we discuss assays for the three viruses (SRV, STLV, and SIV) that, as discussed above, are exogenous retroviruses that can replicate in many different cells of the body during acute infection (we describe a testing regimen for CHV-1 in the next section).

A high viral load can be present in peripheral blood cells as well as in saliva, urine, and feces in animals with little or no antibody response. This possibility of viral shedding without noticeable antibody leads to the following recommended testing regimen:

1. All new animals entering a colony, regardless of age, should have two consecutive negative tests (at least 6 weeks apart) for all three assays (SRV, STLV, SIV) before their incorporation in SPF colonies. The animals’ housing arrangements and groupings should not be changed during testing.
2. Animals should undergo testing for SRV, STLV, and SIV antibodies, with ELISA or another ligand-binding assay using purified virus and immunoblot confirmation of all samples above background. Virus preparations should contain detectable glycoproteins and major capsid proteins of the viral serotypes most prevalent in the animals to be screened; if there is a question about the serotype, it is advisable to use a combination of purified serotypes containing the most prevalent serotypes and to beware of false negatives.
3. A whole blood method should be used to detect viral DNA or RNA and all serotypes present in the population being screened. If there is uncertainty about the serotype, use a well-conserved section of the genome but take care to avoid sections of SRV homologous with endogenous type D viruses.
4. Coculture of PBMC with Raji or another permissible cell line is a useful method of detection of infectious SRV; the virus normally induces syncytial cytopathic effects (CPE) in Raji cells in 3 weeks or less. Confirm positive or negative cultures through PCR and microscopic examination of cultures for CPE. This extra step of viral culture for SRV is important because of the possibility of latent infection in previously untested animals.

Once animals are resident in an SPF colony and confirmed as negative for all three viruses, it is important to run assays 1 and 2 above routinely, both on the newly arrived animals and on all remaining groups of animals. In addition,

- all animals in the SPF colony should have at least two semiannual virus tests once housed in stable groups or single housing;
- animals should be tested 6 to 8 weeks after any new animals are added to an immediate contact area or after they come in contact with new animals;
- animals that may have been exposed to an animal of unknown status should be tested—feces, urine, saliva, and blood can be vectors of transmission and any exposure to these fluids from animals of unknown viral status should be considered a potential exposure;
- dams and infants should undergo testing 6 to 8 weeks after birth; and
- infants at weaning should be screened before being moved to separate housing and once again 4 to 6 weeks after rehousing (Wilkinson et al. 2003).

Cercopithecine Herpesvirus 1 (CHV-1)

The presence in macaques of herpes B virus, known as Cercopithecine herpesvirus 1 (CHV-1), is a primary occupational health concern for research, veterinary, and husbandry personnel who work with these primates or their tissues. CHV-1 normally infects either the oral or vaginal mucosa and results in a lifelong infection in the trigeminal and lumbosacral sensory ganglia hallmarked by latent periods with no clinical evidence of the virus. The virus is spread both by direct sexual contact and by increased oral contact associated with biting and grooming (Weigler et al. 1995). Serological surveys suggest that CHV-1 is not easily transmitted among seropositive macaques (Weir et al. 1993); rather, many macaques acquire the infection between
the ages of 2 and 3 years, when they become sexually active.

For example, when a group of 152 rhesus macaques from Cayo Santiago were relocated and tested for antibodies to CHV-1, 52% of the yearlings, 65% of the 2-year-olds, and 100% of the 3-year-olds were infected (Kessler and Hilliard 1990). A serological study of rhesus macaques at the California National Primate Research Center (CaNPRC) yielded similar results, with 100% infection in the 3- to 6-year-olds and 7.7%, 12.5%, and 66% seropositivity in three different corrals for 6-month- to 3-year-olds. A preliminary survey in the mid-1990s of SRV-free pigtail macaques at the Washington National Primate Research Center (WaNPRC) Medical Lake Facility also indicated that the occurrence of CHV-1 infection coincided with onset of sexual activity (R. Grant, unpublished data). Other reports confirm that establishing CHV-1-free macaque colonies using sexually active animals is difficult due to intermittently detectable infections (Sauber et al. 1992).

