

This Appendix discusses the biological agents used for research at the existing CRPRC and the naturally occurring disease causing agents present in the animals held in outdoor facilities at the CRPRC. A discussion of control measures for these agents is also included, along with animal and vector control programs that are implemented at the site.

D-1 BIOLOGICAL RESEARCH AGENTS PRESENT AT THE CRPRC

Simian Retrovirus Type D (SRV Type D)

SRV is a retrovirus endemic in Asian macaques in the wild or in captivity. In Asian monkeys the virus can cause dysfunction leading to opportunistic infection (pneumonia, diarrhea), cancers, anemia, and death. Some monkeys show no clinical signs and act as a reservoir for the virus, spreading it throughout the colony. In monkeys, transmission is by direct contact, bite, wound, sexual activity, and possible vertical transmission (parent to offspring).

SRV is not thought to be transmissible to humans under normal circumstances. However, at the CRPRC, increased precautions are taken when handling SRV-positive animals. Type D virus is in a different group of retrovirus from HIV and is not closely related. Type D has been isolated only once in humans and that was in an AIDS patient in Texas. Serological surveys and tests of people exposed to the virus with direct contact have shown some antibody positive individuals but no disease.. The virus was historically studied as a model to understand the natural history of the retroviruses. This virus was previously endemic at the CRPRC breeding colony but has been excluded by CRPRC's SPF program.

Simian Immunodeficiency Virus (SIV)

SIV is endemic to many species of African primates and causes no illness in those species. However, when Asian macaques are infected with SIV, they develop Simian Acquired Immune Deficiency Syndrome (SAIDS). In Asian monkeys the viral infection results in immunodeficiency, opportunistic infections, anemia, and eventually death. Transmission is by direct contact, bite, blood transfusion, and possibly venereal contact.

Currently, there are no known cases of disease transmission from animal to man. However, because of its close relationship to HIV-1 and HIV-2 (discussed below), SIV is treated as potentially infectious to humans. At the CRPRC, animals infected with SIV are managed under Biosafety Level 2 (BSL-2) guidelines. Nationally, there are two cases of human transmission in laboratory environments in 1992. One was from a needle stick contaminated with monkey blood. The other was a laboratory worker with a skin rash handling virus material without gloves. One person had transient antibody with no evidence of persistent infection. The second individual remains antibody positive.

Currently, the primary model for SIV research in primates is work on pathogenesis, vaccine production and therapies. The number of animals currently infected with SIV at the CRPRC is about 180.

Human Immunodeficiency Virus (HIV)

HIV-1 causes AIDS in humans and is the dominant type of AIDS virus worldwide. Some macaques can be infected with HIV-1 and may or may not develop the clinical disease. HIV-2 causes AIDS in humans and is the most common AIDS virus in West Africa. Some Asian

macaques can be infected with HIV-2, and the virus can be cultured from these animals. However, they do not develop the clinical disease.

HIV-1 and HIV-2 are known to be transmitted between humans by direct blood contact, unprotected sex, and needle exchange. There is no evidence of transmission by aerosol. There is no evidence of animal to human transmission; however, blood and tissue fluids from infected animals should be considered infectious. Primates, including chimpanzees (not found at CRPRC), pigtail macaques, and longtail macaques, can be infected with HIV-1 and HIV-2. No illness is evident with HIV-2 but some investigators feel there is disease with HIV-1 in pigtail macaques. Use of Biosafety Level 2 (BSL-2) protective clothing guidelines, sound infection control procedures, needle handling restrictions, and proper personal hygiene methods are used to minimize the risk of exposure.

There are no animals at the CRPRC currently infected with HIV-1 or HIV-2.

Amphotropic Murine Leukemia Virus (MuLV)

This retrovirus is a rodent virus that is commonly used as a vector to transform cells with specific sequences of DNA. This is one approach used in gene therapy because MuLV is able to infect a wide variety of cells in cell culture. Although the virus has not been demonstrated to be infectious to humans, it is treated as a BSL-2 agent and handled as a biohazard. The goal of CRPRC research utilizing MuLV is to study its means of transmitting DNA sequences to cells as a possible treatment for HIV or other types of gene therapy.

Simian Foamy Virus

This retrovirus is in the family Spumaviranae and is one of the first retroviruses isolated in 1955. They are found in many primate species, but have not been associated with any pathogenic process. The main problem the virus poses in the laboratory is the tendency to contaminate and disrupt cell cultures. Because this retrovirus does not cause any health-related problems, it is being evaluated as a vector virus in gene therapy studies.

Rhesus Epstein Barr Virus (EBV) and Rhesus Cytomegalovirus (CMV)

These viruses are primate herpes viruses which appear specific for rhesus macaques. They are the monkey counterparts to human Epstein Barr Virus and Cytomegalovirus. There is no evidence of transmission to humans. Transmission between animals appears to be by direct contact of saliva, urine, and other bodily fluids. If an infected animal is present in group-housed animals, almost all animals are infected by 6 to 12 months of age. Healthy animals display no clinical disease symptoms. Generally only immunocompromised animals (such as the aged or SIV infected) may have clinical problems such as pneumonia or kidney infection.

At CRPRC these viruses are used to study their possible role as cofactors in SIV infections. The hypothesis is that somehow these agents might enhance the effect of infection with a retrovirus. One area of research is to breed animals free of EBV and CMV to study SIV in the absence of these viral agents.

Escherichia coli (E. coli); K-12, recombinant DNA

E. coli is a bacteria that lives in the intestines of healthy humans and animals. Some of its strains like E. coli 0157:H7 produces powerful toxin that can cause severe illness in humans, like

bloody diarrhea, and occasionally kidney failure. Transmissions to humans appears to be by ingestion of infested food and water. Although the number of organisms required to cause disease is not known, it is suspected to be very small. Proper personal hygiene and housekeeping methods are used to minimize the risk of exposure.

Vaccinia Virus

Vaccinia Virus is a poxvirus that was used as a live vaccine agent to immunize people around the world to smallpox and eliminated the deadly disease by the 1980s. People vaccinated with vaccinia develop a small lesion or "pox" at the vaccination site. If the scab is removed, this site can then shed virus for several days, which could result in infection of other individuals. Clinical disease is not manifested by immunized individuals.

In monkeys the vaccinia virus is genetically modified to have surface or core viral antigens from SIV or HIV so that vaccination with the vaccinia will produce an antibody response to these agents. There has been no evidence of spread from animals to humans. Vaccinated animals do have a scab which contains infectious material. Infection on the skin would produce a skin vesicle at that site. The only other risk to the animal would be the infection of the eye which could damage the cornea. Approximately 40 animals at CRPRC may be vaccinated in this manner. CRPRC research with this virus is intended to investigate a possible means of vaccinating for HIV.

CRPRC personnel working with the Vaccina Virus or with animals inoculated with modified Vaccina Virus are required to have current vaccination at Occupational Health Services.

HTLV 1 and STLV1

HTLV 1 is Human T lymphotropic virus that was isolated from certain island populations in Japan. In humans, HTLV is associated with leukemia. Research in monkeys utilizes studies of STLV 1 (Simian T lymphotropic virus) which can cause illness in baboons but apparently not in macaques. There is no indication of animal to human spread or spread between humans. Transmission between animals appears to occur by direct contact of blood, saliva, or other body fluids. The primary goal of CRPRC research is to eliminate this virus in the macaque population so its presence will not affect study results.

