A Guide to Integrating PCR Rodent Infectious Agent (PRIA) Panels in Rodent Quality Control Programs

Areas of expertise include:

- Integrating PRIA into your Health Monitoring Program
- Selecting the Appropriate PRIA panel
- Sample Collection and Submission
- Sample Processing and Results Interpretation
- Charles River PCR “TAQ”nology

Kenneth S. Henderson, PhD
Director, Molecular Diagnostics and R&D
Research Animal Diagnostic Services
Charles River
ken.henderson@crl.com

Charles B. Clifford, DVM, PhD, DACVP
Director, Pathology and Technical Services
Charles River
charles.clifford@crl.com

charles river

I. Introduction

Historically, valuable methods such as gross pathology, parasitology and bacteriology continue to be widely used in monitoring the health of laboratory rodents. The other major method, serology, came on the horizon in the middle of the last century to detect antibodies elicited as a result of an infection. The newest approach, polymerase chain reaction (PCR), has been evolving rapidly in recent decades since its invention in the mid 1980s. Using the Charles River PRIA system, it is now possible to screen for essentially all infectious agents of interest in laboratory rodents using only fecal samples, often supplemented with fur or dust swabs and, occasionally, oral swabs.

Charles River Research Animal Diagnostic Services pioneered the development of the PCR Rodent Infectious Agent (PRIA) array to address apparent shortcomings in traditional bedding sentinel use. The recent development of high-density real-time PCR arrays, which can screen samples for large numbers of agents, has brought the power of miniaturized assays to PCR testing just as the miniaturized antibody assays in our Multiplexed Fluorometric ImmunoAssay® (MFIA®) has advanced serology testing. Because of the unique attributes inherent to the real-time PCR array offered by our diagnostic laboratory, more and more institutions are joining the many who have already adopted PRIA panels for detection of rodent infectious agents. Some of the many common uses include quarantine, routine health monitoring, and environmental monitoring. PRIA panels covering all commonly excluded or reported rodent pathogens have been developed to address different testing needs; custom panels are also easily created to fit the requirements of every laboratory rodent facility. Most of the strategies presented in this manual help to resolve the problem of monitoring individual ventilated cage (IVC) racking systems. These systems successfully reduce cage-to-cage spread of infectious agents, but thereby also make it challenging to detect many infectious agents in bedding sentinels. This manual reviews the different applications of PRIA, as well as recommended sample types and strategies to optimize the screening process.

While advances in diagnostics are commonly associated with increased sensitivity and specificity, PRIA additionally has the rare distinction of contributing to increased animal welfare and reduction or replacement of animal use.

II. Integrating PRIA into your Health Monitoring Program

At Charles River, we have integrated PRIA into our health monitoring programs. In our own rodent production facilities, PRIA is used in the routine direct testing of barrier room animals and elite and foundation colonies, for monthly environmental monitoring of barrier rooms, and a smaller panel set is used to screen other production isolators. Further, PRIA is used as a biosecurity surveillance tool; specifically, PRIA is used to test swabs and feces collected from bait stations on the premises of Charles River facilities, to test wild rodents captured or found on Charles River premises, and for epidemiological testing.

Our Genetically Engineered Models and Services group utilizes PRIA to screen our client isolators. PRIA is used to shorten quarantine for facilities that have limited or lack their own quarantine rooms, or when receiving mice from questionable origin. PRIA may also be used to screen rodents upon arrival for projects or prior to departure when shipping to clients or for routine pathogen screening of isolators.

The following sections describes the application of PRIA as a component of a rodent health surveillance program in research facilities.

A. Animal quarantine

PRIA can greatly improve quarantine testing by relying solely on samples collected directly from incoming rodents. A weakness of using the older methods dependent on exposure of sentinels via soiled bedding is that many agents do not transfer or transfer poorly to bedding sentinels (Henderson et al., 2013). In contrast, collection of feces and body/oral swabs directly from quarantined mice or rats within a few days of arrival improves detection of the poorly-transferred agents as well as current infection with all other agents in the panels. Also, PRIA can detect less than the amount of virus required to infect an animal, i.e., < 1 ID (Blank, 2004), so virus that is being shed at levels too low to infect bedding sentinels can still be detected.

Another advantage is that PRIA on samples directly from incoming animals greatly shortens quarantine time. PRIA-based quarantine can be completed in less than 2 weeks post-arrival. Compare this to a typical bedding sentinel-based quarantine, where 6-8 weeks of bedding sentinel exposure is followed by 1-2 weeks for shipment, testing, and reporting.

Not only does the shortened PRIA quarantine get younger research models to the investigators much more quickly, but by reducing quarantine from 8+ weeks to only 2 weeks, the same quarantine space can process 4-fold as many animals. Investigators will appreciate getting younger animals with more breeding life remaining, the possibility to begin breeding in quarantine, and have more time with the animals while the current grants are active. Other advantages of a shorter quarantine period are the possibility to detect and eliminate infected mice more quickly so there is less chance of spread in the vivarium, and reduction in labor and other per diem costs. Additionally, as with other PRIA-based programs, the considerable expense of shipping animals to a diagnostic laboratory is avoided.

Although most agents can be detected in feces, the exact samples needed for testing depend on the pathogens being tested (Appendix A). Sampling strategies are described under "IV. Sample Collection and Submission."

B. Routine vivarium screening

PRIA can be used in several different ways to improve routine screening of rodent facilities; the following sections explain each application in detail.

1. Bedding sentinels

PRIA can be used to eliminate the need to ship live rodents to diagnostic laboratories for routine health screening, avoiding both the cost and the animal welfare concerns associated with shipping. As in conventional sentinel programs, soiled bedding is transferred to bedding sentinel rodents, commonly for 3 months. However, instead of shipping the live sentinels, PRIA panel testing only requires submission of feces and swabs collected from the sentinels.

