

ROUTINE HEALTH MONITORING OF ISOLATOR-REARED IMMUNODEFICIENT RATS AND MICE

This letter is to familiarize you with the isolator-based production and health surveillance of immunodeficient mice and rats for Charles River Research Models and Services North American operations. Charles River produces these animals in flexible-film and semi-rigid isolators, which provide the high-level biosecurity environment required for animals lacking normal host-defense mechanisms. In particular, isolators are used to limit exposure to opportunistic bacteria, including bacteria commonly found in or on humans, as some of these organisms could pose unwanted variables in certain research studies.

Charles River currently maintains well over 1,000 isolators at multiple locations across North America to produce immunodeficient mice and rats. These animals, while maintained under a more microbiologically controlled environment, are not axenic; they are not free of all known organisms. Moreover, while each isolator is initiated with defined flora animals, our policy is to continue operating individual isolators if an occasional mold, yeast, or common nonpathogenic environmental bacterium is detected.

In assessing the health and bacteriologic profiles of the isolators, it is important to understand that each individual isolator functions as a small barrier production room containing between 30 and 75 cages with open (wire) tops. Depending upon the specifications for any given order, mice from multiple isolators may be required to fill that order, and different groups of isolators may fill repeat orders in subsequent weeks. Since each isolator has a slightly different profile in terms of additional organisms beyond the Charles River Altered Schaedler Flora (CRASF), the discrete bacterial profile may vary between individual groups of animals.

Each isolator is cultured (isolator internal surfaces, feces, drinking water) for assessment of bacterial flora at least every 4 weeks. In addition, at least once every 13 weeks animals from one fourth of all of the isolators are collected and screened by serology (Assessment Plus profile), microbiologic culture of skin, respiratory and gastrointestinal systems, complete parasitology, a full necropsy with histopathology of any lesions, and PCR for *Helicobacter*, *Streptobacillus moniliformis*, *Corynebacterium bovis* and (mice only) *Pneumocystis* spp. Four animals are sampled from each isolator for this intensive screening process.

Health monitoring reports are provided by Charles River on our website (www.criver.com), and are continuously updated as new results become available. These reports document the status of the composite of all the isolator colonies for that immunodeficient species located at a given site. Given the large number of isolators maintained, screening all isolators on a more frequent basis, or taking larger numbers of samples/animals would greatly increase the cost of the animals, yet would not contribute commensurately to the health monitoring program. In the thousands of isolator-years we have produced immunodeficient animals in isolators for commercial sale, we have never detected a contamination from an organism on our VAF[®] profile list.

The attached list of infectious agents is categorized by the action that will be taken by Charles River if the organisms are ever detected in an isolator of immunodeficient rats and mice. As can be seen from the chart, confirmation of any Category I agent would initiate an immediate notification of ALL customers that had received animals from any of the isolators in the facility. In keeping with our policy of open communication, all Charles River customers regardless of whether or not they received isolator-reared animals would also be notified. These organisms would be cause for immediate recycle of a standard barrier production room and would clearly result in cessation of shipment of animals from affected isolators and the immediate recycle of the affected isolator(s). Charles River considers each isolator as a microbiologic unit, and will not "test and cull" individual cages within an isolator. The circumstances involving the contamination would also be thoroughly investigated and reported.

A finding of a Category II organism would also result in immediate recycling of the contaminated isolator. Customers receiving animals from that isolator during the period since the last negative screening will be

notified. The entire customer base would not be notified, nor would customers that did not receive animals from the contaminated isolator be routinely notified unless requested. Additional information is available through the Charles River Technical Services Department.

Clearly, management of a very large group of production isolators poses a difficult task both from a production and a health monitoring standpoint. We feel that it is important that our customers understand how to interpret the health monitoring data we provide regarding these animals. In developing this production system, we have had to balance the level of risk to the health of the animals inherent in this production system with the amount, frequency, and completeness of the information generated, as well as with the information our customers require and expect. We are committed to providing our customers with all of the information required to make animal health management decisions, and in this regard we would appreciate your comments. Should you require any additional information or have specific requests, please contact your nearest Charles River Technical Services group at 877-274-8371 (877-CRIVER1) or send an e-mail to askcharlesriver@crl.com.

Sincerely,

Charles B. Clifford, DVM, PhD
Director, Pathology and Technical Services

**CHARLES RIVER GUIDELINES FOR THE RECYCLING AND CUSTOMER NOTIFICATION
OF PRODUCTION ISOLATORS FOR IMMUNODEFICIENT RATS AND MICE**

| <u>Category/Action</u> | | <u>Organisms</u> | |
|---|-------------|------------------|-------------------------------------|
| CATEGORY I. | <u>MICE</u> | <u>RATS</u> | <u>MICE and RATS</u> |
| <ul style="list-style-type: none"> • STOP SHIPMENT FROM ISOLATOR • IMMEDIATE RECYCLE • NOTIFY ALL CUSTOMERS | EDIM | RMV | CAR bacillus |
| | MPV | KRV | Tyzyzer's Disease |
| | MHV | RPV | <i>Corynebacterium kutscheri</i> |
| | MVM | H-1 | <i>Mycoplasma pulmonis</i> |
| | TMEV (GD-7) | RTV | <i>Citrobacter rodentium</i> (mice) |
| | MNV | SEND | <i>Helicobacter hepaticus</i> |
| | SEND | PVM | <i>Salmonella</i> spp. |
| | PVM | REO | <i>Streptobacillus moniliformis</i> |
| | REO | SDAV | Helminths |
| | ECTRO | MAV | Pathogenic protozoa |
| | MAV | ECUN | External Parasites |
| | K | HANT | |
| | POLY | LCMV | |
| | MCMV | | |
| | ECUN | | |
| | HANT | | |
| | LDV | | |
| LCMV | | | |
| MTLV | | | |

| <u>Category/Action</u> | | <u>Organisms</u> |
|--|---|--|
| CATEGORY II. | | <u>MICE and RATS</u> |
| <ul style="list-style-type: none"> • STOP SHIPMENT • IMMEDIATE RECYCLE • NOTIFY CUSTOMERS THAT RECEIVED ANIMALS FROM THE ISOLATOR | | <i>Pneumocystis</i> spp. β-hemolytic <i>Streptococcus</i> spp. Coagulase-positive <i>Staphylococcus</i> spp. including <i>S. aureus</i> <i>Corynebacterium bovis</i> (immunodeficient only) |
| | <ul style="list-style-type: none"> • PROVIDE INFORMATION TO OTHER CUSTOMERS ON REQUEST | |