

Murine Norovirus

1. What is murine norovirus (MNV)?

MNV is a recently discovered non-enveloped RNA virus, although similar viruses are known in many mammals. In fact, other noroviruses cause most of the non-bacterial food-borne gastroenteritis in humans. For example, the human norovirus, Norwalk Virus, has caused the famous recent outbreaks of disease on cruise ships. Noroviruses are not known to cross species, though, and there is no indication that noroviruses native to mice can infect humans.

The first scientific publication on murine norovirus was, Karst et al., STAT1-Dependent Innate Immunity to a Norwalk-Like Virus, *Science* 2003 299: 1575-1578. The major distinction of MNV-1, aside from being the first norovirus found in mice, is that it is the only one which can be grown in tissue culture.

2. Is MNV a pathogen?

No complete answer to this question is available. There is no evidence that MNV is a pathogen except in a few strains deficient in innate immunity. Current evidence indicates that in immunocompetent mice, MNV causes persistent infection, with no clinical signs or other evidence of pathogenicity. Even mice deficient in acquired immunity, including athymic nude mice, scid mice and RAG1 $-/-$ mice, also have persistent infection without any clinical disease. However, high mortality was observed in double knockout mice with severe deficiencies in both acquired (RAG1 $-/-$) and innate immunity (STAT1 $-/-$, or IFN $\alpha\beta\gamma$ R $-/-$).

3. Can MNV interfere with my research?

There is no direct evidence that subclinical MNV infection causes any interference with research. However, the virus replicates in cells of the macrophage

line, and host resistance to MNV apparently requires activity of interferon-dependent systems. These observations leave open the possibility that MNV could impact research into macrophage function or interferon-dependent pathways.

4. What is Charles River Laboratories doing?

Charles River's Serology Department has developed highly sensitive and specific recombinant Multiplexed Fluorometric ImmunoAssay™ (MFIA™) and enzyme-linked immunosorbent assay (ELISA) tests that are used as primary testing methods for MNV detection, and we've developed an indirect fluorescent antibody (IFA) assay that is used to confirm equivocal or positive results. These robust assays detect all known strains of MNV and are fully validated to Charles River's standards. Because our results, as well as the results of other rodent diagnostic laboratories, indicate that MNV is the most prevalent virus of contemporary laboratory mice, our MNV MFIA™ assay has been included in all standard mouse serology panels.

Charles River's Molecular Diagnostics Department has partial or complete genomic sequences from over 40 different genetic variants of MNV found in both North America and Europe. This extensive sequence information was used to develop a very sensitive and robust TaqMan® PCR assay that detects all known strains of MNV. In fact, the MNV PCR assay is so sensitive that we recommend pooling mouse fecal samples up to 10:1 for routine screening. Infection studies performed using field isolates of MNV in immunocompetent mice at Charles River indicate that MNV was actively shed for the 3 month duration of the study. Additional studies to further explore and understand the pathogenesis of MNV are underway.

5. Are VAF® mice purchased from Charles River free of MNV?

Yes. Charles River has tested all colonies extensively and repeatedly by PCR, and has additionally screened many hundreds of serologic samples by MFIA™, both during assay development and prior to commercial launch of our assay. All Charles River VAF® colonies are free of MNV. Furthermore, MNV is included on the VAF® exclusion list, meaning that if MNV infection were ever confirmed in a colony, the colony would immediately be terminated and customers would be notified, as is our policy for other viruses. We feel that our MNV-free status is due in large part to Charles River's advanced biosecurity and our policy of starting new colonies from rederived animals, housed in isolators prior to stocking new barrier rooms. These

policies provide customers with a high degree of protection from novel agents such as MNV, as well as from the next, as-yet-undiscovered, virus.

6. What should the investigator or colony manager do?

The high seroprevalence we observed in commercial samples submitted from outside of Charles River, as well as the high prevalence reported by other diagnostic laboratories, indicates that MNV is the most prevalent virus infection in contemporary laboratory mouse colonies. However, several pieces of evidence suggest that MNV has been present in mice for many years, including: the lack of visible effects suggesting that MNV has become well adapted to its mouse host, the high prevalence and widespread geographic distribution of the virus, and the existence (evolution) of many strains of MNV in mice.

Consequently, there is no need to act with great urgency. Remember, the development of a new assay does not change the health status of mice in your colonies from last week, last month, or last year.

It is appropriate, however, to develop a testing strategy for MNV, which Charles River Laboratories Technical Services (1.800.338.9680 or comments@crl.com) will be happy to discuss with you.