Using PRIA-based testing on soiled bedding sentinels has advantages and disadvantages. Significant advantages are not needing to ship live animals, enhanced sensitivity for some agents present at low levels (e.g., mites, pinworms) and the possibility to pool samples from more than one rack. For most agents, PRIA-based testing of sentinels can provide diagnostic sensitivity equivalent to, or better than, bedding sentinels evaluated by traditional diagnostic methods. The chief disadvantage is that because it relies on soiled bedding sentinels, it has most of the drawbacks of the traditional soiled bedding sentinel program. Additionally, some viruses that are shed for only brief periods may be missed if the sentinels were infected early in the health monitoring period. This is especially true for rotavirus and coronavirus. This disadvantage, brief shedding of some viruses, can be avoided by using immunodeficient sentinels such as nude mice in a PRIA-based program, as immunodeficient mice shed virus persistently. In addition to the immunodeficient sentinel, some institutions add an immunocompetent cage mate to the sentinel cage to provide serological confirmation if a virus is detected, and also to perhaps improve detection of fur mites. However, fur mites are best detected by swabs of the plenum if using IVC racks; plenum swabs are discussed below.

Because only sentinels are evaluated in this particular scenario, then pooling could be used to test across multiple racks. Samples from a maximum of 10 sentinel rodents can be pooled so depending on if one or two sentinel rodents are used per study rack, samples representing up to 5 - 10 study racks could be pooled and tested as one sample.

2. Bedding sentinels plus select cages from rack

An alternative to testing only the bedding sentinels is to also collect samples from study cages on the rack. Because of the exquisite sensitivity of PRIA, samples from up to 10 animals can be combined (pooled) into one test sample. This means that combining samples from the sentinel(s) with samples from multiple cages on the rack will increase the chance of finding any contamination without increasing the cost of testing. Many excluded agents, primarily parasites and bacteria, do not transfer to bedding sentinels. By including samples from study cages, the detection of these agents will be greatly improved over just testing samples from the bedding sentinels. On racks where more than one genetic line of rodent is being housed, it is important to try to include samples from as many different lines as possible since many agents that do not transfer well to bedding sentinels are likely to stay within a genetic line and not spread to other lines on the same rack.

3. Environmental sampling of air (dust)

PCR on airborne particulate matter (i.e., dust) is not a novel idea for rodent pathogen detection and has been previously reported (Compton, 2004). However, current PCR technology has improved both sensitivity and specificity and current high-density assay platforms have made it a practical method for high-throughput routine screening of large numbers of pathogens in dust samples. Fur mite detection by PCR testing of swabs of horizontal plenum has been reported (Jensen, 2013). The number of agents detected by plenum sampling has now greatly expanded to include many agents which are not readily transmitted to bedding sentinels; although early, we have so far found no situation where an agent was present in sentinels or rack mice and not immediately found in plenum swabs. A very attractive advantage of using swabs of plenum or rack HEPA filters is that it completely eliminates the requirement for bedding sentinels. In addition to horizontal plenum testing as described by Jensen, other sampling sites, which include HEPA and pre-filter swabs, pre-filter sections, and room filter swabs or sections, have been evaluated and could potentially be used to monitor entire racks or rooms of animals. Another value of environmental dust sampling is that it is a "historical" sample; rodent pathogen DNA will remain long after an infection has cleared.

Although highly promising, many unknowns remain about air sampling that need to be taken into consideration when implementing dust sampling as part of your health monitoring program. Not all IVC racks are the same; there is tremendous variation among rack design that could influence the amount of pathogen debris collected, not limited to air flow pattern, location of dust build up, filter size/consistency/ location, sampling access, cage level filters (which could impede particle flow, etc. Therefore, it is important to **identify the location on the IVC were dust tends to aggregate** or is concentrated, such as the end of a horizontal plenum or onto a filter. There is every indication that we can detect all agents by air sampling, although we do not know the impact of prevalence, time between sampling or lapsed time post-infection could have on the sensitivity. If racks are not decontaminated and cleaned properly, residual pathogen material from rodents previously housed on the rack may still be present on the racks and could contribute to a positive finding. A baseline testing of racks after cleaning and decontamination and prior to populating with rodents may be important to demonstrate that racks are free of residual pathogen material.

If you do not have IVC racks, sampling surfaces in rooms that contain open-top cages may or may not work as efficiently as plenum or filter testing of IVC racks, but testing areas of dust concentration have been useful for detecting infectious agents. Exhaust air vent grills or the floor of the exhaust duct just behind the grill are often areas that collect dust that are useful for testing. A swab sampling of the cage racks from multiple locations within a room could be pooled as one sample allowing a large representation of the room to be tested.

Static micro-isolator caging presents a challenge because the caging is designed to keep pathogens from spreading outside the cage around the room and there is currently no way to easily test all the cages on a rack using a single sample, although some limited data has suggested that surface swabbing of cage interiors and filters can be useful for fur mites and pinworms. The use of a cage changing station would minimize the amount of pathogen material in the room. Although pathogen material may be limited, the pre-filter of the changing table may serve to concentrate the material that has escaped into the room, and exhaust vents may also provide a minimal material collection site.

4. Combined approach with bedding sentinel plus environmental (dust) sample

This hybrid program retains the use of bedding sentinels, but also incorporates a dust sample to cover pathogens that do not transfer to bedding sentinels. Combined approaches may bring many of the advantages of PRIA, while retaining some of the familiar aspects of other methodologies. One example of a combination program over four quarters would be to submit live bedding sentinels for one quarterly monitoring period for complete whole-animal screening by traditional methods. For the remaining three quarters, PRIA samples from sentinel mice or dust samples could be submitted for an abbreviated PRIA panel. This would reduce animal shipment while improving screening for agents that do not transmit to bedding sentinels. Optionally, serum could also be collected and submitted with PRIA samples to look for short-lived prevalent virus infections if immunodeficient sentinels are not used.

Table 1: Sampling Options Based on Rodent Housing Type

Caging System	Limiting Criteria	Sample Option(s)					
		Survival sentinel samples*	Survival sentinel plus study rodent sampling*	Room filter (swab or section)	Rack filter swab or pre-filter section	Horizontal plenum swab	Room exhaust vent swab
Individually-Ventilated cages (IVC)	Room-level fitration	●	●	●			
	IVC self-contained filtration	●	●		●	●	
	No access to plenums	●	●		●		
Static cages	N/A	●	●				
Open-top cages	N/A	●	●				●

* A single filter swab, room swab or filter section can be pooled along with survival samples from the sentinel or sentinels plus study rodent samples to increase sensitivity of pathogens.

5. Housing considerations

Rodent housing in research animal facilities can vary. However, PRIA can be applied in any scenario. Table 1 shows the sampling options based on each typical housing type. Contact Charles River at *askcharlesriver@crl.com* to discuss your options for sampling based on your specific housing configuration.