Cytomegalovirus (CMV)

CMV is a member of the herpesvirus group. It is endemic in primate species. It is also presents in most adults and juveniles. This virus has the ability to remain dormant within the body over a long period. For most healthy persons who acquire CMV after birth there are few symptoms and no long-term health consequences. Some persons with symptoms experience a mononucleosis-like syndrome with prolonged fever, and a mild hepatitis. Recurrent disease rarely occurs unless the person's immune system is suppressed. The virus is transmitted between animals by direct contact, bite wound, sexual activity, and vertical transmission. Though no evidence exists of transmission from nonhuman primates to humans, CMV is treated as potentially infectious to humans. Protection measures consist avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Listeria

Listeria monocytogenes is a bacteria that can be carried by animals without appearing ill and can contaminate foods of animal origin such as meats and dairy products. A person with listeriosis can develop mild disease as fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. In infected women, listeriosis can cause abortion, infection of the new born, or even stillbirth. Transmission from animal to humans is by direct contact with the animal or body fluids. Protection measures consist of avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Borna Virus

The Borna virus is a negative-stranded RNA virus that produces severe encephalopathy. The virus is used in pilot research projects in neurological studies. Transmission experiments on nonhuman primates showed that nonhuman primates are moderately susceptible to the Borna virus infection. Transmission between animals is by direct contact with saliva, nasal, or conjunctival secretions. Though no evidence of transmission from animal to human exists, humans are expected to be susceptible to the Borna virus infection by direct contact with the animal or body fluids. Protection measures consist in avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Measles

The measles virus is a paramyxovirus, genus Morbillivirus with a single-stranded RNA. A dramatic increase of human cases occurred from 1989 to 1991. Measles is a human disease with no known animal reservoir. In humans, measles produces respiratory disease and rash. Transmission from person to person is via aerosol droplets. Protection measures consist of avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Polio vaccine

The poliovirus is a member of the enterovirus subgroup, family Picornaviridae, with an RNA genome. The polio vaccine is used as a vector in research. In nonhuman primates, the polio virus produces no effect except in rare cases some clinical signs. In humans, the polio virus affect the spinal cord that leads to paralysis. Transmission from nonhuman primates to humans may occur by ingestion or inoculation. Protection measures consist of avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Human herpes simplex

The human herpes simplex is part of the herpesvirus group, which has the ability to remain dormant within the body over a long period of time. The herpes virus is used in vaccine development. In rhesus macaques, there is typically no clinical disease. Transmission from nonhuman primates to humans may occur by direct contact. Protection measures consist of avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Adenovirus vectors

The adenoviruses are medium-sized (90-100 nm) nonenveloped icosohedral viruses containing double-stranded DNA. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body. Adenoviruses are used in gene therapy studies. No or mild effects are caused by adenoviruses in primates. In humans, adenoviruses cause respiratory illness, and depending on the infecting serotype, they

may also cause other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash. Transmission from nonhuman primates to human is by airborne, and direct contact. Protection measures consist in avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Pig Virus

The pig virus is a pig endogenous retrovirus. It is found in the DNA of most pigs and never causes a problem to the pig or anyone around the pig or even if the pig is used for food. It is being used at the CRPRC on nonhuman primates to determine if pig tissue containing the virus would be infectious to immunosuppressed transplant patients. The concern is that if pig organs are placed in transplant patients, the virus may cause health problems. There is no indication that it can infect primates and there are no disease conditions associated with it. The CRPRC conservatively treats it as BL S 2 + pathogen.

D-2 ENDEMIC DISEASE-CAUSING AGENTS, CHARACTERISTICS, AND CONTROL MEASURES AT CRPRC

Nonhuman primates can carry diseases that are potentially infectious to people and conversely people can carry diseases that are highly contagious to monkeys (e.g., measles, influenza). Protective measures taken at the CRPRC are based on the type of potential infectious agent involved, the mode of transmission and the potential risk associated with the infection to people and animals. Disease-causing agents that may exist in the nonhuman primate population can come from surrounding environmental conditions, or they may be endemic in the animal population itself.

Gastrointestinal diseases are the most prevalent cause of morbidity and mortality in colony-managed and zoo-housed nonhuman primates. Diarrhea, either acute or chronic, is the major manifestation of disease. Several pathogenic agents of diarrheal disease in simian primates have been well characterized. Some infectious agents and inflammatory disorders are less well understood. Disease-causing agents can be viruses, bacterial diseases, protozoan diseases, or helminths.

VIRAL DISEASES

Herpesvirus simiae (Herpes B virus)

Herpes B is a naturally occurring herpesvirus in many primate species, particularly macaque species. It is the monkey equivalent of human herpes simplex virus in that it rarely, if ever, causes disease problems in the host species, macaques. Animals that are antibody positive may shed (release virus) at any time asymptotically. Epidemiologic studies of naturally infected rhesus indicate that a single animal may shed for up to 8 hours during any one year. For this reason all macaques are treated as being potentially infectious at any particular time, so the staff at the CRPRC wear gloves and masks when handling any animal. During the past 50 years that Herpes B has been recognized as a human pathogen, there have been only 40 cases of human Herpes B. Over 20 of these people have died, ultimately from fatal encephalitis. Herpes B appears very difficult to catch but very difficult to survive once contracted. For this reason, if any CRPRC worker is involved in an animal-related accident, bite, scratch, or needle stick, the animal is immediately tested by culture and antibody tested for the presence of shedding Herpes B. If an animal is found positive, the worker would be immediately started on antiviral agents

which have been shown to be effective in inhibiting the virus. Other controls that are implemented at CRPRC per CDC guidelines include those found in "Guidelines for Prevention of Herpesvirus Simiae (B Virus) Infection in Monkey Handlers" from *Perspectives in Disease Prevention and Health Promotion*, Health Service, 1987:35-6; DHHS publication no. (CDC) 00-5101; as follows:

- Macaque monkeys should be used for research purposes only when clearly indicated.
- When feasible, monkeys should be maintained under conditions to assure their Herpes B virus-free status.
- All monkeys not known to be free of Herpes B virus infection should be regarded as infected. Direct handling of macaques should be minimized.
- Macaque handlers, when needing to remove physically active animals, should wear specified personal protective equipment.
- Cages and other equipment that may be contaminated with virus should be free of sharp edges and corners. Cage housing should be arranged to minimize cuts, scratches, bites etc.
- Routine screening of macaques for evidence of Herpes B virus infection is not recommended because it is not considered effective.
- Persons handling macaques must be trained on the facility's prevention, reporting, and treatment program.
- All potentially contaminated wounds incurred from macaques or from cages must be treated and referred immediately in accordance with the facilities accident/incident program.

Measles

Measles is a highly contagious paramyxovirus which can affect both man and monkey. In the 1970s and early 1980s, public health officials believed that the measles vaccination would totally control, if not eliminate, measles. Inadequate vaccination of school children and apparent vaccine failure resulted in an increase of measles in the late 1980's that at times required entire high schools or colleges to shut down. In 1987, a staff member or student brought measles into the CRPRC colony. The colony had not been vaccinated because, like the public health officials, CRPRC officials felt measles was on the decrease. The disease outbreak affected 147 animals, and about 70 died. Four staff people were infected with measles at that time. Employees may also have caught the disease from the monkeys; however, there were measles cases in the local community at the time. Since 1987, all employees have been required to have evidence of measles vaccination or previous infection. All monkeys received by quarantine are vaccinated and all monkeys born at the Center are vaccinated by 6 months of age. The CRPRC vaccine program is currently a better program than that available to school children in the United States.

Filoviruses

Filoviruses represent a small group of viruses that contain two of the deadliest viral agents known to man, Marburg virus and Ebola virus. These viruses can both cause a severe

hemorrhagic disease for which there is no treatment and can result in 50-80 percent mortality. Ebola occurred spontaneously in villages in Africa and was never associated with an animal vector. Marburg was first recognized in Germany in 1967 when animals in an imported shipment of African Green Monkeys were found to be infected with Marburg virus. They had not gone through quarantine, and tissues were handled by a variety of people. Since then Marburg has occurred again in isolated incidents in Africa, but once again was not associated with an animal vector.

In 1989, an outbreak of Simian Hemorrhagic Fever (SHF) and Ebola virus was found by CDC and the Army Disease Lab at Fort Detrick in a group of an Asian species, long tailed macaques, at a quarantine facility in Reston, Virginia. The fact that Ebola virus had only been seen before in Africa and that the test used to detect it is very sensitive but not very specific, made the analysis of this outbreak difficult to verify. CDC had also tested people who had contact with the monkeys and found some people antibody positive, including one person who had punctured himself in the hand while performing a necrosy (dissection of an animal body after death). None of these people ever showed any signs of clinical disease. At the time, CDC tested 50 longtailed macaques at the quarantine facility used by CRPRC and volunteer employees from CRPRC's animal care staff.

