Document Number: ANP028	Title: VIVARIUM MICROBIOLOGICAL MONITORING AND	Effective Date: MAY 2019
Section: Animal Research	QC PROGRAM	Supersedes Date:
Subsection:	<u>CONFIDENTIAL INFORMATION</u>	Page:
Procedure	MOLECULAR MEDICINE RESEARCH INSTITUTE	1 of 3

1.0 OBJECTIVE

1.1 The objective of the Standard Operating Procedure is to describe procedures and policies for rodent health monitoring.

2.0 SCOPE

2.1 This procedure applies to all rodents in the MMRI Animal Facility and covers all procedures necessary to evaluate the health status of the rodents housed therein.

3.0 POLICY

3.1 It is the policy of MMRI, Inc. to establish written and approved procedures to assure that health of the animals is monitored and maintained.

4.0 **RESPONSIBILITIES**

- 4.1 It is the responsibility of the Manager of Animal Research to coordinate and oversee the implementation of the health status of the rodents and microbial program. Animal Facility personnel will be responsible for ordering, housing and exposing the rodents, collecting the samples and preparing the samples for evaluation.
- 4.2 It is the responsibility of the Manager of Animal Research and the IACUC to review this procedure annually and revise it if necessary.

5.0 REFERENCES

- Ref 1. M Mahler, et. al., Working Party Report: "FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units", Laboratory Animls (2104), Vol 48(3), P178-192.
- Ref 2. ANP012, Collection of Blood, Urine and feces
- Ref 3. <u>https://www.criver.com/products-services/research-models-services/animal-health-surveillance?region=3601</u>
- Ref 4. https://www.idexxbioanalytics.com/animal-health-monitoring

Document Number:		Effective Date:
ANP028	QC PROGRAM	MAY 2019
Section: Animal Research		Supersedes Date: November 2015
Subsection: Procedure	<u>CONFIDENTIAL INFORMATION</u> MOLECULAR MEDICINE RESEARCH INSTITUTE	Page: 2 of 3

- Ref 5. Product insert: BD BBL[™] Prepared RODAC[™] Plates BBL[™] Trypticase[™] Soy Agar with Lecithin and Polysorbate 80
- Ref 6. Pryor, A. K., and C. R. McDuff. 1969. A practical microbial surveillance system. Exec. Housekeeper, March.
- Ref 7. Dell, L. A. 1979. Aspects of microbiological monitoring for nonsterile and sterile manufacturing environments. Pharm. Technol. 3:47-5

6.0 PROCEDURE

6.1 Animal Health Testing (Direct Sampling).

Animal health monitoring is to be carried out according to FELASA guidelines (Federation of European Laboratory Animal Science Associates) (Ref 1). Animals from breeding colonies and study animals procured from commercial vendors are used for sampling. Serology and PCR testing is to be conducted quarterly. These services are provided by Charles River Laboratories or other reputable vendors (e.g. IDEXX). Charles River Laboratories offers both serology and Pria® PCR panels. The following procdures descibe materials used for collection and shipping of samples to Charles River Laboratories for testing, but other reputable testig laboratories may be used.

- 6.1.1 <u>Materials</u>: Sampling materials such as Hematips, sticky swabs, fecal pellet collection tubes are procured from Charles River Laboratories to avoid any contamination.
- 6.1.2 <u>Sampling zones</u>: Sampling zones assigned to the caging systems are designated as Sample #1, Sample #2, Sample #3 and Sample#4. Additional zones may be added when needed.
 - 6.1.2.1 Example

Sample #1 is Side B of mouse innovive rack with immunocompromised animals ; Sample #2 is Side B+A of rack with immunecompetent breeding colonies; Sample #3 is Side A of rat rack ; Sample #4 is Side B of the rat rack or as directed by the attending veterinarian.

6.1.3 <u>Labeling:</u> Label the hematips and collection tubes with sample IDs prior to starting the sample collection.

Document Number: ANP028	Title: VIVARIUM MICROBIOLOGICAL MONITORING AND	Effective Date: MAY 2019
Section:		Supersedes Date:
Subsection	CONFIDENTIAL INFORMATION	November 2015
Procedure	MOLECULAR MEDICINE RESEARCH INSTITUTE	Page: 3 of 3

- 6.1.4 <u>Serology</u>: Blood samples for serology are collected via saphneous vein blood draw. Samples from multiple animals are pooled to assess the general health of animals in the vivarium. Five different animals from five different cages (including both sexes and different age) are used for sampling. Saphneous vein is pricked with a 25 gauge sterile needle and a drop of blood is collected on heamtip. Blood samples obtained from all 5 animals are collected and combined on the same hematip. The hematip is allowed to dry then placed in the shipping container provided by testing laboratory.
- 6.1.5 <u>PCR Testing</u>: Fecal pellets, oral swabs and body fur swabs are used for PCR testing.
 - 6.1.5.1 Fecal Sampliing:

Fecal pellets collected from ten individual cages are collected for each sample. For example, ten cages from side B of mouse rack with immunocompromised animals will be used for sampling the fecal pelletes.

6.1.5.2 Oral Sampling:

Oral swabs are collected from 8-10 animals and pooled as a sample. Swabs are collected from buccal cavity and tip is cut and plced in a tube for testing. A fresh swab is used for each animal and up to 10 swabs can be pooled in one tube.

6.1.5.3 Swab Sampling:

Body swabs are collected by rolling pink sticky swabs (provied testing laboratory) in the direction opposite to the fur growth. Up to 10 swabs can be pooled as a single sample.

6.1.6 Shipping:

Samples are shipped at room temperature for analysis.

6.2 Microbial Environmental Monitoring

- 6.2.1 Sampling for microbial monitoring will be done after standard sanitization procedures.
- 6.2.2 <u>Materials</u>: RODAC[™] plates (e.g. BD Catalog # 215275) or normal bacteriological media agar plates will be used for microbial monitoring.

Document Number:	Title:	Effective Date:
ANP028	VIVARIUM MICROBIOLOGICAL MONITORING AND OC PROGRAM	MAY 2019
Section: Animal Research		Supersedes Date: November 2015
Subsection: Procedure	<u>CONFIDENTIAL INFORMATION</u> MOLECULAR MEDICINE RESEARCH INSTITUTE	Page: 4 of 3

- 6.2.3 RODAC[™] or bacterilogical media plates must not be opened prior to sampling and complete sterility must be maintained.
- 6.3.4 Sampling:
 - 6.3.4.1 RODACTM plates:

Samples from multiple areas of the vivarium will be collected by touching the surface of be samples with the dome shaped media of RODAC plates.

6.3.4.1 Bacteriological Plates:

If bacteriological agar plates are used for microbial monitoring, swabs from desiugnated area will be collected using sterile cotton swabs. A small region on the sampling area will be swabbed with the sterile swab and the swab will be immediately streaked onto the plate in a laminar hood.

- 6.3.5 Incubation: Plates will be incubated at 35°C for 72 hour.
- 6.3.6 <u>Interpretation of Results</u>: The number of colonies on each plate will be counted and compared with standard guidelines for judging RODAC counts (Ref 6 and 7)