C. Diagnostic cases

Diagnostic cases are another consideration in rodent health monitoring. Most health testing today is for subclinical infections where laboratory testing of clinically normal animals is the only way to monitor for the agents. However, if infection is suspected as a cause of lesions or other anomalous observation, PRIA can effectively screen for common rodent pathogens. Animals sick from infection often have not yet produced antibodies so serology may not be a good diagnostic approach. Animals may also be too sick to ship, but samples can still be collected, shipped and evaluated by PRIA. Note that we also recommend histopathology as an important component of diagnostic evaluation of sick animals, as it can help localize tissue involvement and provide necessary context to PCR findings.

D. Biosecurity risk management

Wild rodent populations harbor a variety of pathogens that can be introduced into your facility. Samples from wild rodents or collected from contaminated traps and bait stations can be tested by PRIA to assess the presence of pathogens in rodent populations in your facility area. For Biosecurity, it can help you to assess the potential risk to your facility and identify threats so you can plan and deploy commensurate biosecurity countermeasures. Sequencing of detected viruses can help to determine if surrounding populations were the source of infection for any recent breaks in your facility. Samples can include swabs of bait traps and fecal pellets, which can be combined as one sample so that a single assay could give a good idea of pathogen "pressure" in the environs of the entire facility, even if no wild rodents are actually captured

III. Selecting the Appropriate PRIA Panel

Charles River developed real-time PCR assays to accommodate a wide range of rodent pathogen screening needs for an extensive list of viruses, bacteria, protozoa and metazoan parasites. These assays have been grouped into panels based on common needs and utility, with the aim to simplify sample collection processes by using common sample combinations. Custom panels based on your specific monitoring and exclusion goals can easily be created. Selecting the appropriate PRIA panel may depend on your exclusion list, health concerns and surveillance practices. Since these can vary by facility, we provide one-on-one consultation to help you select the appropriate standard panel or develop customized panels based on your requirements. Please contact *askcharlesriver@crl.com* to begin this discussion.

Table 2: Summary of Charles River Standard PRIA Panels for Direct Sampling from Mice and Rats

	PRIA Panels for Direct Sampling from Mice or Rats							
	Enteric	Prevalent	Fecal	Surveillance	Surveillance Plus	FELASA Basic	FELASA Complete	Bacteria Only
Agent Category								
Viruses	●	●	●	●	●	●	●	
Bacteria	●	●	●	●	●	●	●	●
Parasites/Protozoa/Fungi	●	●	●	●	●	●	●	
Sample Type(s) - ALL checked boxes are required								
Feces	●	●	●	●	●	●	●	●
Body Swab	●	●		●	●	●	●	●
Oral Swab					●		●	●
Lung		●*			●		●	

* Lung required for rat panel to optimize detection of *Pneumocystis*.

A summary of the PRIA panels for direct sampling from mouse and rat infectious agents is shown in Table 2; the agent list for each panel can be found in Appendix B. In addition to these panels, PRIA panels for guinea pig, hamster, gerbil and rabbit are available; contact Charles River for details. Each standard Environmental PRIA Panel includes assays to detect viruses, bacteria and parasites/protozoa/fungi (see Appendix B for details). For environmental PRIA, there are multiple sample options; either submit a surface swab, a filter section, a pool of surface swabs and feces, or a pool of a filter section and feces.

IV. Sample Collection and Submission

A. What sample types should be submitted?

Collection and shipping of the optimal samples is vital to having confidence in the results.

1. Testing rodents in quarantine or sentinels

The typical set of samples collected from rodents for a PRIA testing includes fecal pellets, where most pathogens can be found; oral swabs for a few rare respiratory viruses and bacteria; and a body swab to detect fur mites and *C. bovis* and improve detection of pinworms. As an alternative, fecal pellets and plenum swabs can be collected, pooled and tested. Optimal and permissible sample types for each pathogen are listed in Appendix A and it is important you include the correct sample type for each pathogen when submitting for PRIA testing. Optimal samples are those where the highest organism number is usually found and should be used when the status of an individual animal must be known with high confidence. Permissible samples may be more convenient to collect and are sufficient for determination of the status of entire groups of animals. Do not hesitate to contact us for recommendations in specific situations.

2. Quarantine

Even if a group of animals in quarantine is infected, not all individuals may be shedding; some may be uninfected and others may have cleared infection. Therefore, it is important that all rodents in a quarantine group are sampled, or at least a large representative proportion. For groups of rodents greater than 20, we suggest sampling as many cages as possible (preferably all), especially animals across the 4-8 week-old range where endemic virus and parasite infections are most often active. To mitigate PCR-associated inhibitors and sample dilution, our process has been optimized for pools of up to 10 of each of these three sample types (feces, oral swabs, fur swabs) so that one pooled sample submission can represent up to 10 quarantined rodents. Since small groups of animals are typical in quarantine, one set of pooled samples is usually sufficient for the incoming group.

B. How do I collect samples?

Samples for PRIA testing may be collected directly from animals or from places in the environment where nucleic acid (in dander, dust, etc.) can be found. General instructions for sample collection are provided in this section and additional details and sample collection procedures are available by contacting Charles River at *askcharlesriver@crl.com.*

