

ROUTINE HEALTH MONITORING OF CHARLES RIVER BARRIER PRODUCTION COLONIES OF RABBITS IN NORTH AMERICA AND EUROPE

This letter describes health monitoring program for rabbits raised in Charles River barrier production colonies in North America and Europe. The terms, VAF/Plus[®] and SPF, are used synonymously in North America and Europe, respectively, to refer to the list of pathogens for which these rabbits are routinely monitored and of which they are found free. The definition of these terms is discussed further below.

Every 13 weeks (quarterly), we evaluate 8 animals, 4 rabbits 3 - 5 months old and 4 at least 6 months of age (usually ex-breeders) from each production colony by a comprehensive health monitoring program which includes bacteriology, parasitology and gross pathology, as well as serology (Assessment Profile). Rabbit colonies are also screened quarterly for all *Helicobacter* species infection by polymerase chain reaction (PCR) methods. In addition, each colony is tested annually by PCR for an extensive list of bacteria and some parasites and fungi, using the Charles River PRIA PCR panel.

Monthly, between quarterly submissions of live animals, we screen the colonies by serology and parasitology. Serum samples from 8 rabbits are screened by the Tracking Profile. Interim parasitology samples (fecal pellets from 8 rabbits) are processed by density separation and concentration methods, and are examined for the presence of helminth eggs, as well as for any protozoal trophozoites or cysts, including all *Eimeria* spp.

We report the health status of our rabbit barrier production colonies through our website, www.criver.com, where colony health status reports are continuously updated as new results become available. These health reports include not only the pathogens that comprise the VAF/Plus[®] and SPF profiles, but also certain other agents not considered of colony health significance, but about which we occasionally receive inquiries. The VAF/Plus[®] and SPF profiles are identical and include those agents for which a colony would be terminated. It is the absence of those agents that defines the animals as VAF/Plus[®] or SPF. In addition to the agents on the VAF/Plus[®] and SPF profile, Charles River provides the status of our colonies with regard to additional organisms which do not pose a threat to the health or general research usefulness of the animals. On the website, agents are separated into several categories: those monitored by serology, those monitored by bacteriology (culture and/or PCR), and those monitored by parasitology. Within these categories, attention should be paid to the letters in brackets following the name of the agent; these letters indicate which actions Charles River would take if the agent were detected and also the frequency of monitoring for that agent.

Included below is a Rabbit Health Monitoring Summary which generally describes the methods used to monitor Charles River production colonies. Colonies are screened for the listed microorganisms and parasites by a combination of serological, bacteriological, parasitological and pathological procedures. In rabbits, Charles River uses the sensitive Multiplexed Fluorometric Immunoassay[™] (MFIA[™]) as a primary test for detecting serum antibodies to most agents. Charles River Laboratories also maintains the capability of performing alternate procedures to enable confirmatory testing in order to corroborate unexpected positive results.

The described health monitoring procedures and results are regularly reviewed by our professional staff, and revised as needed to ensure that Charles River colonies are evaluated in a scientifically rigorous, valid and cost-effective manner. It remains Charles River's policy to inform our customers of any breaches in health or genetic integrity of our animals, and in this regard, we have developed an email notification system as well as other means of communication to provide this important health care information to our customers in a timely manner. You should direct your inquiries or requests to your local Charles River Technical Services office or by e-mail to comments@crl.com for any assistance required in gaining more specific information on our monitoring procedures, for additional data on our animals or for assistance in interpreting the monitoring information. Please also request that your name be added to our professional contacts list if you want to receive additional health monitoring information and other information important in making health care decisions.

LABORATORY RABBIT HEALTH MONITORING SUMMARY

SEROLOGY AGENT	FREQUENCY weeks	METHOD	
		PRIMARY	ALTERNATE
Cilia-Associated respiratory bacillus (CARB)	4	MFIA	IFA, PCR
<i>Encephalitozoon cuniculi</i> (ECUN)	4	MFIA	IFA, PCR
<i>Treponema cuniculi</i> (TREP)	4	RPR	None
Parainfluenzavirus 1 (PIV-1, SEND)	13	MFIA	IFA, PCR
Parainfluenzavirus 2 (PIV-2)	13	MFIA	IFA, PCR
Reovirus type 3 (REO)	13	MFIA	IFA, PCR
Rotavirus (ROTA)	13	MFIA	IFA, PCR
<i>Clostridium piliforme</i> (CPIL)	13	MFIA	IFA, PCR, Histopathology
Lymphocytic choriomeningitis virus (LCMV)	13	MFIA	IFA, PCR
<i>Toxoplasma gondii</i>	52	MFIA	IFA, PCR
Rabbit Hemorrhagic Disease Virus	4, 52 ¹	ELISA	None

METHODS: MFIA = Multiplexed Fluorometric Immunoassay
RPR = Rapid Plasma Reagin
IFA = Indirect Fluorescent Antibody test
PCR = Polymerase Chain Reaction

PARASITOLOGY (Monthly)

Some of the agents for which we screen are listed in the attached table.

