Any product that comes from an animal is considered a biological. Biologicals include cells, tumor lines, serum, viral stocks, ascites fluid, or other animal products. Biologics are a threat to rodent colonies because they potentially carry microbial agents that are unwanted in animal facilities. Unwanted microbial agents that can contaminate biologicals include ectromelia virus, rodent parvovirus, rodent coronavirus, lymphocytic choriomeningitis virus, lactic dehydrogenase virus, hantavirus, reovirus, and Mycoplasma sp. This Bulletin will discuss each of these agents and their consequences on biomedical research.

Ectromelia virus is the cause of mouse pox which is the most devastating viral disease of mice. If we consider the sources of ectromelia virus outbreaks in the United States over the past 30 years, all the have been associated with contaminated biologicals. The most recent outbreak was in 2003 and was associated with contaminated mouse serum from China. An outbreak in the early 1980s cost millions of dollars and resulted in the loss of many valuable mouse strains. Ectromelia is one of the few viral infections that leads to significant morbidity and mortality in addition to alteration of immunological parameters.

Rodent parvovirus is another frequent biological contaminant. Unlike carnivore paroviruses, rodent parvoviruses in general do not cause clinical disease. Rather, they confound experiments by altering cell responses, particularly T-lymphocyte responses, tumor kinetics, and immunologic parameters. When cells are established in culture, rodent paroviruses have the perfect opportunity to multiply and destroy cells. Rodent paroviruses are the bane of laboratory animal veterinarians. Once they are established in a rodent colony, they are difficult to eliminate because they are difficult to detect and are present at low levels.

Rodent coronaviruses such as mouse hepatitis virus and rat coronavirus are also common contaminants of rodent biologicals. These viruses are the most contagious rodent viruses and will quickly spread through rodent colonies if rodents are housed in conventional non-microisolator cages. In addition to causing morbidity and mortality, rodent coronaviruses have numerous effects on experimental parameters including immunosuppression, blood dyscrasias, and increased tumoricidal activity of macrophages.

Lymphocytic choriomeningitis virus (LCMV) is another common contaminant of rodent biologicals. This zoonotic agent can cause flu-like symptoms in people, so it is of major concern for rodent colony managers. LCMV is very unlikely to cause natural disease in rodents, but experimental infections cause choriomeningitis when injected cerebrally into immunocompetent mice and hepatitis if injected parenterally into immunocompetent adult mice. Subclinical persistent viral infection occurs after inoculation of young mice, with wasting syndrome as a long term
consequence. LCMV alters tumor kinetics and immunologic parameters in infected mice.

Lactic dehydrogenase virus (LDHV) is another virus that can cause persistent viral infection and is the most frequent biological contaminant. This virus is a laboratory phenomenon that can only spread by parenteral injection or by oral inoculation. Like LCMV, it is an experimental infection. Unlike LCMV, LDHV infected mice show no clinical signs. The virus is named for its ability to alter the kinetics of lactic dehydrogenase enzyme in blood, resulting in an 8 to 11-fold increase above normal blood values. There is no serologic test for this agent because mice do not produce antibody against the virus. Historically, enzyme levels in blood are used as a measure of viral presence. LDHV alters tumor kinetics and immunologic parameters in infected mice.

The source of an outbreak of hantavirus in Japan, which sickened 126 people and killed one, was due to subclinical hantavirus infection in rats. Human infection was due to improper processing of contaminated rodent tissues. The virus that caused this epidemic belongs to a family of related viruses that are major zoonotic concerns necessitating biological testing. Research personnel have reported illnesses in Belgium, England, and France. Hantaviruses are enzootic in wild populations of rodents and do not cause clinical disease in the natural host. There have been no reports of the effects of these agents on experimental data.

Reoviruses can also contaminate biologicals. These viruses are fairly ubiquitous and can infect multiple species. Reoviruses are not significant pathogens in contemporary mouse colonies. They have been reported to interfere with ascites tumor studies, enhance tumor specific immunity, and reduce pulmonary clearance of Staphylococcus aureus.

Mycoplasmal biological contamination can be from human or animal source, usually from contaminated culture material. Mycoplasma contamination confounds research results by altering lung function and immunologic parameters of infected rodents.

In order to prevent contamination of UIC rodent colonies, it is necessary to screen biologicals for pathogens. In addition, Food and Drug Administration guidelines dictate that monoclonal antibodies and other biological products must be demonstrated to be free of viral contamination before their use for therapeutic or diagnostic purposes. In order to protect UIC rodent colonies, question 9,b Form A in the ACC protocol asks whether biologicals will be used in rodents, and whether the veterinary staff has been contacted to arrange testing of the biological.

There are two methods to test for microbial contamination of rodent biologicals, antibody production tests and PCR tests. MAP, RAP, and HAP are acronyms that describe the antibody production (AP) test method. The M, R, and H stand for mouse, rat, and hamster, respectively. These tests involve the exposure of immune competent animals to the biological in question via various routes that include intraperitoneal, oral, and intranasal administration. There are two major concerns with these tests. The first is that the biological may not contain an infectious dose and could yield a false negative result. The second concern is that viral infection could cause the rodent to become immunoincompetent and thus
be incapable of seroconversion to the contaminating agents. Typically five animals are inoculated with 0.5 ml intraperitoneally, 0.05 ml intranasally, and 0.05 ml orally, and another five animals are inoculated by the same routes with a 10-fold dilution of the test material. Antibody production tests typically involve inoculating an animal with the test product, measuring LDH levels in the blood four days post-inoculation, and measuring antibody titer to agents of interest 28 days after inoculation. The antibody production test takes longer than PCR methodology, but is less costly.

PCR test methods require $10^7$ cells, 30 mg of tissue, or 400 ml of serum or ascites fluid. This method is preferable if the quantity of biological is limited. It is a highly sensitive test and is able to detect as little as two copies of nucleic acid in a sample. The TaqMan PCR technique (real time PCR) utilizes a fluorogenic 5'-nuclease assay, which has the advantage over conventional PCR testing in being more sensitive and thus is a preferred method. In addition, PCR screening eliminates the need to use animals to screen for murine viruses in biological material.

Both antibody production tests and PCR tests can have false positive and false negative results. Both tests may give false results if the contaminating viruses are divergent or newly emerging strains. Commercial laboratories have extensive experience in serologic and PCR testing for rodent infectious agents and have developed highly accurate tests. We recommend taking advantage of these services.

Another practical matter to discuss is how biologicals become contaminated in the first place. Most contaminated biologicals are derived from contaminated rodent sources.

The incidence of reported contaminated biologicals has gone from 70% in the early 1970’s to less than 10% today. This decrease in contamination prevalence coincides with the reduced rate of viral infections in animal colonies which is a result of stricter biosecurity measures throughout the laboratory animal field. However, contamination can occur in the laboratory through sloppy sterile technique at the bench, during storage, or during shipment. Prior to opening vials of biologicals, the exterior of the vial should be thoroughly cleaned and disinfected. Passage of cell lines in non-permissive hosts will eliminate viral contaminants and has been used as a method to clean cell lines. Typically a mouse biological will be administered to an immunoincompetent rat such as a nude rat, or a rat, hamster, or guinea pig biological will be administered to an immunoincompetent mouse such as a nude mouse. The recipient species is not permissive to viral growth and contaminating agents will be eliminated.

In summary, all biological materials need to be tested for infectious agents if they are from an unknown source. If they are derived from a known source, please consult with the veterinary staff about the need for testing.

ANNOUNCEMENTS