Table 3: Whole-Animal PRIA Sample Collection

Sample Type	Sample Shipping Materials	Collection Process	Storage and Shipping Conditions
Feces	Vials, return shipping box *For up to 10 mouse pellets: one 2 ml snap-top vial* *For up to 10 rat fecal pellets: one 5 ml snap-top vial (filling the vial no more than 75% full; if necessary, send rat fecal pellets in a 15 ml conical tube)*	Collect fresh fecal pellets from each animal, so you know each animal is sampled, with no bedding material. For many purposes, fecal pellets can also be collected from the soiled bedding; however, copy number can decrease with time and it is impossible to know which or how many animals are being sampled.	Store: Room temperature Ship: Overnight at ambient temperature *Note: Store at -20 °C if not shipped immediately; can be shipped on cold packs or dry ice if it is necessary to pack with other sample types.*
Body Swab	Swabs, vials, return shipping box *For submission of a single swab: one 2 ml snap-top vial* *For up to 10 swabs: one 5 ml snap-top vial (filling the vial no more than 75% full)*	With the provided swab, swab the entire body going against the direction of hair growth.* If multiple animals will be screened together as one sample pool, either use one swab for up to five animals, or swab each animal with individual swabs and pool the swab tips. Clip the swab head and place in the provided vial; discard the remainder of the swab. Up to 10 swabs can be pooled and submitted in one vial. Please note that the vial should be no more than ~75% full.	Store: Room temperature Ship: Overnight at ambient temperature *Note: Store at -20 °C if not shipped immediately; can be shipped on cold packs or dry ice if it is necessary to pack with other sample types.*
Oral Swab	Swabs, vials, return shipping box *For submission of a single swab: one 2 ml snap-top vial* *For up to 10 swabs: one 5 ml snap-top vial (filling the vial no more than 75% full)*	Use the supplied dry non-alginate swab with no transport media to swab the oral cavity.* If multiple animals will be screened together as one sample pool, either use one swab for up to five animals, or swab each animal with individual swabs and pool the swab tips. Once swabbing is completed, cut off the swab tip from the shaft and place into the provided vial; the remainder of the swab should be discarded. Please note that the vial should be no more than ~75% full.	Store: Room temperature Ship: Overnight at ambient temperature *Note: Store at -20 °C if not shipped immediately; can be shipped on cold packs or dry ice if it is necessary to pack with other sample types.*
Lung	Vial, return shipping box	In the vial supplied, send approximately 5 mm of tissue from a single animal; lung tissue from multiple animals cannot be pooled.	Store: -20 °C Ship: Overnight on dry ice

* See Appendix C for photographs of the body and oral swab collection technique.

1. Sampling from animals

We suggest collecting samples from each incoming rodent in quarantine about 2-4 days after arrival. Even if animals first became infected during transit, the common infectious agents begin to be shed very quickly.

Table 4: Environmental PRIA Sample Collection

Sample Type	Sample Shipping Materials	Collection Process	Storage and Shipping Conditions
Swab*	Swabs (pink "sticky"), vials, return shipping box *For submission of a single swab: one 2 ml snap-top vial* *For up to 10 swabs: one 5 ml snap-top vial (filling the vial no more than 75% full)*	Use the pink "sticky" end to swab the surface;* do not use the white tip. Clip the swab head and place in the provided vial; discard the remainder of the swab. Up to 10 swabs can be pooled and submitted in one vial. Please note that the vial should be no more than ~75% full.	Store: Room temperature Ship: Overnight at ambient temperature *Note: Can be shipped on cold packs or dry ice if it is necessary to pack with other sample types*
Filter	Zip-top bag (not supplied), return shipping box *For submission of a single swab: one 2 ml snap-top vial* *For up to 10 swabs: one 5 ml snap-top vial (filling the vial no more than 75% full)*	Submit a single filter section, 1-2 inches in size, of the dirtiest filter material, placed in a zip-top bag.	Store: Room temperature Ship: Overnight at ambient temperature *Note: Can be shipped on cold packs or dry ice if it is necessary to pack with other sample types*

* Swab the plenum,** IVC rack, HEPA filter or another surface. Areas of dust concentration can be selected, such as the grates on cage-changing stations, exhaust vents or bedding vacuum hoses. "Dust is a must" — finding dusty areas is more important than the area being sampled.
** See Appendix C for photographs of plenum swab collection technique.

2. Sampling from the environment

Locations where dust and dander accumulate are also excellent places to screen to assess the infection status of the entire rack, room, etc. In general, these are more useful for routine vivarium monitoring than in quarantine, but can be used in many situations to supplement or even replace samples collected directly from the animals.

C. Sample pooling

Using the PRIA system, our laboratory is routinely able to detect agents such as *P. pneumotropica, S. aureus, S. pneumoniae* and others within fecal pellets. One key to the sensitivity of the PRIA system is the limit on the number of fecal pellets processed in one sample.

Ten (10) is the magic number. Assay sensitivity, PCR inhibition avoidance and processing volumes have all been optimized based on a maximum of 10 fecal pellets, 10 oral swabs, 10 body swabs or 10 plenum swabs. If you put in 11 or 12 specimens instead of 10, we will likely still be able to process them, but if you stray far outside the specification, it can compromise the stringent nucleic acid extraction and concentration procedures, compromising assay sensitivity, so adhering to sample collection limits is very important.

When pooling samples, each sample type must be pooled in separate appropriate containers labeled with the same sample ID (i.e., pool feces with feces, body swabs with body swabs) and then each group must be pooled prior to receipt by Charles River; see Appendix D for example pooling scenarios from either animals, the environment or a combination.

D. Sample submission to Charles River

The swabs and other sampling and shipping materials described in this document are carefully selected and provided free of charge from Charles River Research Animal Diagnostic Services (RADS). For example, we have found that using the appropriate swab can significantly enhance sensitivity of detection for some agents, such as fur mites. Additionally, the tubes provided have been incorporated into our initial processing procedures; by sending samples in the tubes provided, it allows for the most streamlined processing since samples need not be transferred to another tube, and it reduces the risk of sample cross-contamination. To submit samples, visit *www.criver.com/ltm*, where you can request shipping materials, search the catalog and create an order online. Samples for PRIA testing do not need to be scheduled in advance. If you have any questions regarding any aspect of sample submission, please contact Charles River at *askcharlesriver@crl.com*.

V. Sample Processing and Results Interpretation

A. Sample processing and timing

The entire process typically takes about 5 working days after sample receipt; confirmation testing of unexpected results may increase turnaround time. Your use of the recommended swab and container types, as well as appropriate packaging, will facilitate sample processing. A unique order number is assigned to each sample. If at all possible, a portion of the original sample is retained for possible retesting prior to nucleic acid extraction of the remainder of the sample. The samples are processed and the results interpreted by our staff. All samples are tested in duplicate and most yield a clear positive or negative result. Those that are equivocal are retested for clarification before the results are sent to you. Results are then released via email.

B. Results report and interpretation

Because it is important to submit the appropriate sample types for each test panel, the sample types received by the laboratory are listed on your results report. If any were missing, we will note it in the comments section of your report.

If you receive an unexpected result, please contact us immediately. We will review the raw data and may resubmit the retained sample for evaluation at no charge and may additionally approve further sampling to resolve the issue.

