

An Overview of Diagnostic Tests for MHV Detection

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The term MHV refers to a spectrum of enveloped, ssRNA Coronaviruses that manifest substantial variation in their tissue tropism and pathogenicity. Polytypic biotypes of MHV express a diversity of tissue tropisms and can disseminate from the initial site of replication in the respiratory epithelium to neural, hepatic, vascular, and lymphatic tissues. Polytypic MHV viruses are also more likely to cause more severe disease with clinical signs of illness. Enterotropic strains of MHV are generally limited to replication within the intestinal tract. The course of enterotropic MHV infection is often mild, usually without clinical signs of disease. The exception is epizootic outbreaks in naïve colonies in which highly susceptible neonatal and infant mice can become diarrheic and die from infection. Enterotropic strains of MHV can infect naïve animals of any age (Barthold et al, 1993). Once the virus establishes an enzootic infection cycle in a colony, susceptibility is generally limited to immunocompromised mice and newly introduced naïve animals (Homberger, 1997). Enterotropic biotypes of MHV are considered to be more contagious than the polytypic biotypes and are now the most prevalent MHV strains associated with infections in laboratory mouse colonies (Homberger and Barthold, 1995).

As mentioned above, the enterotropic biotypes of MHV generally do not cause clinical signs of illness in mice except in naïve mouse colonies. Therefore, you cannot rely on finding sick mice as an indication of MHV-induced disease. Furthermore, once the enterotropic viruses becomes enzootic within a mouse colony,

infant mice no longer manifest signs of illness since they are protected from infection by maternally derived antibodies (Homberger and Barthold, 1992). The best way to detect the presence of enterotropic MHV biotypes is to include an appropriate and sensitive screening test in your routine health surveillance program.

Diagnostic Tests for MHV Detection

There are several options with regard to screening mice for detection of MHV. Serologic tests are the most popular screening tools for MHV in use today and represent the best option for detecting the presence of the virus in large animal colonies. The enzyme-linked immunosorbant assays (ELISA) and Indirect Immunofluorescent Assays (IFA) are the most common serologic methods used. An additional test, the Immunocomb, offered by Charles River Laboratories is another enzyme immunoassay used to detect antibodies to MHV, however, unlike ELISAs it is performed as a solid phase, not liquid phase assay. Western blots offer a highly sensitive and specific method to demonstrate the presence of antibodies to MHV; however, the methodology does not lend itself to use as a screening tool and is more useful for research.

Currently, the ELISA is the preferred test to screen for MHV because of its high sensitivity (a measure of the test's inherent ability to detect positive serum samples from a truly infected population), high sample capacity, ease of use, and rapid turnaround time. The IFA is mostly used as a confirmatory test, rather than a screening test, because of its high specificity (a measure of the inherent ability of the test to detect negative samples from a truly negative population), limited sample capacity, and requirement for observation under a fluorescent microscope. It is important to point out here

that most commercial serologic assays for MHV employ antigens from polytropic biotypes such as the A59 strain, however, there is sufficient immunologic cross-reactivity between the polytropic and enterotropic biotypes that these antigens will bind antibodies to either biotype (Homberger, 1997). When a serum sample from a mouse is found to be positive for the presence of antibodies to MHV by a serologic test, the result should be confirmed prior to taking an action step within the mouse colony. An ELISA result can be confirmed by IFA, however, additional confirmatory tests should also be performed so that there is little doubt that the diagnosis is correct. Moreover, care should be taken in the interpretation of serologic tests for MHV, in that non-specific reactions can occur in which antibodies bind to both the virus antigen containing well and the tissue culture lysate control well. In these cases tests must be repeated and the results confirmed by a second test method.

Molecular testing methods such as the reverse transcriptase PCR (RT-PCR) provide very sensitive tests that can be used as a screening tools, or confirmatory tests. Either way, the utility of the RT-PCR entirely depends on the animal being actively infected at the time of sampling. Therefore, in mouse colonies that have had positive ELISA results for MHV, it would be best to use the RT-PCR assay as a confirmatory test on very young mice, immunocompromised mice, or recently introduced, naïve adult animals, as these have the greatest likelihood of being actively infected. It is noteworthy to state here that there has been no known attempts made to standardize the laboratory methodology among the many laboratories that utilize RT-PCR for the purpose of diagnosing MHV infections of mice. For example, the sequence and sensitivity of the primers, and the protocols for thermal cycling and gel analysis of the PCR product

may substantially differ between laboratories. This is important when you are sending tissue samples out for diagnostic evaluation by RT-PCR. You need to inquire from each laboratory about the reliability of their test and consider sending the sample to more than one laboratory to confirm the results.

Despite the limitations of the current use of RT-PCR for MHV detection, the future looks bright for molecular detection methods for MHV. These tests are very sensitive, capable of detecting minute quantities of RNA, and additionally, they offer the opportunity to detect an infection before mice demonstrate evidence of seroconversion to the virus (Compton and Jacoby, 1998).

Histopathology is another diagnostic tool that is very useful for initial diagnosis or confirmation of an MHV infection. Lesions that are indicative of active enterotropic MHV infection such as syncytia and giant cell formation in the mucosal epithelium of the intestines should be corroborated to by evidence of the virus within the tissues. Immunocytochemical tests to detect viral antigens in the histopathologic lesions, or *in situ* hybridization to identify viral RNA in these lesions would confirm a diagnosis of MHV. It should be pointed out, that finding syncytia in the intestinal mucosa of a mouse that has antibodies to MHV is strongly suggestive of an MHV infection, however, it is not definitive proof that the two findings are related. Therefore, you must make a judgement about whether you want additional testing done on your mice, and what the testing method will be. The major drawbacks of histopathology as a means of screening for MHV infections in mice, is that it is a very time consuming process, requires high levels of technical expertise, as well as the expertise of a board certified veterinary pathologist. Nonetheless,

histopathology is an excellent means of confirming suspected cases of MHV in a mouse colony.

Electron Microscopy (EM), like histopathology, requires tissues, fecal samples, or cell culture samples in which to look for evidence of MHV infection. EM can detect single virions within a sample, but the virions must be specifically identified to species using additional techniques such as immunoelectron microscopy using labeled (usually gold) anti-MHV antibodies. These antibodies bind to the virus and are also electron dense, thus they will specifically label the virus, and also enhance its visibility in the electron micrograph. EM, because of its technical sophistication and the time required to process samples is best suited as a method for confirmation of suspected cases of MHV infection.

The methods that you chose to initially test your mouse colonies for the presence of MHV should be sensitive, reliable and cost effective, and most importantly, appropriate for your individual situation. Be certain that you are comfortable with the interpretation of preliminary tests that indicate the possibility that your colony is infected with MHV. If you are not, retest the samples at more than one outside laboratory. Always get confirmatory results using a second testing methodology. The cost in dollars and time that it takes to get reliable confirmatory test results is well worth it if you have a high level of confidence in the results. Ultimately, these results will be used to make some important decisions concerning the welfare of the animals in your charge.

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