Loyola University Chicago
Comparative Medicine Facility

Standard Operating Procedure

SOP Number: **02-18-5631**
Service: **Research**
Operating Section: **Diagnostic Laboratory**
Unit: **CMF**
Title: **Environmental Monitoring Program**

Purpose:
To describe the monthly program for monitoring sanitization and sterilization procedures and water quality which, if poor, could potentially affect the health and well-being of animals housed within the CMF.

Procedure:

1) General
   a) Samples for the Environmental Monitoring Program will be collected routinely on a monthly basis according to the CMF Environmental Monitoring Schedule. The test sights listed in the schedule are typical (but not exhaustive) and may be randomized to avoid prediction on the part of the sanitizer.
   b) Additional testing will be done as needed or upon request.
   c) ATP testing swab sampling will be the primary testing method.
   d) Samples for microbiology will be collected on standard culturette swabs, Rodac plates, or with water quality filters to further identify organisms in high contamination sites as needed.
2) Water ATP sampling
   a) Select water samples will be taken from various areas according to the CMF Environmental Monitoring Schedule.
   b) Samples from lixit valves will be taken from an unused cage slot (preferably one with an unused sterile cage).
   c) Samples from the wall will be taken from the quick connect (after first sanitizing the connector).
   d) Samples from the water filling station will be taken directly from the filler output.
   e) For any nonsterile water sample, the water will first be allowed to run (approximately ten milliliters). Then a few milliliters will be collected into a sterile collection container for sampling.
   f) Sterile water will be tested from the clean cagewash area according to the CMF Environmental Monitoring Schedule. A single bottle will be requested for sterilization prior to testing so as not to contaminate an entire rack of bottles. The used bottle will be emptied and returned to the clean bottle supply.
   g) The ATP water sample swab testing procedure will be executed as follows:
      i) Allow water swab test devices to equilibrate to room temperature before use.
      ii) Forcefully flick device in a downward motion to shake liquid extractant from collection dipper to bottom of tube.
      iii) Holding swab tube firmly, twist and pull collection device out of tube.
      iv) Submerge sample collection dipper in water sample for 1-2 seconds (a quick 5-count).
      v) Lift sample collection device up vertically and reinsert in test tube.
      vi) Gently shake device for 1-2 seconds (a quick 5-count) to release water sample from collection tip and to mix sample with extractant at bottom of test tube.
      vii) Activate by holding tube firmly and using thumb and forefinger to break the valve by bending bulb forward and backward. Squeeze bulb twice, expelling all liquid into tube.
      viii) Shake for 3-5 seconds (a quick 15-count) to mix sample.
      ix) Insert entire swab device into luminometer, close lid and press "OK" to initiate measurements. (Device should be measured within 15 seconds of activation.)
3) Hard surface ATP sampling
   a) If testing a sanitized site, swabbing should be done as soon after sanitizing as possible.

b) If testing a suspect (nonsanitized or visibly dirty) site, area and suspect conditions should be noted when swabbing.
c) The ATP surface swab testing procedure will be executed as follows:
   i) Allow ATP surface swabs to equilibrate to room temperature before use.
   ii) Holding swab tube firmly, twist and pull top of swab out of swab tube.
   iii) Thoroughly swab a standard 4 x 4 inch area for a typical flat surface. For irregular surfaces, ensure swabbing technique remains consistent for each test and swab a large enough area to collect a representative sample. Rotate swab while collecting sample to maximize sample collection on swab tip and apply sufficient pressure to create flex in swab shaft. Swab in a crisscross pattern vertically, horizontally, and in both diagonal directions.
   iv) After swabbing, replace swab back in swab tube.
   v) To activate device, hold swab tube firmly and use thumb and forefinger to break valve by bending bulb forward and backward. Squeeze bulb twice, expelling all liquid down swab shaft.
   vi) Bathe swab bud in liquid by shaking for 5 – 10 seconds (a quick 15-count).
   vii) Insert entire swab device into luminometer, close lid and press "OK" to initiate measurements. (Device should be measured within 15 seconds of activation.)

4) Microbiological sampling
   a) Using aseptic techniques, samples may be collected by wiping surface with a culturette swab. Alternatively, Rodac plates may be pressed firmly against the surface to be tested.
   b) Swabs are placed into tubes of broth. Tubes or Rodac plates are incubated at 37° C for at least 24 hours.
   c) Following tube incubation, the broth cultures are sub cultured to Plate Count Agar or other general purpose agar plates using the isolation technique. Plates are incubated at 37° C for at least 24 hours.
   d) For organism identification, refer to procedures in CMF Laboratory SOP, "Lab5628 Aerobic Cultures".