1. I never had positive results before. Why do I have them now when I use PRIA?

Unlike serology, where most viruses are no longer commonly found, we often find multiple agents in samples submitted for testing that are not viruses. Direct testing of rodents with PRIA is more sensitive than conventional screening methods for many agents, so as a result, PRIA usually has more positive findings. This is especially true for many opportunistic organisms commonly found in the 1-10 copy range, which are missed by conventional methods, but even for PCR approach may come close to the limit of detection. An increase in positive results can sometimes be associated with cross-contamination from other samples, which can be one of the challenges for infectious agent PCR. This is because some of these agents are highly prevalent or found in high copy numbers, and sometimes both; fortunately, we can mitigate this because we use real-time PCR that helps us estimate the copy numbers and rule out low copy numbers that are not consistent with a real infection. Equivocal samples are retested to also confirm or dispute an initial finding when the copy numbers are off. Most importantly, when determining our cut-offs used for scoring test results as -, +/- or +, we will bias more towards a false-positive than a false-negative result because the outcome of missing a true-positive outweighs a false-negative result (you can always retest to confirm an unexpected positive); therefore, we strongly recommend that unexpected results be discussed with RADS so we can authorize follow-up testing to confirm or dispute an initial finding.

2. What does +/- mean?

A +/- on the results means some target nucleic acid was detected. However, the amount of target nucleic acid (copy number) was very low and/or the copy number was not consistent with the number range usually associated with active infection for that agent. Unexpected +/- results very often require some additional investigation and we recommend you call us to discuss.

3. What to do with a positive?

If an unexpected positive result is received, we strongly recommend that you contact us immediately and, almost always, confirm with additional sampling prior to any colony culling.

C. Retesting

Retained portions of samples are held for one month. Because the extraction process for swabs uses the entire swab, additional swabs may need to be resubmitted.

If you have any questions about the testing process or your results, we are here to help you. Simply email us at *askcharlesriver@crl.com* and you will be placed in touch with the appropriate staff member.

VI. Charles River PCR “TAQ”nology

PCR is playing an increasing role in research animal diagnostics. PCR detects DNA or RNA from infectious organisms currently present in the submitted samples, in contrast to methods such as serology, which detects previous exposure. Since the PCR technique detects current infection and does not depend on an antibody response, it is particularly suited to screening immunodeficient animals as well as incoming (quarantine) animals or animals in the acute phase of infection that might not have had time to produce antibodies. PCR is also used to screen research biologics (e.g., cell lines) for infectious agents. PCR is an appropriate screening tool for both rodent and human cell cultures (i.e., cells and supernatant).

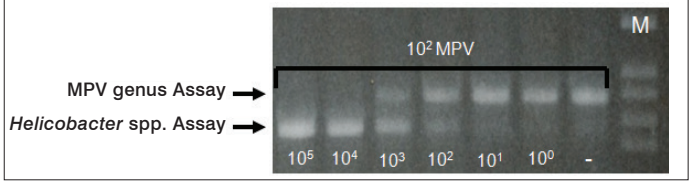
Not all PCR testing is the same, and PCR technology has evolved well beyond the gel-based PCRs primarily used back in the 1980s and 90s. Utilizing new assays and technologies developed in-house, our goal is to provide you with the most precise results possible while saving you time and money. For all PCR performed at Charles River, including our PRIA panels, we only use real-time TaqMan® PCR technology. Further, our PRIA panels are performed using the OpenArray® platform technology.

A. Why TaqMan®?

TaqMan® is a method of PCR which utilizes a special fluorogenic DNA probe to detect and measure amplification. It builds on the benefits of traditional gel-based PCR with increased analytical sensitivity and specificity. All our assays are singleplex, which is superior to multiplex. Multiplex PCR, the combining of multiple assays into a single reaction mixture, can suffer from competitive inhibition leading to false-negative results. (Hamilton, 2002, *J.Clin.Microbiol.*; Whiley/Slooth, 2005, etc.). This flaw in multiplex assays is especially apparent when there is a large disparity in target copy number among different agents. The example of competitive inhibition shown in Figure 1 demonstrates how a constant amount (100 copies) of a parvovirus template cannot be detected as the amount of a *Helicobacter* template increases in the duplex PCR.

Figure 1: Competitive Inhibition with Multiplexed PCR Assays

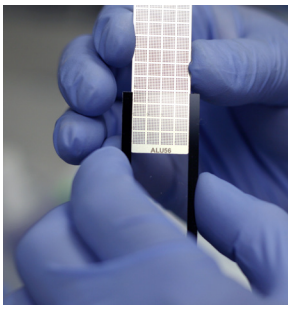
Multiplexing combines one or more assays within the same PCR reaction. Multiplexing is prone to competitive inhibition, which can yield false negative results.



Real-time TaqMan® PCR permits us to estimate the nucleic acid target copy numbers, and it is sensitive to the single copy range, which can improve the accuracy of detection. TaqMan® technology is ten to one hundred times more sensitive than traditional gel-based qualitative PCR, and the use of a fluorogenic DNA probe that binds to a specific sequence between primers provides incomparable specificity. This technology allows samples to be analyzed without opening reaction tubes, which prevents the release of potentially contaminating PCR products, a common downfall associated with gel-based PCR assays.

B. Why OpenArray®?

Figure 2: OpenArray® chip



The OpenArray® is a real-time PCR microarray platform that permits the simultaneous amplification of over 2,600 individual tests on a PCR plate the size of a microscope slide; this slide is called the OpenArray® "chip" (Figure 2). Each reaction "hole" targets just one specific target sequence — assays are not multiplexed. Using the OpenArray® platform makes it possible to efficiently screen samples for large numbers of infectious agents without compromising analytical sensitivity and specificity. For more information on the OpenArray®, visit www.criver.com/dx.

C. Assay development

Our Research & Development (R&D) team's sole job is to research emerging diseases, develop novel assays and continually refine methodologies. In recent years, our PCR R&D group has developed more than 160 TaqMan® assays to screen for disease in over 10 host species, including mammals, birds and fish. The continued development of assays and application of quantitative PCR methodologies permits common adventitious agents to be detected sensitively and specifically in samples taken noninvasively from rodents. The adoption of the OpenArray® platform, combined with experience in the practicalities of pooling total nucleic acid from a range of sample types, has created the possibility to effectively sample sentinel or principal (index) animals housed in individually-ventilated cages in a cost-effective manner. It is now possible to generate meaningful health monitoring data starting from simple clinical samples and avoid shipments of live animals and the potential welfare hazards thereof.

