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Expression of Recombinant Proteins from Rodent Infectious Agents for use in the Enzyme-Linked Immunosorbent Assay (ELISA)

Health surveillance is an important part of a laboratory animal quality control program. This includes gross and histopathology, parasitology, bacteriology, serology, and more recently, molecular diagnostics.

Serology is used to detect serum antibodies to a defined list of infectious agents, principally viruses, that animal breeders and researchers wish to exclude from their breeding colonies or animal research facilities. The presence of antibodies in an animal's serum indicates that the animal has been infected with the agent. The sera from representative animals or sentinel animals are routinely monitored by the ELISA to detect conversion from sero-negative to sero-positive. To detect antibodies against an infectious agent, a protein must be present in the well, which contains epitopes that are antigenic (elicits production of antibodies) within a host.

WE ARE PLEASED to announce that on April 1, 1998, rodent parvovirus ELISA plates will finally be available! This assay utilizes baculovirus-expressed, recombinant non-structural protein as the antigen. This protein (NS-1) is highly conserved among rodent parvoviruses, and the assay can detect MVM and MPV in mice and KRV, H-1 and RPV in rats. The use of histidine tag and nickel-coated microplates enhances the sensitivity and specificity of the assay.

Traditional Production Methods

Traditional production of antigen has been accomplished by culturing, preparing and purifying the infectious agent. The disadvantages of this method include working with zoonotic agents, multiple cell lines or growth conditions, and multiple methods of purification. Background may be highly variable from batch to batch and is minimized by further treatment or purification of the antigen.

Molecular Production Methods

Current molecular techniques permit the cloning of specific DNA sequences into vectors for expression in bacteria, yeast and eukaryotic cell lines. Viral or bacterial genes expressing an immuno-dominant protein can be cloned and regardless of the source of the gene, the same or similar methods for protein expression and purification can be used.

Viral Vector Production Methods

CRL is currently using an expression system that utilizes an insect virus called baculovirus. A recombinant gene can be cloned into a transfer vector DNA to make a recombinant virus that produces significant quantities of the expressed protein.

Our goal is to use this system to create a library of baculoviruses that can be used to express immuno-dominant antigens for all of the agents that we are testing for by the ELISA. All of these antigens can be produced in the same cell line and the background in most cases is lower than traditionally produced antigens. We are now adding histidine tags to the recombinant proteins, which can assist in purification of the protein via metal chelating chromatography. More recently we have investigated a way of bypassing column purification by adding cell lysates from infected cultures directly to ELISA plates that are nickel coated.

Infectious Agents

There are several infectious agents of concern for the research community working with laboratory animals. As studies and long-term projects rely on healthy animal models for meaningful data, it is important to routinely screen for the presence of infectious agents. Early detection of accidental infections is important to avoid wasting time, money and animals.

Charles River's laboratory animal customers have their own health surveillance programs; some clients decide to submit samples to our laboratories for Comprehensive Health Monitoring, Serology or MAP/RAP/HAP testing. Others have elected to perform the work in their laboratories using our serology reagents or the Murine Immunocomb™.

In this and future issues of The Sentinel, we will provide information about the infectious agents Charles River tests for. In this issue we discuss the important zoonotic agent lymphocytic choriomeningitis virus (LCMV).

Lymphocytic Choriomeningitis Virus

Agent: Enveloped RNA virus, family Arenaviridae, genus *Arenavirus*

Characteristics of Infection: Wild mice are the principal reservoir hosts, but laboratory animals and humans are susceptible to the virus. Rodent infections are typically asymptomatic, but disease signs vary depending upon the virus strain, the mouse strain and the age of mouse at the time of infection. Only mice and hamsters readily transmit LCMV to other species, shedding virus in the urine, saliva and milk. In mouse colonies, transmission is initially horizontal, but once infection is enzootic, the intrauterine route is used exclusively, and with near total efficiency.

Public Health and Research Implications : LCMV infection can cause serious and rarely, fatal disease in people. LCMV has been found to contaminate biologic materials, including transplantable tumors; cell lines; virus stocks including leukemia, distemper, rabies and mouse poliomyelitis virus.

Diagnosis: As natural rodent infections are generally asymptomatic and human infections result in nonspecific clinical signs, they are best diagnosed by virus isolation/ detection and serology. Specimens for virus isolation include blood, body fluids and tissues, especially kidney and urine when testing hamsters. Virus isolation should be performed on colonies that are seldom monitored and may be enzootically infected.

Serology is most effective for routine monitoring of rodents and the diagnosis of LCMV in humans. Methods include complement fixation (CF), neutralization test (NT), indirect immunofluorescence assay (IFA), radioimmunoassay (RIA), and ELISA . ELISA and IFA have replaced the less sensitive CF and NT. RIA is the most sensitive, but not routine.

Eradication: The entire stock of animals should be destroyed and incinerated; cages and other equipment exposed to the animals should be autoclaved; the animal room should be fumigated with formalin or paraformaldehyde and allowed to remain vacant for 7-10 days.

If you would like further information about laboratory animal Health Monitoring programs, please call Charles River's Technical Assistance Department at 800-338-9680.