Screening procedures are also capable of detecting a wide variety of nonpathogenic and opportunistic organisms whose presence is recorded. This information is available upon request. Some of the agents are listed on our standard health reports while findings on other less common agents can be obtained upon request.

GROSS PATHOLOGY (Every 13 weeks)

The major internal organs are examined grossly during necropsies performed as a part of the comprehensive HM program. If gross lesions are present, affected organs/tissues are histologically evaluated.

¹ Screened monthly in Europe and annually in North America

Charles River Prevalent Rodent Infectious Agents (PRIA) PCR Panel

(Conducted annually on all Charles River rabbit colonies as an additional screen for bacteria, fungi and protozoa)

PARASITES

Pinworms (*Aspiculuris* and *Syphacia*)

Cryptosporidium

Giardia (*G. lamblia* and *G. muris*)

VIRUSES

Mouse adenovirus (MAV-1 & MAV-2)

Mouse hepatitis virus (MHV)

Mouse parvoviruses* (MPV-1, MPV-2, MPV-3, MPV-4, MVM)

Mouse rotavirus (MRV/EDIM)

Mouse norovirus (MNV)

Rat coronavirus (RCV, SDAV)

Rat parvoviruses* (RPV, KRV, RMV, H-1)

Rat theilovirus (Theiler's-like virus of rats [RTV])

Theiler's murine encephalomyelitis virus (TMEV [GDVII])

BACTERIA / FUNGI

Bordetella bronchiseptica

Campylobacter spp.

Cilia-associated respiratory bacillus (CAR Bacillus)

Citrobacter rodentium

Clostridium piliforme

Corynebacterium bovis

Corynebacterium kutscheri

*Helicobacter** (Genus, *H. hepaticus*, *H. bilis*)

Klebsiella oxytoca

Klebsiella pneumoniae

Mycoplasma pulmonis

Pasteurella pneumotropica (Jawetz and Heyl biotypes)

Pneumocystis spp.

Pseudomonas aeruginosa

Salmonella spp.

Staphylococcus aureus

Staphylococcus xylosum

Shigella spp.

Streptobacillus moniliformis

Streptococcus (Beta-hemolytic; Group B)

Streptococcus (Beta-hemolytic; Group C)

Streptococcus (Beta-hemolytic; Group G)

Streptococcus pneumoniae

CONTROLS

Positive Template Control

Negative Template Control

Spike Control

Nucleic Acid Recovery Control (NARC)

*Strain determination assays are performed on all positive results.

LABORATORY RABBIT HEALTH MONITORING SUMMARY

MICROBIOLOGY AND PARASITOLOGY²

	FREQUENCY	TOPOGRAPHY	AGENTS
Microbiology	13 weeks	Respiratory Tract (nasal aspirate and bronchial wash)	<i>Bordetella bronchiseptica</i> , beta hemolytic <i>Streptococcus</i> spp. (Serologic Groups B, G, and other), <i>Streptococcus pneumoniae</i> , <i>Pasteurella multocida</i> , <i>P. pneumotropica</i> , other <i>Pasteurella</i> spp., <i>Klebsiella pneumoniae</i> , <i>K. oxytoca</i> , <i>Pseudomonas aeruginosa</i> , other <i>Pseudomonas</i> spp., <i>Staphylococcus aureus</i> .
	13 weeks	Gastrointestinal Tract	<i>Salmonella</i> spp., <i>Pseudomonas aeruginosa</i> , all other <i>Pseudomonas</i> spp.
	13 weeks	Fecal Pellets	<i>Helicobacter hepaticus</i> , <i>Helicobacter bilis</i> , all other <i>Helicobacter</i> spp.
Parasitology	Monthly	Gastrointestinal Tract Metazoan Endoparasites	All helminths, including but not limited to: <i>Passalurus ambiguus</i> , <i>Obeliscooides cuniculi</i> , trichostrongyles, cestodes and cestode larvae.
	13 weeks	Skin/Pelage and ears: Ectoparasites	All ectoparasites, including but not limited to: <i>Psoroptes cuniculi</i> , <i>Cheyletiella parasitovorax</i> , <i>Listrophorus gibbus</i> , <i>Sarcoptes scabiei</i> , <i>Notoedres cati</i> , trombiculid mites, ticks, lice, fleas.
	Monthly	Gastrointestinal Tract Protozoan Endoparasites	All GI protozoa, including but not limited to: <i>Eimeria stiedae</i> , other <i>Eimeria</i> spp., all Trichomonads (<i>Trichomonadaceae</i>), <i>Giardia</i> spp., <i>Hexamastix</i> spp., <i>Entamoeba</i> spp., <i>Enteromonas</i> spp.

² Does not include the additional annual PCR screening using the PRIA panel above.