References

Blank, W. A., K. S. Henderson, and L. A. White. “Virus Pcr Assay Panels: An Alternative to the Mouse Antibody Production Test.” [In eng]. Lab Anim (NY) 33, no. 2 (Feb 2004): 26-32.

Compton, S. R., F. R. Homberger, F. X. Paturzo, and J. M. Clark. “Efficacy of Three Microbiological Monitoring Methods in a Ventilated Cage Rack.” Comp Med 54, no. 4 (2004): 382-92.

Elnifro, E. M., A. M. Ashshi, R. J. Cooper, and P. E. Klapper. “Multiplex Pcr: Optimization and Application in Diagnostic Virology.” Clin Microbiol Rev 13, no. 4 (Oct 2000): 559-70.

Henderson, K. S, C. L. Perkins, R. B. Havens, M.-J. Kelly, B. C. Francis, V. S. Dole, and W. R. Shek. “Efficacy of Direct Detection of Pathogens in Naturally Infected Mice by Using a High-Density Pcr Array.” J Am Assoc Lab Anim Sci 52, no. 6 (2013): 1-10.

Jensen, E. S., K. P. Allen, K. S. Henderson, A. Szabo, and J. D. Thulin. “Pcr Testing of a Ventilated Caging System to Detect Murine Fur Mites.” J Am Assoc Lab Anim Sci 52, no. 1 (Jan 2013): 28-33.

APPENDICES

Appendix A: Sample Type(s) per Mouse and Rat Pathogen Assay in the PRIA Array

AGENT	Survival Sample			Post-Mortem Samples†	Transmission to Bedding Sentinel	Relative Prevalence
	Feces	Body Swab	Oral Swab			
Viruses						
Parvovirus (MVM, MPV, H-1, KRV, RPV, RMV)	●			MLN	Good	Common
Murine norovirus	●			FC	Inconsistent	High
Rodent coronavirus (MHV, RCV, SDAV)	●			MLN	Good	Sporadic
Group A rotavirus (MRV, EDIM)	●			MLN	Good	Sporadic
Theilovirus (TMEV, GDVII, RMV)	●			MLN	Good	Sporadic
Adenovirus type 1 (FL) & 2 (K87)	●			MLN	Poor	Rare
Reovirus type 1, 2, 3, 4	●			IL/MLN	Good	Rare
Pneumovirus of mice			●	NPW	Poor	Rare
Sendai virus			●	NPW	Poor	Rare
Ectromelia - (mousepox)	●	●		SK	Poor	Rare
Seoul (Hantavirus)	●			KD,LU	Not reported	Rare
Lymphocytic choriomeningitis virus	●	●	●	MLN, KD	Not reported	Rare
Bacteria						
Helicobacter‡	●			SAS	Inconsistent	High
Citrobacter rodentium	●			SAS	Not reported	Rare
Mycoplasma pulmonis		✶	●	NPW	Poor	Sporadic
Streptobacillus moniliformis	●		✶	SAS	Not reported	Rare
P. pneumotropica (Heyl & Jawetz)	●	✶	✶	FC,NPW	Poor	High
Clostridium piliforme	●			IL	Good	Sporadic
CAR bacillus			●	LU	Poor	Rare
Pseudomonas aeruginosa	●	✶	✶	SAS	Not reported	High
Salmonella	●			SAS	Not reported	Rare
Campylobacter	●			SAS	Inconsistent	Sporadic
Bordetella bronchiseptica	●		✶	SAS	Poor	Sporadic
Bordetella hinzii	●			NPW,BW	Not reported	Common
Corynebacterium kutscheri	●			SAS	Not reported	Rare
Corynebacterium bovis	●	✶		SAS	Good	Common
Staphylococcus aureus	●			SAS	Inconsistent	High
Streptococcus pneumoniae	●			SAS	Not reported	Sporadic
Klebsiella pneumoniae	●			SAS	Inconsistent	High
Klebsiella oxytoca	●			SAS	Inconsistent	High
Beta-hemolytic *Streptococcus* group B	●			SAS	Not reported	High
Beta-hemolytic *Streptococcus* group C, G	●			SAS	Not reported	Sporadic
Proteus mirabilis	●			SAS	Not reported	Common
Parasites/Protozoa/Fungi						
Fur mites (*Myobia, Myocoptes, Radfordia*)		●		SAS	Poor/Delayed	Common
Pinworms (*Aspiculuris, Syphacia, Passalurus*)	●	●		SAS	Inconsistent	Common
Giardia	●			SAS	Poor	Sporadic
Spironucleus muris	●			SAS	Poor	Common
Cryptosporidium	●			SAS	Poor	Sporadic
Entamoeba	●			SAS	Poor/Delayed	Common
Pneumocystis				LU	Poor/Delayed	High

† Up to four post-mortem sample types can be pooled for one rodent when testing by PRIA; MLN = mesenteric lymphnode, IL = ileum, KD = kidney, NPW = nasopharyngeal wash, BW = bronchial wash, LU = lung, FC = feces, SK = skin, SAS = same as survival samples

‡ Prevalence in laboratory rodents: High > 5%, Common 1-5%, Uncommon 0.5 - 1%, Rare < 0.5%

✶ Add for increased sensitivity, especially for individual animal testing

APPENDICES

Appendix B: Charles River PRIA Panels for Mouse and Rat Infectious Agents (2014 Calendar Year)