In 1990 CDC stated that 15 of CRPRC's monkeys were antibody positive and 2 of the animal care staff were positive for the antibody. Press reports were released regarding the deadly monkey virus. At this same time, CDC performed an epidemiology study of members of the general population by collecting, storing, and analyzing serum from several places including such diverse areas as Eskimo serum from Alaska. CDC concluded that their test had a high number of false positives, and that some of the positives were cross reactions with other viruses such as adenovirus and measles. CDC then reported to the CRPRC that the animals were actually negative for Ebola virus. It was not until three years later, in February 1993, that CDC confirmed that CRPRC's employees were also negative for the virus. This finding has not yet been reported in the media to assure the public that a "filovirus outbreak" did not occur. However, there is now CDC documentation to confirm this fact. CDC did require routine testing in quarantine for a brief period. However, routine testing has been discontinued. It appears now that there is another simian filovirus, which is not Ebola, and does not cause disease in man, but can kill monkeys.

These cases show that transmission from natural reservoir to human is unknown, however, once a human is infected, person-to-person transmission is the means by which further infections occur. Specifically, transmission involves close personal contact between an infected individual or their body fluids, and another person.

Prevention consist in minimizing direct contact with primates and use of appropriate protection measures.

Hepatitis A

In humans Hepatitis A is a liver disease caused by the hepatitis A disease. Animals may show non-specific gastrointestinal illness and elevation of liver enzymes. Hepatitis A is rarely a cause of death, and is generally spontaneously resolve. Short and long term vaccines are available. The primary route of transmission is fecal oral, infection by drinking or eating contaminated water or

shellfish is also possible. Prevention measures include use of gloves, prevent fecal-oral contact, good personal hygiene, and vaccination of animals and workers.

Monkey Pox

Monkey pox is a disease that affects infected animals, which develop fever and cutaneous pox lesions of 1-4 mm in diameter. Animals can also develop coughing and nasal discharge. Serious Monkey Pox cases can be fatal. Transmission between animals is by direct contact and aerosol droplets. Since this disease is not endemic at the CRPRC, prevention measures consist of quarantine of all animals coming from other facilities. Since the incubation period is of 3-4 days any cases of monkey pox would be identified and treated in quarantine. Effects and transmission from non-human primates to humans is not known, but basic prevention measures such as gloves, prevention of fecal-oral contact, and good personal hygiene are used.

Tanapox

Tanapox is a disease that affects infected animals, which develop fever and cutaneous lesions of 1-4 mm in diameter. Lesions may be mild swellings or show ulceration. Infected animals can also develop coughing and nasal discharge and will typically recover after 1-2 weeks. Transmission between animals is by direct contact, aerosol droplets and vectors (mosquitoes). Since this disease is not endemic at the CRPRC, prevention measures consist of quarantine of all animals coming from other facilities. Since the incubation period is of 3-4 days any cases of tanapox would be identified and treated in quarantine. Effects and transmission from nonhuman primates to humans is not known, but basic prevention measures such as gloves, prevention of fecal-oral contact, and good personal hygiene are used.

Rabies

Rabies virus belongs to the order *Mononegavirales*, viruses with a non-segmented, negative-stranded RNA genome. Infected animals show signs of salivation, paralysis and sudden death. Animals may present with either silent rabies or furious rabies. Transmission between animals is by bites. Since this disease is not endemic at the CRPRC, prevention measures consist of keeping outdoor primates behind a perimeter fence and trapping and relocating pests and feral wildlife.

Parainfluenza virus

This is a human disease which is a risk to monkeys. Workers wear face masks to protect the animals.

BACTERIAL DISEASES

Campylobacter species

Campylobacter is the most commonly isolated enteric organism associated with diarrhea in humans and captive primates. The role of campylobacter in nonhuman primate gastrointestinal disease is unclear. Campylobacter has been isolated from animals with watery or bloody diarrhea as well as animals with normal stools.

Of the eight named types of campylobacter, two isolates of primary concern are:

- *Campylobacter coli* - This is the most common isolate in nonhuman primate colonies. It causes proliferative ileitis.
- *Campylobacter jejuni* - This is most common in nonhuman primates although many laboratories have not distinguished between *C. coli* and *C. jejuni*.

Reservoirs of campylobacter are found in domestic animals, nonhuman primates, and wildlife. Domestic animals, particularly dogs, are the major cause of bacterial diarrhea in children. A survey of newly captured cynomolgus monkeys showed them to be free of this organism. The organism is probably endemic in all group-housed captive nonhuman primates. In several surveys at primate centers, 80-90 percent of the animals were stool culture positive. In the CRPRC colony, 67 percent of a small group of healthy rhesus macaques carried *Campylobacter*. *Campylobacter* is transmitted by surface waters not subject to chlorination. The mode of transmission is probably fecal-oral in nonhuman primates. In humans, raw milk, poultry, and contaminated water are common sources. Transmission is controlled by preventing fecal-oral contact by using good personal hygiene and by wearing gloves.

Shigella species

Shigella enteris can range from mild diarrhea to dysentery. Dysentery is defined by Dorlan's Medical Dictionary as inflammation of the intestines, especially the colon, accompanied by abdominal pain, and frequent stools containing blood and mucus. In 1973, a CDC survey of major primate centers determined that shigellosis was the principal known cause of digestive disease and was endemic to their institutions. Approximately, 15 percent of diarrheas at primate centers are due to shigella.

Shigella is transmitted by food and waterborne routes. Pet monkeys pose a hazard to children. Human cases in primate facilities have been rare. Between nonhuman primates, direct fecal-oral contact is the most common route of transmission (i.e., fecal material present on cages or floors as a means of reinfection, as well as contaminated hands, food, or water). Flies can also spread the organism. Asymptomatic carrier animals (studies show from 5 to 67 percent) are usually the source of the organism. Stress may initiate disease in these animals. The immune status of the host plays a role in the development of clinical disease. During a 1987 outbreak of measles at CRPRC, which caused immunosuppression, *Shigella* was a major component in morbidity and mortality. *Shigella* organisms are susceptible to drying, heat, and disinfectants, and these characteristics are used as a basis for control technologies. For indoor housing conditions, animals with clinical disease are isolated, good sanitation with disinfectants is instituted, splashing is minimized during hosing, and animal carrier contact is decreased.

Once the disease becomes endemic in animals housed outdoors, no effective control measures are known, although rigorous quarantine screening of initial stock has been attempted. Treatment of asymptomatic carriers appears superfluous but may be indicated for new animals coming into quarantine.

Yersinia species

Yersinia species cause diarrhea that is sometimes bloody, with fever and abdominal pain. *Yersinia* in outdoor animals is associated with wet weather in California. The CRPRC has between 0 and 10 cases per year, when outbreaks are experienced during winter and spring months. It is transmitted between animals most commonly by fecal contamination but can also

be transmitted by contaminated food. Human-to-human cases have been documented. Birds and rodents act as reservoirs for the bacteria. Depending on the location of the colony, varying numbers of feral animals carry *Yersinia*, again in winter and spring months.

Control at the CRPRC is primarily centered on pest control, housekeeping and flood prevention around animal enclosures.

Salmonella species

Salmonella, of which 2000 serotypes are identified, is an organism extremely resistant to environmental factors and capable of survival for long periods of time. The organism has an incubation period of 8 to 48 hours if foodborne, with illness that lasts from 2 to 14 days. It produces fever with headache, abdominal pain, bloating and constipation, giving way to loose watery diarrhea that may have mucus and blood. Two-thirds of people have a cough arising from lung involvement. There may be nonspecific problems with or without these gastrointestinal signs.

Although many species of animals are susceptible, *Salmonella* has occurred rarely in most primate facilities. CRPRC has seen four cases in 12 years. *Salmonella* is transmitted in three major ways:

- Ingestion of contaminated food or water.
- Contamination from wild rodents and birds.
- Carrier animals and humans. Stress may precipitate active disease.

Salmonella outbreaks are controlled by determining the source of the organism and eliminating it (e.g., diet, water, rodents). Asymptomatic carrier animals and humans can be identified and isolated.

