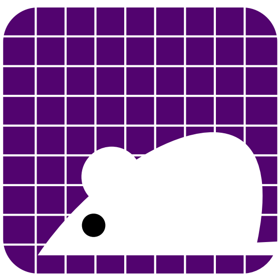


THE sentinel

The sensitivity and specificity of our state-of-the-art molecular methods, combined with fast turnaround time and Charles River's industry leading service, all add up to make our molecular diagnostic services an important part of your comprehensive health monitoring program.

Molecular Diagnostic Testing for Rodent Pathogens

Dramatic advances in molecular biology have allowed for the creation of new, more sensitive tests for the detection of rodent pathogens in clinical specimens. Polymerase Chain Reaction (PCR) is currently the best developed and most widely used of the molecular-based assays. Since 1995, Charles River Laboratories has commercially offered PCR testing for the detection of rodent pathogens because of its speed, sensitivity and specificity. Meticulously designed and continuously refined, our assays provide the most accurate and comprehensive molecular-based testing available in the marketplace today.

PCR- An Overview

In a typical PCR, two primers are designed to bind in opposite directions to complementary strands of infectious agent DNA. The sequence between the two primer-binding sites (less than 500 base pairs) is amplified exponentially with each PCR cycle, which consists of three steps. In the first step, nucleic acid isolated from the clinical specimen is denatured at a high temperature (e.g., 95°C). In the second step, primers anneal to their complementary amplification target sequences at a lower temperature (e.g., 55°C), but not so low as to permit non-specific binding to other sequence locations. In the final step, the reaction tube is heated to temperatures (e.g., 72° C) optimal for DNA polymerases, which synthesize copies of the target sequences by extending the primers. A PCR assay consists of 30-50 cycles, each lasting little more than a minute, that are performed automatically by a programmable heating block called a thermocycler. Each cycle generates copies of the target sequence exponentially, resulting in detectable levels of transcript.

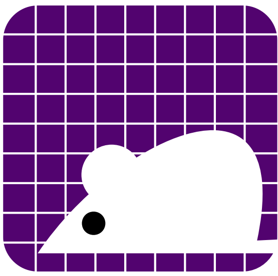
Fluorescent-Based PCR Detection

Charles River Laboratories offers a new, more advanced diagnostic PCR assay utilizing fluorescent probe technology. This system combines the sensitivity of PCR with the specificity of probe hybridization. An oligonucleotide probe, labeled with both a reporter and quencher dye, anneals specifically to template DNA between the forward and reverse PCR primers. During the extension phase of the PCR cycle, the annealed probe is separated from the quencher dye generating a sequence-specific signal. The sequence specific signal is read with a flourometer immediately after completion of amplification. Post-PCR processing is performed without opening the reaction tube eliminating the release of contaminating PCR amplicons into the test environment. The probe adds a second level of specificity to the assay, eliminating false positives based on non-specific PCR products. Charles River Laboratories utilizes this fluorescent-based PCR-probe technology for the development of all viral and bacterial PCR assays.

Value-Added Testing Controls

Amplification inhibitors are a major problem in diagnostic PCR assays, often giving rise to false negative results. We have seen evidence of PCR inhibition with Helicobacter spp. test articles derived from the rodent intestinal tract (fecal pellet, cecum and colon). In-house testing revealed that PCR inhibitor activity is concentration dependent and by serially diluting our test articles we are able to restore template amplification. Although, inhibition is not a new phenomenon, controls detecting inhibition were not routinely performed as part of the infectious disease-PCR process. In an effort to recognize inhibition and restore amplification, we have implemented sample and system suitability controls for all diagnostic PCR testing. Based on our findings, we modified our DNA extraction procedure by reducing the sample amount by ¼ and increasing the DNA resuspension volume by 2000%. Experiments using mice that were positive for H. spp. indicate assay sensitivity is not compromised by the extraction modifications.

CHARLES RIVER LABORATORIES' DIAGNOSTIC PRODUCTS & SERVICES NEWSLETTER
FALL 2000



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Inquires may be addressed
to Lisa White, Editor

Spiked-well control samples are included for each test article to demonstrate amplification competence. The spike (target nucleic acid) should amplify if PCR inhibitors are not active in the test article. Our spiked-well controls allow us to rule out the possibility of false negative results due to amplification inhibition. Also, mock-spiked wells containing non-target nucleic acid are utilized to detect any false positive results due to assay contamination.

Testing Services

Let Charles River Laboratories bring your testing to the next level. We offer PCR combined with fluorogenic probe technology for the detection of rodent viruses and bacteria. Fluorogenic PCR panels are a quick and relatively inexpensive alternative to MAP testing of biologics. Our panels are composed of problematic laboratory animal pathogens, including zoonotic agents. The Essential Panel will be available in January 2001 while the Comprehensive Panel will roll out later that year. PCR testing for all agents in the Essential Panel are available NOW on an individual assay basis.

VIRUSES

*Viral Agents**	*Essential*	*Comprehensive*
MHV	X	X
MVM	X	X
MPV	X	X
LDV	X	X
LCMV	X	X
EDIM	X	X
TMEV	X	X
ECTRO	X	X
REO-1	X	X
REO-3	X	X
HTN	X	X
SEO	X	X
POLY		X
SEND		X
PVM		X
K Virus		X
MCMV		X
MTLV		X
MAV		X

**A non-fluorogenic PCR assay for Mycoplasma can be added by request*

BACTERIA

*Helicobacter**	*Service 1*	*Service 2*
H. species	X	X
H. hepaticus	X	X
H. bilis		X

**We also maintain the capability to perform microaerophillic culture methods for confirmatory testing*

OTHER

Corynebacterium bovis
Mycoplasma species
(non-fluorogenic assay)

For pricing and sample submission information please call our Technical Assistance Department at 1-800-338-9680.