MOUSE PANELS (DIRECT ANIMAL SAMPLING)	Enteric	Prevalent	Fecal	Surveillance	Surveillance Plus	FELASA Basic (3 month)	FELASA Complete (Annual)	Bacteria Only
Viruses								
Mouse parvoviruses (MVM/MPV)	●	●	●	●	●	●	●	
Murine norovirus	●	●	●	●	●	●	●	
Mouse coronavirus (MHV)	●	●	●	●	●	●	●	
Mouse rotavirus (EDIM)	●	●	●	●	●	●	●	
Mouse theilovirus (TMEV, GDVII)	●	●	●	●	●	●	●	
Adenovirus type 1 (FL) & 2 (K87)			●	●	●		●	
Reovirus type 1, 2, 3, 4			●	●	●		●	
Pneumovirus of mice					●		●	
Sendai virus					●		●	
Ectromelia (mousepox)			●	●	●		●	
Lymphocytic choriomeningitis virus			●	●	●		●	
Bacteria								
Helicobacter	●	●	●	●	●	●	●	●
Citrobacter rodentium			●	●	●		●	●
Mycoplasma pulmonis					●	●	●	●
Streptobacillus moniliformis			●	●	●		●	●
P. pneumotropica (Heyl & Jawetz)	●	●	●	●	●	●	●	●
Clostridium piliforme			●	●	●		●	●
CAR *bacillus*					●			●
Pseudomonas aeruginosa					●			●
Salmonella			●	●	●		●	●
Campylobacter			●	●	●			●
Bordetella bronchiseptica					●			●
Bordatella hinzii			●	●	●			●
Corynebacterium kutscheri			●	●	●		●	●
Corynebacterium bovis			●	●	●			●
Staphylococcus aureus			●	●	●			●
Streptococcus pneumoniae			●	●	●	●	●	●
Klebsiella pneumoniae			●	●	●			●
Klebsiella oxytoca			●	●	●			●
Beta-hemolytic *Streptococcus* group A					●	●	●	●
Beta-hemolytic *Streptococcus* group B			●	●	●	●	●	●
Beta-hemolytic *Streptococcus* group C, G			●	●	●	●		●
Proteus mirabilis			●	●	●			●
Parasites/Protozoa/Fungi								
Fur mites *(Myobia, Myocoptes, Radfordia)*		●		●	●	●	●	
Pinworms *(Aspiculuris, Syphacia*)*	●	●		●	●	●	●	
Giardia			●	●	●	●	●	
Spironucleus muris		●	●	●	●	●	●	
Cryptosporidium			●	●	●	●	●	
Entamoeba		●	●	●	●	●	●	
*Pneumocystis***					●			
Sample Types Required								
Fecal Pellet	●	●	●	●	●	●	●	●
Body Swab	●	●		●	●	●	●	●
Oral Swab					●		●	●
Lung**					●		●	

* Although it has been found in feces, a body swab (with focus on perianal area) is required for optimal detection.

** Although *P. murina* has been detected in oral swabs of immunodeficient mice, a lung sample is required for better detection confidence.

APPENDICES

Appendix B

RAT PANELS (DIRECT ANIMAL SAMPLING)	Enteric	Prevalent	Fecal	Surveillance	Surveillance Plus	FELASA Basic (3 month)	FELASA Complete (Annual)	Bacteria Only
Viruses								
Rat parvovirus (H-1, KRV, RPV, RMV)	●	●	●	●	●	●	●	
Rat coronavirus (RCV, SDAV)	●	●	●	●	●	●	●	
Theilovirus (RMV)	●	●	●	●	●	●	●	
Adenovirus type 1 (FL) & 2 (K87)			●	●	●		●	
Reovirus type 1, 2, 3, 4			●	●	●		●	
Pneumovirus of mice					●	●	●	
Sendai virus					●		●	
Seoul (Hantavirus)			●	●	●		●	
Bacteria								
Helicobacter	●	●	●	●	●	●	●	●
Mycoplasma pulmonis					●	●	●	●
Streptobacillus moniliformis			●	●	●		●	●
P. pneumotropica (Heyl & Jawetz)	●	●	●	●	●	●	●	●
Clostridium piliforme			●	●	●	●	●	●
CAR *bacillus*					●		●	●
Pseudomonas aeruginosa				●	●			●
Salmonella			●	●	●		●	●
Campylobacter			●	●	●			●
Bordetella bronchiseptica					●			●
Corynebacterium kutscheri			●	●	●			●
Staphylococcus aureus			●	●	●			●
Streptococcus pneumoniae			●	●	●	●	●	●
Klebsiella pneumoniae			●	●	●			●
Klebsiella oxytoca			●	●	●			●
Beta-hemolytic *Streptococcus* group A					●	●	●	●
Beta-hemolytic *Streptococcus* group B			●	●	●	●	●	●
Beta-hemolytic *Streptococcus* group C & G			●	●	●	●	●	●
Proteus mirabilis			●	●	●			●
Parasites/Protozoa/Fungi								
Fur mites (*Myobia, Myocoptes, Radfordia*)		●		●	●	●	●	
Pinworms (*Aspiculuris, Syphacia**)	●	●		●	●	●	●	
Giardia			●	●	●	●	●	
Spironucleus muris		●	●	●	●	●	●	
Cryptosporidium			●	●	●	●	●	
Entamoeba			●	●	●	●	●	
*Pneumocystis***		●			●		●	
Sample Types Required								
Fecal Pellet	●	●	●	●	●	●	●	●
Body Swab	●	●		●	●	●	●	●
Oral Swab					●		●	●
Lung**		●			●		●	

* Although it has been found in feces, a body swab (with focus on perianal area) is required for optimal detection.