Enteropathogenic coli (E. coli) species

E. coli is part of normal intestinal flora. There are certain pathogenic strains of *E. Coli* which can produce a watery stool, which rarely contains blood. It is associated with nonhuman diarrhea. The significance of this species is questionable as a disease-causing agent because it can be isolated from the healthy intestinal tract. Transmissions to humans appears to be by ingestion of infested food and water. Although the number of organisms required to cause disease is not known, it is suspected to be very small. Proper personal hygiene and housekeeping methods are used to minimize the risk of exposure.

Listeria

Listeria monocytogenes is a bacteria that can be carried by animals without appearing ill and can contaminate foods of animal origin such as meats and dairy products. A person with listeriosis can develop mild disease as fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. In an infected woman, listeriosis can cause abortion, infection of the new born, or even stillbirth. Transmission from animal to humans is by direct contact with the animal or body fluids. Protection measures consist in avoiding direct contact, wearing appropriate hand, skin, and slash protection.

Tuberculosis

Tuberculosis is caused by the gram positive, acid fast, aerobic, bacillus of the Mycobacterium genera. The most common species of mycobacteria encountered in humans and nonhuman primates is *M. tuberculosis*, and is the one which cause infections. In nonhuman primates and humans, clinical infections can result in severe disease affecting multiple organs systems and is frequently fatal. Tuberculosis is spread through the air. Prevention measures consist of screening all animals in quarantine with Tuberculosis skin tests performed every two weeks over a twelve-week period. Animals in the colony are skin tested for the tuberculosis three times annually. All CRPRC personnel and visitors are skin tested for tuberculosis annually.

Leptospirosis

Leptospirosis is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus *Leptospira*. In primates, clinical infections may be inapparent. In natural infections there may be diarrhea and pregnant animals may abort. Experimental infections of animals may result in fever, malaise, weight loss, and in some cases death. Transmission can be through small lacerations or wounds of the skin, by skin contact especially mucosal surfaces such as eyes or nose, or by contact with food or water contaminated by urine. The bacteria does not survive in dry environments. The disease is not known to be spread from person to person. Basic prevention measures consist in use of gloves, prevention of fecal-oral contact, and good personal hygiene.

Melioidosis

Melioidosis is an infectious disease caused by the bacterium *Burkholderia pseudomallei*. In primates, some cases can present as a subclinical chronic case, and may present as a broncho-pneumonia or as a subcutaneous disease. The disease can result in death. Transmission by soil and contaminated water is the typical source of the causative agent, *Burkholderia pseudomallei*. Typically associated with Southeast Asia, the organism has been cultured from soil worldwide. Infections occurs by ingestion, inhalation, or percutaneous. Prevention measures include quarantine of all animals and use of gloves when handling primates.

Mycobacterium avium

Primates infected with avian TB typically are asymptotic. Animals that are immunocompromised may present with a chronic enteric infection with continuous or intermitent diarrhea. Several cases of avian TB have been observed in animals infected with Simian Immunodeficiency Virus. Wild bird act as a vector for this agent and may contaminate the environment organisms. Infection is typically by ingestion or inhalation. Prevention measures for humans consist in wearing gloves and mask when treating animals.

Pseudomonas species

Pseudomonas has been isolated from nonhuman primates with gastrointestinal disease and from animals considered to be carriers.

PROTOZOAN DISEASES

Giardia lamblia

Giardia lamblia is a parasite transmitted by contaminated water or food by cysts which encapsulate the protozoal cells. It causes an explosive, foul smelling, watery diarrhea with intestinal cramps, flatulence, nausea, and malaise. *Giardia* is common in many natural bodies of water due to wildlife and can be found in many household pets. It is controlled by preventing fecal-oral contact and wearing gloves.

Entamoeba e. histolytica

Entamoeba e. histolytica is a parasite transmitted by contaminated water and sometimes food. Cyst germination leads to dysentery-type diarrhea and severe intestinal cramps that if untreated can migrate to other organs. It is controlled by preventing fecal-oral contact and wearing gloves.

Balantidium coli

Balantidium coli is a parasite of domestic animals but occasionally infects the intestinal tracts of humans. It produces dysentery-like disease similar to *E. histolytica*.

Cryptosporidium

Cryptosporidium is not treatable and may cause diarrhea.

Trypanosoma cruzi

T. cruzi is a blood parasite that is found in South and Central America that causes Chaga's disease. It is transmitted by the triatomid, or "kissing bug," which bites an individual and passes the parasite in its feces. The long-term effects of the infection can involve the gastrointestinal tract, the brain, and the heart. Disease usually develops over a number of years, even decades, and is untreatable. The parasite is present in millions in South America and has been introduced at a low level into the United States by immigrants giving transfusion. The parasite and closely related nonpathogenic forms are found in different species of New World monkeys (monkeys from South and Central America). CRPRC had observed some of the *Trypanosomes* in the blood of some of its squirrel monkeys and contacted the CDC. Some of the Trypanosomes are of the pathogenic type and others appear harmless. There are triatomid bugs in California but CDC officials said that the type of bug present in Northern California is incapable of transmitting the parasite. To make sure that there is no health threat to employees, CRPRC worked with CDC to test volunteers from its animal care staff for the parasite. All tested personnel, including some people who have worked with monkeys for 30 years, were negative. It appears that the primary risk is direct blood contamination from a needle stick or other accident. The CRPRC no longer houses squirrel monkeys.

Plasmodium

Blood parasites of the genus *Plasmodium* cause the Malaria disease. Malaria symptoms in nonhuman primates can vary from very mild to severe depending on the species of *Plasmodium* involved. Animals with severe disease can develop severe anemia, shock dehydration and depression. The malaria parasite is transmitted into the host by the female mosquito when she feeds on the mammalian host. Different species of malaria will use different mosquito species as vectors with the genus *Anopheles* transmitting most forms. Therefore, potential transmission exists anywhere mosquitoes have access to infected animals. Prevention measures consist in controlling flying insects in all primate facilities. At the CRPRC all animals entering the colony are treated for malaria.

HELMINTHS

Trichuris

These organisms are parasites that may or may not be associated with disease in man or monkeys. Transmission is by direct fecal-oral contact and is easily prevented by the use of gloves, hand washing, and good hygiene practices.

Oesophagostomiasis

The causative agents are *Oesophagostomum stephanostomum*, *bifurcum*, and *aculeatum* which cause a nodular intestinal worm infection. The parasite lives in the intestines of various primates and sometimes humans. These definitive hosts can sometimes serve as intermediate hosts. In animals, mild infection is subclinical. Abdominal pain follows more severe infection with diarrhea or even dysentery. In humans infection goes unnoticed but sometimes abdominal pain, bleeding and even peritonitis occur. Transmission is by eggs passed in feces that release larvae which infect the definitive host on ingestion. Prevention measures consist of testing animals for helminth parasites during quarantine and treating them, preventing fecal-oral contact, wearing protective clothing and gloves, and good personal hygiene.

Strongyloidiasis

Strongyloidiasis is caused by infection with *Strongyloides stercoralis*. The condition is an infection of humans but primates have been found naturally infected. Symptoms include dermatitis at sites of larval penetration, diarrhea, epigastric pain, nausea, malaise, weight loss, and coughing. Transmission is by skin contact. Prevention measures include fecal exams of monkeys in quarantine, and rigid attention to hygienic habits, including use of footwear in endemic areas.

Bertielliasis

Bertielliasis are found endemically in nonhuman primates with no symptoms. They acquire the parasitosis by ingesting mites infected with cysticeroid larva. Humans can become infected accidentally by ingesting food containing the mites. In humans the infection is usually asymptomatic, but cases with recurrent abdominal pain, vomiting, anorexia, constipation, and intermittent diarrhea have been observed. Prevention measure consist in controlling mites.

D-3 CONTROL PROCEDURES

Control technologies to protect public health from biological agents at the CRPRC include the control of laboratory procedures and animal care, waste products, disease control in the animal population, worker safety and health protection and physical animal containment. Some control procedures associated with direct transmission from primates were discussed above in Sections D-1 and D-2.