Results:

1) ATP RFU results
   a) Floors, door handles, and temp/humidity controls
      i) 0-40=properly sanitized
      ii) 41-75=warning zone
      iii) >75=dirty
   b) Other hard surfaces
      i) 0-10=properly sanitized
      ii) 11-30=warning zone
      iii) >30=dirty
   c) Any sterile substance or surface
      i) 0-1=sterile
      ii) >1=nonsterile
   d) For results in the warning zone, the person responsible will be informed for future reference. Repeated warning zone results may result in staff retraining.
   e) For dirty results, the person responsible will resanitize and the site retested until adequate RFU levels are reached. Repeated dirty results may result in staff retraining.
   f) For nonsterile results, the individual responsible for the unit will be notified and appropriate actions will be taken to ensure proper use and/or function until adequate RFU levels are reached during retesting. Repeated nonsterile results, if deemed to have been caused by user error, may result in staff retraining.
2) Microbiological results (gram positive cocci and gram negative bacillus will be identified)
   a) Sanitized items/surfaces and nonsterile water
      i) No Growth/Gram Positive Bacillus = Satisfactory
      ii) Gram Positive Cocci = Marginal
      iii) Gram Negative Bacillus = Unsatisfactory
   b) Sterile surfaces and liquids
      i) No Growth = Satisfactory
      ii) Growth = Unsatisfactory
3) All results will be reported to the CMF administration. Unusual or deleterious results will be discussed and a plan of action determined if needed.

Discussion/Background:

Maintaining a routine environmental monitoring program in an animal facility is important in ensuring that sanitation and/or sterilization is being appropriately achieved to ensure that living conditions for the animals are adequately maintained. Because most environments are not sterile and bacteria is ubiquitous, the presence of some types of bacteria (gram positive bacillus, for example) in the environment may be acceptable. The presence of other, pathogenic or opportunistic bacterial types, can indicate that sanitation is not being adequately achieved. The presence of any type of bacteria where sterility is expected (i.e. autoclaved items) is always unacceptable. A routinely performed environmental monitoring program helps detect potential sanitation/sterilization problems, training deficiencies, and equipment failures.

APPROVALS

Responsible Official Signature			**Date** 07/09/2019
QA Signature			**Date** 07/09/2019
Version	**Effective Date**	**Supersedes**	**Original Date**
#3		#2	08/18/10

CMF Environmental Monitoring Schedule

Area	Possible Test Sights	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Large An Surgery	Light Handle Instrument Stand Floor Wall Prep Scrub\Sol		X						X				
LA Surgery Autoclave	Pack Liquid	X				X				X			
Clean Casewash (sanitization)	NHP/Rabbit\GP Rack NHP/Rabbit\GP Pan Rat Cage/Rack Rat\GP Bottle (dry) Rat\GP Sipper Swine\Canine Flooring Enrichment (nonsterile)		X			X			X			X	
Bulk Autoclave Water	Sterile Water Sterile Sipper			X			X			X			X
Bulk Autoclave Caging	Sterile Mouse Caging Sterile Mouse Rack Sterile Enrichment			X			X			X			X
Bottle Filler Water	Water		X			X			X			X	
ABSL Autoclave Caging	Sterile Soiled Caging				X				X				X
CMF Zone 1 An. Housing Rms	Floor Wall Hood	X						X					
CMF Zone 1 Water	Water @ Lab Water @ Quick Connect	X						X					
CMF Rat\Mouse Proc. Rms	Hood Counter				X						X		
CMF Zone 2 An. Housing Rms	Floor Wall		X						X				
CMF Zone 2 Water	Water @ Lab Water @ Quick Connect		X						X				
CMF GP\Swine Proc. Rm	Table Counter				X						X		
CMF Rabbit Proc. Rm	Hood Counter				X						X		
CMF Zone 3 An. Housing Rms	Floor Wall			X						X			
CMF Zone 3 Water	Water @ Lab Water @ Quick Connect			X						X			
CMF NHP Proc. Rm	Table Counter				X						X		
CMF ABSL Gowning	Floor					X						X	
CMF Lab	Micro Hood HP Water	X						X					
CMF Lab Autoclave	Article Liquid	X			X			X			X		
CC Animal Housing Rms	Floor Wall Hoods		X				X				X		
Feed	New bags						X					X	
Bedding	New bags			X					X				

The agenda is a guideline and may be adjusted as needed to accommodate population and sanitization schedules. The test sights listed are typical (but not exhaustive) and may be randomized to avoid prediction on the part of the sanitizer. More information on actual site tested can be found on the results summary log.

Zone 1=rat and mouse rooms 089, 088, 087, and 086
Zone 2=guinea pig and rabbit housing rooms 085, and 084
Zone 3=swine\canine and NHP housing rooms 083, 082, 081, 080, and 079

CMF ABSL, quarantine, and behavioral areas will be tested post-sanitization, prior to occupancy (not on a regular schedule).

Autoclave testing for the purposes of this schedule refers to ATP or microbiological testing and does not include the weekly spore tests performed.