Early attempts to create a CHV-1-free colony by segregating CHV-1 yearlings that remained seronegative using herpes simplex virus (HSV)-based ELISA were successful and have been consistently confirmed by the National B Virus Resource Center, directed by Dr. Julia Hilliard. At this facility, antibody-negative primates do not interact with adult animals and are group housed only with animals matched by age and viral status. As these primates reach sexual maturity they remain seronegative and in turn produce 100% CHV-1-negative offspring. However, careful monitoring and removal of seroconverts is required over time to maintain CHV-1-negative status, as evidenced by a situation that occurred with a WaNPRC SPF-3 group. When routine screening analyses of this colony in 2002 revealed one CHV-1 antibody-indeterminate animal, attempts to maintain an SPF-3 colony proved futile: within nearly 3 years and in spite of corrective actions, over half of the animals had seroconverted, in effect creating an SPF-2 (SRV- and STLV-negative) colony from what had been a productive SPF-3 colony (Table 2). The experience led to increased monitoring frequency of subsequent colonies in order to more rapidly identify and reassign new seroconverters. This example highlights the necessity not only of continued monitoring but also of rigorous animal caretaking standards and procedures.

Diagnostic assays for CHV-1 have taken advantage of the high level of similarity between simian and human alphaherpesviruses (Eberle and Hilliard 1989; Eberle et al. 1989; Hilliard et al. 1989; Katz et al. 1986; W.R.M. communication with Margaret Thouless, University of Washington School of Public Health, 1986). A baboon alphaherpesvirus has also been used to detect antibodies against macaque CHV-1 with good results (Ohsawa et al. 1999). Although the cross reactivity between species-specific herpesviruses is good and extends to most of the major structural proteins, some loss of sensitivity occurs when using human HSV antigens to detect antibodies to simian alphaherpesviruses (Hilliard et al. 1989; Ohsawa et al. 1999). Until a CHV-1-specific diagnostic test is available, it is important that the high sensitivity of the assay equal or exceed the specificity to minimize the incidence of false negatives. The CHV-1 situation is further complicated by this virus’s stringent control as a CDC Select Agent and a level 4 risk group designation for propagation.

It is possible to establish an SPF-3 colony using HSV and HTLV to detect animals exposed to CHV-1 and STLV respectively, and to use groups of presumptive CHV-1- and STLV-negative juveniles that have not been exposed to adult animals as SPF-3 colony founders. Additional measures to maintain production and genetic diversity include regular interval screening and the careful addition only of similarly derived new animals to an SPF-3 group as needed. Most significant, however, is continued monitoring to confirm SPF status and control of all facility-specific operations that may inadvertently provide the means to introduce undesirable viruses into SPF groups.

### Table 2 CHV-1 conversion in an SPF-3 breeding colony

<table>
<thead>
<tr>
<th>Year</th>
<th>Total in colony</th>
<th>CHV-1 positive</th>
<th>Percent positive</th>
</tr>
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<tbody>
<tr>
<td>2002</td>
<td>60</td>
<td>1</td>
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<td>2005</td>
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CHV-1, Cercopithecine herpesvirus 1; SPF, specific pathogen-free.

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## Conclusion

SPF nonhuman primate colonies are increasingly requested for research use as well as for the beneficial effects on both animal health and occupational safety. Although there have been efforts for decades to eliminate tuberculosis from primate colonies, the last 20 years have been witness to an unparalleled activity to create NHP colonies that are free of other pathogens as well. These efforts have been supported by technological advances that enable improved diagnostic assays and by increased emphasis on breeding schemes designed to produce and maintain colonies free of undesired pathogens. AIDS-related research programs have also accelerated the process due to their essential use of immuno-compromised animals.

It is apparent that the dynamic nature of most NHP facilities constitutes the greatest risk to SPF colonies—NHPs from the far reaches of the globe as well as from domestic suppliers are constantly coming and going. With the arrival of each new individual comes the potential of introducing a known or unfamiliar infectious agent capable of jeopardizing any or all SPF programs. Regardless of the
length of a specific pathogen list and the success of creating and maintaining productive NHP SPF colonies, investigators and support staff alike must remain vigilant, keeping in mind that new or newly interesting infectious agents will continue to emerge. An extremely important component of this effort is the careful design of and strict adherence to effective husbandry operational practices that protect SPF colonies. Nonhuman primate colonies of the future will need to be maintained in increasingly stringent conditions to ensure the restriction of pathogens of interest.

**References**


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