Animal Care Wastes

Animal care wastes include animal excreta, bedding and uneaten chow, cage washing solutions, animal carcasses and tissues, worker's disposable protective clothing, sharps, infectious waste and non-infectious waste. Indoor animal cages are hosed daily with a disinfectant solution. The disinfectant solution and urine and feces from the cages entering the sanitary sewer goes to the

campus wastewater treatment plant. Detergent/ disinfectant solutions are believed highly effective against virus and bacteria. In addition, indoor animal cages are cleaned and disinfected every 2 weeks at CRPRC's cage washing facility. In the process, cages are washed down with disinfectant, then steam cleaned with a steam gun or put through a cage washer at an elevated temperature for a given duration. The presence or absence of virus and/or bacteria in this effluent is based on the organism's survivability through cage washing with disinfectant. The effluent is treated further in the wastewater treatment plant.

Animal bedding in the outside cages consists of gravel underneath the perches and around feedpads. The area under the perches is shoveled periodically and sent to the landfill. This bedding material is used by generally healthy nonexperimentally infected animals; however, any virus deposited to the bedding is not likely to survive outside its host. Indoors, dry bedding is used primarily in the nursery areas. This material, and any uneaten animal chow, is also landfilled. If animals are experimentally infected, bedding is autoclaved. Disposable protective clothing used in experimentally infected animal and quarantine areas is also autoclaved and landfilled.

Animal tissue is considered infectious waste and is either disposed of by a licensed facility or autoclaved. CRPRC employees are trained to designate whether individual waste is infectious waste. Infectious waste tissues, including dead animals and necropsy tissues are double-bagged and refrigerated and picked up by a licensed medical waste transport and disposal company for disposal.

Sharps containers are maintained throughout the facility. They are used for all experimental test kits, hospital disposables, TB test kits and serum bank disposables. Containers are picked up by a commercial medical waste service for disposal.

Medical waste includes blood- and/or body fluid-soaked materials primarily from the hospital and infectious animal housing modules. This material is disposed in biohazard-labeled bags which are autoclaved and sent to the landfill when sterilized. Non-hazardous solid waste or non-medical waste is landfilled.

All waste from the Primate Quarantine facility is considered potentially infected and is autoclaved prior to disposal at the campus landfill.

Transmission of Biological Agents

Disease is controlled within and from the animal population by control of five potential transmission vectors: between members of the nonhuman primate population, from nonhuman primates to other wildlife, from other wildlife to nonhuman primates, from nonhuman primates to humans, and from humans to nonhuman primates.

Transmission between members of the nonhuman primate population can occur by several routes:

- **Inhalation.** Inhalation of aerosols generated between nonhuman primates (i.e., sneezing etc.) is not considered a major route of transmission for the majority of experimental viruses at CRPRC which are primarily bloodborne pathogens.
- **Injection or Skin Puncture.** Entry through skin penetration or an open wound is the most likely route of virus transmission between nonhuman primates.

- **Reproduction.** Vertical transmission of viruses (from parent to offspring) is a possible mode of transmission within the nonhuman primate population. However, the population is screened and controlled to minimize this type of transmission because it may disrupt research of other viruses.
- **Dermal Absorption.** Disease transmission through the skin is not considered a significant means of transmission.
- **Ingestion.** Ingestion is not considered a significant mode of transmission of some of the viruses. Ingestion of bloodborne pathogens could possibly result in infection, if cuts or sores were present in the oral cavity. Ingestion of bacteria or parasites following contact with other nonhuman primates is a route of entry primarily for endemic disease-causing agents.
- **Otic/Optic Absorption.** These are routes of transmission of bacteria and some viral species.

Transmission of disease within the nonhuman primate population is controlled first by a thorough quarantine process where animals are held for 90 days at the Quarantine Facility at the CRPRC and tested for retroviruses. Serum (blood after all cells and clotting factors have been removed) is collected initially during quarantine. In the next 18 months, two additional samples are collected. Thereafter samples are collected every 2 years from the animals. All serum is frozen and kept in order to conduct epidemiological studies if needed. Infected animals are housed in designated modules and individual cages to prevent transmission to the general primate population. Animals receive routine health checks and screening to track their wellness and the potential for onset and spread of disease. Full medical profiles for each animal are maintained on computer and in medical records. All animals are vaccinated for measles and outdoor animals are also vaccinated for tetanus. During quarantine they are tested for TB, six times in 90 days.

Transmission from monkeys to other wildlife is not considered a significant threat for the immunodeficiency viruses for two reasons. Most of the retroviruses researched at CRPRC and *Shigella* species of bacteria (see disease-causing agents discussed in Appendix D-2) are species-specific and are currently known to survive only in human and primate species. Also, experimentally infected animals are housed indoors only and there is no opportunity for contact with other wildlife.

Transmission of bacterial or parasitic agents which are not commonly used in research at CRPRC, but which may be disease-causing organisms, can be transmitted from monkeys to wildlife by:

- **Inhalation.** While this is not usually the major route of transmission, some disease agents may be transmitted by sneezing and generation of aerosols from body fluids.
- **Ingestion.** Ingestion of biological agents, following contamination of food or water or bodily contact between animals is the most significant route of entry for disease agents. This type of interaction can occur with animals such as squirrels, rats, rabbits, and birds that have some contact with the monkeys at the outside field cages, particularly at the

feeding pads. Animal access is limited as much as possible by enclosures at the housing areas.

- Skin Penetration. Feral (or wild) animals may be bitten or scratched by monkeys causing staphylococcus or streptococcal infections.

CRPRC personnel regard transmission of disease into its research and breeding colonies as a significant issue. Primate health is of paramount concern for research quality control purposes. In addition to the animal health and quarantine measures used to prevent transmission within the population, a pest control program for the CRPRC is carried out to minimize the primate/wildlife contact in the outdoor cages. Physical barriers such as fencing, cage covers and maintenance of cage integrity minimize contact with larger animal wildlife. CRPRC's pest control program, which includes trapping and extermination, is conducted in conjunction with the County Animal Control Department on the operations area, to limit pests such as rodents and insects (i.e., flies and mosquitos).

Transmission of disease from primates to humans, is primarily a concern for transmission of Herpes B virus by skin penetration from needle sticks, bites, scratches and cuts on contaminated sharp edges. Transmission can also occur from exposure of mucous membrane to animal fluids. Targeted activities include animal and cage handling, surgical procedures and laboratory experimentation. Ocular exposure has also been documented as a route of exposure. Other open wounds in the nose, throat and mouth are a potential route of entry with a much lower probability of occurrence.

Aerosol inhalation from primates to humans is not considered a significant route of entry for the immunodeficiency or Herpes B viruses. The primary agent with potential for inhalation is TB and the vaccinia virus. All animals are vaccinated for measles and tested for TB three times yearly. All personnel are tested annually for TB. Personnel working in rooms where vaccinia is used are vaccinated with vaccinia. Other agents have the potential to be spread by aerosols that can possibly later enter the body by open cuts or wounds. While the life expectancy of these agents outside the body or controlled conditions is extremely limited and not generally considered communicable except by specific means, their transmission is nevertheless controlled with industrial hygiene methods. These worker protection methods which are outlined further in a later section, include such practices as personal hygiene, splash shields, washing and disinfection of cages and surfaces, training and monitoring employees and accident/incident prevention and investigation. Control methods used to prevent transmission between CRPRC primates and between primates and wildlife are protective of workers and the community as well.

Laboratory Wastes

Generated wastes are segregated, handled, labeled, stored, transported, and disposed of to minimize direct or indirect exposures to the public of chemical or biological agents in conformance with prudent biological practices. In general, biological practices used at the CRPRC include the following:

- CRPRC uses the UC Davis Environmental Health and Safety Department (EH&S) for guidance or assistance with the disposal of biological waste materials.

- Waste is segregated as it is generated into clearly marked readily available, containers. Chemical, infectious, ordinary trash (office trash), and recyclable materials (paper, aluminum cans) are collected separately.
- Workers are trained on waste segregation procedures.
- CRPRC and EH&S personnel review literature on biohazard control technologies continuously to maintain adequate control procedures.
- Standard written waste disposal procedures which address whether each waste stream is expected to contain pathogens, and the prevention of improper segregation of wastes.
- The use of the UC Davis EH&S Department to provide oversight of waste collection by trained environmental and industrial hygiene professionals.