** Lung is required for detection.

APPENDICES

Appendix B

MOUSE/RAT PANELS (ENVIRONMENTAL SAMPLING)	Environmental Mouse Prevalent	Environmental Mouse Surveillance Plus	Environtmental Rat Prevalent	Environmental Rat Surveillance Plus	Environmental Mouse/Rat Complete
Viruses					
Parvovirus (MVM, MPV, H-1, KRV, RPV, RMV)	●	●	●	●	●
Murine norovirus	●	●			●
Rodent coronavirus (MHV, RCV, SDAV)	●	●	●	●	●
Group A rotavirus (MRV, EDIM)	●	●			●
Theilovirus (TMEV, GDVII, RMV)	●	●	●	●	●
Adenovirus type 1 (FL) & 2 (K87)		●		●	●
Reovirus type 1, 2, 3, 4		●		●	●
Pneumovirus of mice		●		●	●
Sendai virus		●		●	●
Ectromelia - (mousepox)		●			●
Seoul (Hantavirus)				●	●
Lymphocytic choriomeningitis virus		●			●
Bacteria					
Helicobacter	●	●	●	●	●
Citrobacter rodentium		●			●
Mycoplasma pulmonis		●		●	●
Streptobacillus moniliformis		●		●	●
P. pneumotropica (Heyl & Jawetz)	●	●	●	●	●
Clostridium piliforme		●		●	●
CAR *bacillus*		●		●	●
Pseudomonas aeruginosa		●		●	●
Salmonella		●		●	●
Campylobacter		●		●	●
Bordetella bronchiseptica		●		●	●
Bordetella hinzii		●			●
Corynebacterium kutscheri		●		●	●
Corynebacterium bovis		●			●
Staphylococcus aureus		●		●	●
Streptococcus pneumoniae		●		●	●
Klebsiella pneumoniae		●		●	●
Klebsiella oxytoca		●		●	●
Beta-hemolytic *Streptococcus* group B		●		●	●
Beta-hemolytic *Streptococcus* group C & G		●		●	●
Proteus mirablies		●		●	●
Parasites/Protozoa/Fungi					
Fur mites (*Myobia, Myocoptes, Radfordia*)	●	●	●	●	●
Pinworms (*Aspiculuris, Syphacia*)	●	●	●	●	●
Giardia		●		●	●
Spironucleus muris	●	●	●	●	●
Cryptosporidium		●		●	●
Entamoeba	●	●	●	●	●
Pneumocystis		●		●	●
Sample Type Options					
Surface Swab	●	●	●	●	●
Filter	●	●	●	●	●
Surface Swab + Feces	●	●	●	●	●
Filter + Feces	●	●	●	●	●

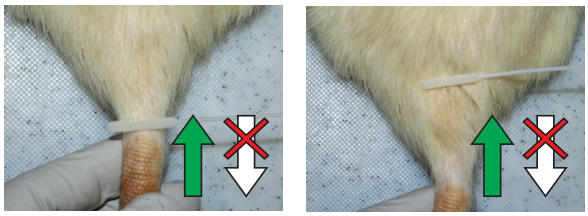
APPENDICES

Appendix C: Body, Oral and Plenum Swab Collection

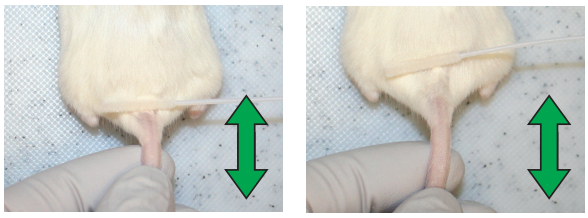
Body Swab Collection

With the pink “sticky” swab provided (not pictured), swab the entire body going against the direction of hair growth. Be sure to swab around ears, under chin, base of neck, inguinal areas, base of tail and perianal area.

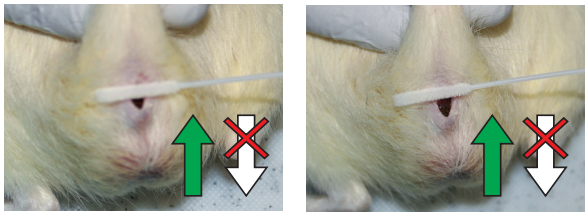
Step 1: Fur Swab Collection for Rat



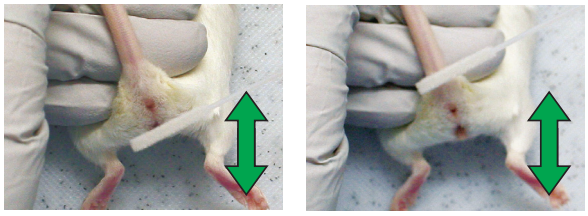
Step 1: Fur Swab Collection for Mice



Step 2: Anal Swab Collection for Rat



Step 2: Anal Swab Collection for Mice

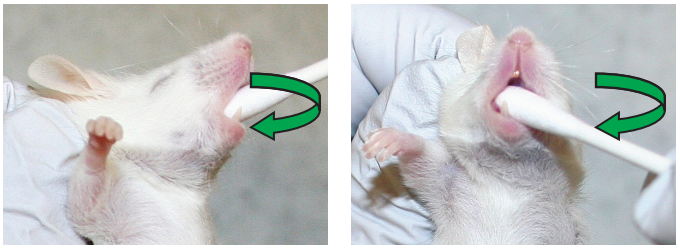


Oral Swab Collection

Use the supplied dry non-alginate swab with no transport media to swab the oral cavity.

Oral Swab Collection for Rat

Oral Swab Collection for Mice



Plenum Swab Collection

Access the plenum and swab using the pink “sticky” end of the swab provided; be sure to sample the dirtiest/dustiest area.

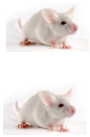
APPENDICES

Appendix D: Sampling Scenarios

Sampling Example 1: Quarantine
(Nine transgenic mice in quarantine)

1. Collect one **fecal pellet** from each mouse and place in one tube.
2. If the PRIA panel requires a **body swab**, collect one from each mouse and place each swab tip in one tube.
3. If the PRIA panel requires an **oral swab**, collect one from each mouse and place each swab tip in one tube.
4. Submit all tubes labeled with the exact same sample ID number to be tested as **one sample.**

Sampling Example 2: Routine Screening
(Two sentinel mice)



1. Collect one **fecal pellet** from each sentinel mouse and place in one tube.
2. If the PRIA panel requires a **body swab**, collect one from each mouse and place each swab tip in one tube.
3. If the PRIA panel requires an **oral swab**, collect one from each mouse and place each swab tip in one tube.
4. Submit all sample types labeled with the exact same sample ID number to be tested as **one sample.**

Sampling Example 3: Routine Screening
(Sentinel-free monitoring)

1. Collect one **fecal pellet** each from up to 10 mice on the rack and place in one tube.
2. Collect **environmental sample** (e.g., one HEPA filter swab, pre-filter section, or pooled plenum swabs) from the IVC rack and place in separate tube/bag.

IVC Rack

3. Submit all sample types for an Environmental PRIA Panel, labeled with the exact same sample ID number to be tested as **one sample.**

Sampling Example 4: Routine Screening
(Sentinel-free monitoring, environmental samples only)

IVC Rack

1. Collect **environmental sample** (e.g., one HEPA filter swab, pre-filter section, or pooled plenum swabs) from the IVC rack and place in separate tube/bag.
2. Submit one sample type for an environmental PRIA panel.

charles river | research animal diagnostic services

askcharlesriver@crl.com
www.criver.com