Laboratory waste products include air emissions, effluent and segregated wastes. Potential for release in air of biological agents from laboratories (as well as animal care facilities) are from biological safety cabinets, autoclaves, fume hoods, downdraft necropsy (dissection of an animal after death) tables, the transport to the waste storage areas, and open stationary sources. The fate of the air exhausted by each of these air handling sources varies with its purpose and necessary level of contaminant control. Biosafety cabinets are HEPA filtered. Some cabinets recirculate air into the room, while others exhaust directly from the building. Laboratory fume hood air is not recirculated, but all vented to the roof. The necropsy downdraft table is vented to the roof. Indoor cage rooms for monkeys receive 100 percent fresh air and are not recirculated. General ventilation systems and fume hoods are tested and certified annually by EHS; this includes filter leak and velocity tests.

Most HEPA filters for biosafety cabinets recirculate air in the lab rooms and are 99.97 effective in screening out particles at 0.3 microns in size. One micron is equal to 1,000 nanometers. Information on laboratory equipment in the *Handbook of Facilities Planning* shows that viruses range in size from 20 to 1,000 nanometers or 0.02 to 0.1 microns. However, airborne viruses do not usually travel alone but on a host aerosol (i.e., droplet or dust) or bacteria for which HEPA filters are very effective. In addition, HEPA filter efficiency does not decrease greatly for particle sizes below 0.3 microns because the particles are collected by a mechanism called diffusion (Harvard School of Public Health 1980). Therefore HEPA filters effectively remove virtually all viral-laden particles.

In general, the fragility of retroviruses are believed to limit the hazards of air emission pathways. Aerosols deposit in relatively short distances from point sources. Potential aerosol emissions, if not controlled by a biosafety cabinet, are controlled by splash guards and decontamination of surrounding work surfaces. Autoclave air emissions are not considered significant sources for living bioaerosols because they destroy biological material by using prolonged elevated temperatures and/or pressure treatment of contaminated material. These methods are known to be extremely effective as sterilization techniques.

Most laboratory tissues, fluids, and cultures are treated as infectious waste. These included cultures and other infectious material that are disposed in biohazard-labeled bags, autoclaved and sent to the landfill once they have been sterilized. CRPRC's waste segregation and disposal plan is carried out under the direction of the UC Davis Environmental Health and Safety (EH&S)

Department that also provides oversight for holding areas on the CRPRC property. Key to this program is the training of workers on proper disposal techniques.

Engineering, Administrative and Personal Protective Equipment Controls

Protection from biological agents is provided to CRPRC employees and the community by a variety of engineering, administrative and personal protective equipment controls. Each type of control is aimed at minimizing a potential route of entry by the agent to the worker. Exposure and subsequent infection with a biohazardous agent can occur by several routes: (1) inhalation; (2) ingestion; (3) skin; (4) conjunctival; 5() penetration through unbroken skin; and (6) reproductive. Traditionally, respiratory exposure is thought accountable for about 65 to 75 percent of all infections ("Biohazards: Reference Manual"). The major potential route of exposure for CRPRC research virus, however, is by direct skin penetration because of the inherent characteristics of retroviruses. Concern still exists for aerosol deposition on surfaces or in the nose and throat region with the subsequent ability of the agent to gain access by open cuts or sores. Typically biological agents are thought to have a limited ability to survive outside the body. There is limited information on this subject but research continues as to the actual survival parameters of these agents under varying conditions. Survival of viruses in the environment is dependent on a complex interaction of factors. To provide a greater margin of safety, control measures are based on conservative estimates of maximum periods that viruses can remain infectious (i.e., a worst-case basis). Among the survival factors are ambient temperature and relative humidity, texture and composition of the substrate, exposure to sunlight, presence of other organic material and for aquatic environments, the chemical and physical properties of the water (i.e., pH, dissolved solids, hardness, etc.). Generally under dark, sterile conditions, SIV/HIV are still detected for 1 to 2 days although greatly reduced in magnitude. However, disinfectants inactivate the virus in a matter of minutes.

Engineering Controls

Feasible engineering controls of worker exposures are preferred by Cal-OSHA. Engineering controls provide a degree of containment of biological agents and minimize personnel's contact with the agent. These safety features are "built in" to facility, and equipment design and operation. The most significant engineering control that can be implemented is observation of the correct Biosafety Level (BSL) criteria for laboratory and equipment design. CRPRC utilizes BSL-2 for most of its laboratory and animal handling facility applications. Biosafety Level 2 is suitable for experiments involving agents of moderate potential hazard to personnel and the environment. Laboratory personnel have specific training in handling pathogenic agents and are supervised by competent scientists. Access to the laboratory is limited when experiments are being conducted and procedures involving large volumes or high concentrations of agents or in which aerosols are likely to be created are conducted in biological safety cabinets or other physical containment equipment. BSL 2+ (additional protective clothing and safety procedures) is used for infectious disease rooms and quarantine. Higher levels of biosafety are required for Herpes B only if large quantities are cultured or if animals are infected experimentally. This is not done at the CRPRC.

Other engineering controls that are available at the CRPRC include:

- Biological safety cabinets and a variety of enclosed containers (i.e., for centrifuges) to provide containment of infectious aerosols generated by many microbiological procedures.
- Construction that facilitates cleaning and prevents accumulation of contamination (i.e., prevents crowded workspace areas).
- Separation of administrative, laboratory, animal handling and cage washing facilities in different buildings to prevent adjacent cross-contamination problems.
- Containment of research-infected animals in separate modules with locks and anteroom chambers.
- Access to laboratory and animal care areas that is limited.
- Laboratory doors with viewing ports that are kept closed when experiments are in progress and animal doors that are self-closing with viewing ports to minimize entry.
- Animal facility, cage washing, laboratory, and laboratory fume hood ventilation that is not recirculated to other rooms before being exhausted.
- Mechanical pipetting devices that are used to prevent mouth pipetting.
- Medical care and laboratory devices that are designed to prevent needlesticks and unwarranted cuts or punctures.
- Worker eating/rest areas, handwashing, shower and change area facilities that are clearly defined from work areas.
- Substitution of less hazardous agents in a research experimentation when possible.
- Consideration of animal security and safety provisions in the arrangement of cages in a room.
- Animal security provisions are built into building, cage designs, means for transferring animals between cages and performing medical treatment. All animals that are experimentally infected with retroviruses are housed indoors.
- An insect and rodent control program that is well implemented.
- Splash shields on fume hoods or equipment to prevent or minimize aerosol exposures to workers.

Physical Control of Animals

Physical control of animals applies to keeping monkeys within the enclosures and cages on the operations area. The concern is that monkeys may be released from the operations area by break-outs or intruder break-ins, have the opportunity to travel, interact with, and possibly infect wild or domestic animals or humans in the community. Potential routes of transmission and the agents involved are discussed earlier in this appendix. A summary of these discussions generally indicates that:

- Escaped monkeys, like most escaped animals, tend to remain in close proximity to their enclosures (even indoor cages), as this is where the greatest activity is and their homes are.
- CRPRC's concern for maintaining a healthy animal population against possible incoming diseases serves as an added incentive to minimize any type of disease transmission from its primates.

An extensive security program is in place at the CRPRC operations area to prevent break-outs and break-ins. All facilities are linked to the campus alarm system and the entire operations area is fenced and locked.

Passive locator security measures are also in place to monitor animals. In the outdoor field cages, a census is conducted three times per year. Newborns are counted daily. Routine monitoring and maintenance of enclosures is performed to assure that breaks and tears do not occur in fencing. For indoor animals, census flags are given to all animals inoculated with HIV. All animals inoculated with live virus are tracked on CRPRC's computer report.

General areas on the CRPRC operations areas are labeled to warn trespassers. All entry doors and biohazardous containers are also appropriately labeled as to the type and degree of hazard in accordance with regulatory requirements. Labeling is provided appropriate to the degree of hazard. Warnings are progressively more descriptive as the proximity to the hazard increases. General warnings are appropriate for trespassers. Explicit warnings with necessary precautions on entry doors or containers are appropriate for workers. Overzealous labeling can lead to confusion by workers who rely on this information or become apathetic if they believe the hazard to be everywhere and difficult to control.

Infected animals are maintained in facilities that are locked at all times. Access is given only to designated and trained individuals. These facilities have anterooms to serve as secondary holding areas in case a monkey escapes from a locked cage and the cage room area. Animals are moved between cages using transfer cages or between buildings in accordance with written procedures. Infected animals are typically anesthetized for handling or transport. All doors are self-closing. Doors of interior cage rooms have viewing ports for observation of animal activities. Other hospital and laboratory areas also have locked or restricted entries as well.

Animal control concerns during potential emergency response scenarios are addressed in CRPRC's Emergency Action Plan. These plans address the potential for fire/explosions, earthquakes, personnel accident/injury (minor or severe), bombs or threat of violence, trespassers and animal escape. As with any emergency scenario, the consequences of emergencies are best prevented or minimized with emergency preplanning and drills.

Administrative Controls

Administrative controls provide protection to workers by providing information that can be used to reduce potential exposures. The most beneficial types of administrative controls that minimize exposure are those implemented to comply with Cal-OSHA Injury and Illness Prevention (IIPP) Standard, the Laboratory Safety Standard, and the Bloodborne Pathogens Standard. The elements of the IIPP standard include a written program assigning an individual with responsibility and authority for worker health and safety, a Job Hazard Analysis and Standard Operating Procedure for job tasks, a self-inspection program, a discipline/incentive

program for workers, a means of communicating employee concerns (Cal-OSHA prefers a Safety Committee), worker health and safety training programs and a standard reporting and investigation procedure for follow up of injuries or illnesses that occur in the work place. The elements of a written Laboratory Chemical Hygiene Plan include a person with daily authority for implementing the program, a hazard assessment of laboratory exposure, general safety guidelines for the laboratory, implementation and safety review of standard operating procedures, routine ventilation survey of laboratory hoods and employee training. CRPRC's Exposure Control Plan describes the types of potentially infectious materials that employees may encounter at the facility, the job classification, and tasks. It contains a schedule for implementation. It specifies methods of compliance that are both universal and unique to the facility: engineering controls, work practice controls, personal protective equipment, handling of contaminated equipment and waste, pre- and post-exposure incident procedures, training, and recordkeeping. Other administrative controls that are implemented at the CRPRC include:

- Workers are provided with the Job Hazard Analysis of their work tasks which includes potential worker-related hazards and methods of protection.
- Workers are trained with the Primate Center Occupational Health Guidelines, a Primate Handling Videotape, CRPRC's Exposure Control Plan, Infection Control Plan, Radiation, Safety and Animal Care classes given by UC Davis, Environmental Health and Safety Department.
- Pesticide safety training required for antimicrobials by UC Davis.
- Standard operating procedures that address waste disposal handling of diseased animals; transporting animals; laboratory, surgical and routine medical procedures; reporting spills and accidents; and treating accidental cuts, bites or scratches with a potential for exposure. For example, no animals for infectious disease studies are handled unless sedated. If, by experimental design, sedation is not possible, a restraint chair is used.
- Incoming animals from any other facility are quarantined for observation to identify potential diseases and control their spread.
- A full time safety officer to oversee and implement the CRPRC Safety Program.
- Application and posting of labels and warning signs to warn others of potential risks and methods for protection.
- Close interaction with the campus EH&S to periodically audit work practices, perform monitoring and outline recommended practices.
- CRPRC's medical surveillance of workers includes providing medical preplacement screening or test, and inoculations (such as measles, tetanus, TB, etc.) to prevent the transmission of disease into the facility; and annual serum testing of workers. Worker medical surveillance parallels the animal medical surveillance program, already discussed. These services are conducted in addition to any medical surveillance performed in response to either unexpected human or animal symptomology. Physical plant workers are also required to be TB tested and have their serum drawn prior to working at the Center.

- A written Protective Clothing and Service Reporting Program for physical plant employees that come to perform maintenance on CRPRC facilities. This includes baseline medical screening of workers, a notification of potential hazards, a check-in with the Animal Care Facilities (ACF) Management Office to get information on potential hazards and appropriate protective measures, and a method for logging maintenance activities performed on the operations area.
- A housekeeping program is in place to limit physical clutter, control contamination, and facilitate the efficient use of chemical disinfectants. Housekeeping is a daily routine when working with biohazards. Counter surfaces and work areas are disinfected at regular intervals and at the end of every shift. Laboratories are directed to follow the EHS General Guidelines for Chemical Laboratories.
- Designated work surfaces are disinfected daily after work is performed in a biosafety cabinet, hood or workbench and after each spill of viable material. Shutdown of a laboratory and complete cleaning is typically done when a laboratory is transferred from one investigator to another.
- A well-defined accident/incident reporting and investigation procedure. Any employee injury is reported to ACF Management and evaluated by Employee Health Services within 24 hours. An accident form is completed. Accidents involving an animal-related injury are evaluated for contributory conditions and changes are implemented if necessary. All accidents and injuries are reviewed with the employee by the Safety Officer.

Personal Protective Equipment Controls

Personal protective equipment (PPE) provides worker protection when engineering and administrative controls are not feasible or when work is temporary in nature. PPE includes appropriate gloves, face and eye protection, outer wear, National Institute of Occupational Safety and Health (NIOSH)-approved respirators and other gear necessary to the laboratory or animal handling task. Examples of some of the PPE applications at CRPRC are:

- Designated work uniforms and shoes are worn for work around animals and animal cages. Leather gauntlets are worn if hand capture is necessary. Masks, gloves and eye protection are worn in animal rooms. Long sleeves are worn when handling animals or hosing cages.
- Laboratory coats in designated laboratory areas with upgrade to latex gloves, safety glasses and/or splash shields for certain operations.
- Laboratory clothing, face masks, gloves and booties for entry to the controlled area.
- Double gloving, tyvek gown or coveralls, face mask, head cover, goggles or face shields and booties are worn for work in quarantine or BSL 2+ animal housing.
- Rubber boots, eye protection, masks, gloves, uniform and tyvek sleeves for cage washers.

- Personal protective equipment provided to physical plant workers, appropriate to the job task. Training by CRPRC is given to physical plant workers prior to each job as to appropriate protective measures.

D-4 ANIMAL CARE

The ability of biomedical scientists to enhance the well-being of humans and animals depends directly on advancements made possible by research, much of which requires the use of experimental animals. The scientific community has long recognized both a scientific and an ethical responsibility for the humane care of animals, and all who care for or use animals in research, testing, and education must assume responsibility for their general welfare. It is especially important to recognize that the intent of research is to provide data that will advance knowledge of immediate or potential benefit to both humans and animals. Scientists have developed, and should continue to develop and use, scientifically valid adjunctive or alternative methods to animal experimentation. However, when needed, proper care and humane treatment of animals used in research, testing, and education requires scientific and professional judgement, which is based on knowledge of the husbandry needs of each species and the special requirements of research, testing, and educational programs (NIH 1985).

To encourage optimal care for laboratory animals, various types of accreditation and professional societies have been created to provide a mechanism for peer evaluation of animal care programs by the scientific community. Humane treatment of laboratory animals, protection of personnel from hazards associated with the use of laboratory animals, and control of variables that could affect animal research adversely are among the principles objectives of these programs (AAALAC undated). Nothing in these activities is intended to limit the freedom of scientific inquiry or to limit the investigators' obligation to conduct experiments in accord with humane principles.

Regulatory Setting

Regulators, agencies and professional organizations that guide and determine the appropriateness of animal care at the CRPRC research facility are provided in Table 3.1-5. Most inspections that occur are closely connected to the funding of animal research itself. However, some of the most beneficial guidance can be provided by institutional policies or campus animal care committees that routinely interact with researchers. NIH recommendations for the responsibilities of such a committee include:

- meeting at regular intervals, appropriate to program needs but not less than twice per year;
- ensuring a mechanism exists to review the appropriateness of animal care;
- providing a written report, at least two times annually, to the responsible administrative official on the status of animal care;
- performing other functions to meet the needs of federal, state, and local regulations and policies

The primary source for guidelines used by the agencies is the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Since its inception in 1965, AAALAC has been recognized by the National Institutes of Health (NIH) as a means of complying with NIH policies, contained in the "Guide for the Care and Use of Laboratory Animals." A summary of issues addressed by this guide includes animal care concerns that are described as follows:

- **Laboratory Animal Husbandry.** A good husbandry program provides a system of housing and care that permits animals to grow, mature, reproduce and maintain good health. It also minimizes animal variation to experimentation. It includes a comfortable, escape-proof, dry and clean housing system that takes care of animal biological needs, protects animals from known hazards, provides adequate activity for the species, and avoids unnecessary physical restraint. Housing should meet minimum space, humidity, ventilation, illumination, temperature, and noise recommendations. Animals should receive adequate food, bedding, sanitation, waste disposal and vermin control attention in addition to emergency, weekend and holiday care.
- **Veterinary Care.** Adequate veterinary care consists of observing all animals daily to assess their health and welfare, using appropriate methods to prevent, control, diagnose and treat diseases and injuries, providing guidance to users regarding handling, immobilization, anesthesia, analgesia and euthanasia, and monitoring surgery programs and post surgical care. Adequate identification and records on each animal is paramount to assuring quality care.
- **Physical Plant.** The physical condition and design of animal facilities determines the efficiency and economy of their operators. In addition to meeting applicable state and local building codes, animal facilities should be separate from personnel areas such as offices, conference rooms, and most laboratories. They should provide for special activities, quarantine, animal housing, personal hygiene and hazardous agents or service equipment. Corridors, doors, windows, floors, drains, walls, ceilings, power, storage and sanitizing areas must meet construction guidelines.
- **Personnel Qualifications and Occupational Health.** Adequate number of full-time personnel technically qualified in animal care should be employed on staff. The occupational health guides are as protective to housed animals as to the staff itself.

Existing Animal Care Practices at the CRPRC

The CRPRC facilities house a total of about 3,800 monkeys, 1,800 of which are housed outside for breeding. There are three species maintained onsite: Rhesus Macaques, Long-tailed Macaques, and Titi Monkeys. The CRPRC facilities could house any primate except the Great Apes.

Health care and medical recordkeeping for each primate begins at birth. Because of the additional breeding and reproductive research concerns of the CRPRC, a significant amount of prenatal care and monitoring is provided. The CRPRC tracks the lineage of each population member along with significant family history. Each monkey receives routine medical checkups and is included in a preventative medicine program. Medical treatments and observations are documented historically and maintained in a medical folder for each animal as well as a computerized health record system.

Outdoor facilities consist of the breeding corals and circular outdoor enclosures with a cone-shaped roof (called corncrubs). Animal care and veterinary staff work on a rotating schedule to provide coverage 365 days a year. The entire CRPRC staff is utilized for emergency or urgent care conditions. Security personnel are present during off-hours and have a home-phone list of CRPRC personnel to notify during an emergency. Outdoor housing areas are fenced and covered grounds that provide considerable room for monkey play, socialization, reproduction, climbing, etc.

Indoor facilities where primates are housed include two animal wings, 12 modular buildings, One Butler building, and a quarantine facility. The average number of animals infected or treated with radiation, the types of research conducted and their applicability to human and animal concerns, are described in Appendix Section D-1. Cage size and design specifications, illumination, ventilation, animal handling and testing procedures, transport, restraint (when necessary), disease prevention, and worker protection conditions and operating procedures are described in Appendix Section D-3. These conditions have been audited by and are in compliance with AAALAC, USDA, CDC, and NIH guidelines. Provisions for and maintenance of housing conditions are available in written CRPRC program documents.

An average of 116 animals per year are received by the CRPRC from outside sources. The remainder are bred at the operations area itself. State and Federal regulations require incoming animals to be quarantined for 31 days. All animals are quarantined at the existing quarantine facilities for a period of 90 days before becoming accessible to the CRPRC colony. The quarantine facility meets or exceeds state quarantine regulation requirements for the California State Department of Health Services and CDC. These requirements are specific as to animal holding facility construction, infection control programs, occupational health programs and interim guidelines for transit and quarantine to detect or manage ebola-related filovirus infections.

Animal handling and transport guidelines are outlined in written CRPRC procedures. The objective of these procedures is to minimize animal contact and protect the safety and health of workers by minimizing their contact with the animals as well as to provide humane treatment for animals. Animals in quarantine are not to be handled directly by workers in an active state. They may be treated by remote means, while limited in their movements within their cage or while sedated for more difficult procedures. All infected animals must be sedated for transport between buildings on the operations area. Other animals can be transported within locked cages. CRPRC written procedures detail methods for physical and chemical restraint to maximize employee safety and optimize animal health.

Animal care history at the CRPRC includes some accidents, escapes and incidents of trespassing. Although there are not written records to confirm most escapes, according to Dr. Jeff Roberts, chief veterinarian at the CRPRC, approximately 133 animals may have escaped since CRPRC's inception in 1961, with one animal unaccounted for. Reports of primates usually turn out to be opossums or other wildlife.

The following are the documented escape incidents: (1) On March 9, 1967, when the primates were housed at the Putah Creek facility, records indicate some monkeys escaped to the Milk Farm in Dixon. These monkeys were eventually captured and returned to the center. (2) In September 1977, five tamarins, a small South American monkey, escaped from the South Field Corral but not from the CRPRC. All were recaptured without incident. (3) The only incident in

which an animal remained unaccounted for was when approximately 15 animals escaped in the summer of 1987. When the animals were returned to their cage, a census was taken and there was one animal that was not accounted for. The possibility exists that this animal had been an unreported death prior to the escape and capture.

Escapes on the average are infrequent, less than 3-4 per year. Experience at the CRPRC is that the animals remain around their housing area, even if young animals can escape due to their relatively small size. The majority of the animals were born and raised at the CRPRC and consider the cages their natural territories. They remain in proximity to these cages, where food and water are provided and where their family members are located.

Cage security has improved over the years. In the outdoor corrals, a census is conducted three times a year. Newborns are counted daily. Routine observation and maintenance of fencing minimizes or eliminates breaks that occur from wear or animal activity. The addition of outdoor canopies has also lowered the escape rate.

The last incident of trespass onto the CRPRC was in 1992 when one outdoor cage was clipped on a weekend. Eleven animals got out of their cage and were put back in by handlers. This is the only incident of known vandalism to the CRPRC animal facilities. Security measures include outdoor animal handling staff that patrol field areas during the day and campus police patrol of the grounds at night. Security for animals housed indoor is discussed further in Appendix D-3 and includes locks and alarm systems.

Animal-related emergencies at the CRPRC include a fire, a freezing incident and an alleged filovirus outbreak. A fire broke out in the corncrubs on July 16, 1990 when CRPRC was designing new types of forced air heating systems to provide additional heated space in outdoor enclosures. A sheet of plexiglass adjoined to plywood in the enclosure came into direct contact with the heater and caught fire. Eleven juvenile long tailed macaques were killed either directly by the fire or by smoke inhalation.

On December 19, 1990, a sudden cold snap produced by an Arctic front resulted in the death of four monkeys: two long tailed macaques and two bonnet macaques. The overnight storm brought outside temperatures into the sub-zero range. Animal care staff found the four animals alive but extremely hypothermic in their cages that morning. They were immediately transported to the animal hospital and received emergency treatment, but did not survive. All remaining long tailed macaques were moved inside, as were other aged rhesus macaques. Additional wind protection and heating was provided to the bonnet macaques and to other rhesus housed outdoors. Since these incidents, additional time and research at CRPRC has been devoted toward finding better means of providing warmer conditions to the monkey population when temperature extremes occur.

The alleged filovirus "outbreak" of November 1989 to Spring 1990 is described in detail in Appendix D-2. In brief, CDC recognized a new simian "Ebola-like" filovirus in monkeys quarantined at Reston, Virginia. CDC conducted initial surveys of CRPRC animals and employees that indicated positive for the virus for some primates and employees. However, the test was subsequently discovered to be extremely unreliable and follow-up testing by CDC showed that all of the original "positive" tests were negative. CRPRC has the CDC letter documentation that indicates the false results.

The facility is also completely inspected annually by the UC Davis Fire Department, at a minimum, for adequate fire prevention and suppression systems. There are more frequent checks of alarms and safety facilities. CRPRC management and staff are prepared to mobilize quickly if animal care needs require urgent attention.

