FAU FLORIDA ATLANTIC UNIVERSITY

Division of Research
Comparative Medicine

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SOP # 115	**Title: Microbiological Monitoring Using Microbial Agar**

SCOPE: This SOP is applicable to all Veterinary Services personnel

SOP OWNER: Certified Veterinary Technician (CVT)

PURPOSE: To ensure the microbial cleanliness and effectiveness of sanitation procedures by monitoring bacterial levels and types at all CM managed Vivaria using D/E Neutralizing Microbial Agar

LOCATION: All Vivaria

Approved by: Sylvia Gografe, D.V.M., Ph.D. Director Veterinary Services

I. References

1. *Guide for the Care and Use of Laboratory Animals*. National Research Council; National Academy Press, Washington, D.C., 2011
2. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, CDC/NIH, 2009
3. SOP 114 AccuPoint Use
4. SOP 213 Workflow In and Between Vivaria
5. CM#50 Environmental Monitoring Sample Log

II. Responsibilities

1. Laboratory Animal Technician (LAT)
   a. Adhere to procedures as outlined in this SOP.
   b. Perform only procedures for tasks which have been trained.
2. Certified Veterinary Technician (CVT)
   a. Adhere to procedures as outlined in this SOP.
   b. Provide appropriate training including hands-on sessions to personnel helping with sampling and other monitoring procedures outlined in this SOP.
   c. Manages the Environmental Monitoring Program (EMP).
   d. Reviews results and suggests measures to improve outcome of sanitation procedures based on test results to the AV and FM.
3. Facility Manager (FM)

a. Adhere to procedures as outlined in this SOP.
b. Ensure procedures are followed as outlined in this SOP.
c. Ensure appropriate SOP training is provided to particular personnel depending on function/job description in concert with the CVT.
d. Directs implementation of measures to improve outcome of sanitation procedures.

4. Director/Attending Veterinarian (AV)
   a. Adhere to procedure as outlined in this SOP.
   b. Ensure appropriate training is provided to particular personnel and necessary resources are available.
   c. Oversees EMP, reviews all results and establishes acceptability limits (i.e. pass/marginal/fail parameters). Assures implementation of measures to improve outcome of sanitation procedures.

III. General Information

1. Quality assurance practices set forth in this SOP and SOP 114 (Microbiological Monitoring Using ATP) will ensure that sanitation and sterilization of equipment in cage wash, animal housing rooms and procedure rooms in the vivarium yield an adequate reduction or total absence of surface contamination as appropriate for the particular surface/item.
2. Both microbial growth and organic residues exist as a barrier for effective sanitation
3. D/E Neutralizing Microbial Agar neutralizes a broad spectrum of antiseptic and disinfectant chemicals, which are commonly used in sanitation processes in vivaria. Difco HYcheck™ contact slides with D/E Neutralizing Agar is used for the isolation of microorganisms from environmental surfaces measured in Colony Forming Units (CFU).
4. Testing of hard surfaces will be organized using both ATP (see SOP 114) and HYcheck™ testing in all five (5) CM managed centralized vivaria (i.e. 71, 35A, 35B, MC17 and MC19).
5. On average, the quarterly number of microbiological samples collected will be 68 HYcheck™. See Table 1 for quarterly distribution of HYcheck™ samples between the different vivaria.

IV. Procedure

1. <u>General</u>
   a. Testing will be organized using the Environmental Monitoring Sample Logs, ATP Neogen AccuPoint Data Manager test plans and the HYcheck™ Microbial Sample Chain of Custody Form(s). These logs/plans/forms can be edited based on test results and/or the need to improve sanitation procedures and will outline sample sites to be collected from each of the vivaria at any given time.
   b. All Difco HYcheck™ plates for quarterly testing:
      i. Should be ordered with additional media to take into account for controls, defects, errors, etc.
      ii. Should be ordered from the commercial laboratory to have the same lot number, if possible, to eliminate the need to split buildings/testing sites and/or the need for additional controls.
      iii. Must be kept refrigerated and checked for defects before use.
   c. Equipment and room surfaces must be cool and dry prior to testing.

2. Materials Needed
   a. Pen/pencil
   b. Environmental Monitoring Sample Log (See CM#50)
   c. Difco HYcheck™ Chain of Custody Form(s) (quantity dependent on number of sample sites per Environmental Monitoring Sample Log)
   d. ATP Neogen AccuPoint Data Manager test plans
   e. Difco HYcheck™ plates (quantity dependent on number of sample sites per Environmental Monitoring Sample Log; additional plates needed for controls with 2 per Lot #)
   f. Facility PPE
   g. Shipping box with cooler (large enough to fit all Difco HYcheck™ plates and ice packs)
   h. Ice packs (quantity dependent on size of cooler and number of plate boxes)
   i. Large plastic bags to fit Difco HYcheck™ plate boxes and Chain of Custody Form(s) (multiple bags if necessary)

3. Sampling Procedures
   a. Review Environmental Monitoring Sample Log and Table 1 to ensure all sample sites are included; revise as needed.
   b. Pre-fill and print the Difco HYcheck™ Chain of Custody Form(s) if possible; including:
      i. Sample date and time
      ii. Printed name and signature of person sampling
      iii. Difco HYcheck™ ID # (letters)
      iv. Expiration date
      v. Lot #
      vi. Location (campus)
      vii. Building
      viii. Room #s (multiple if needed)
      ix. Customer (FAU)
      x. Contact name and phone number (CVT)
      xi. Contact email address for report (CVT)
      xii. Sample locations (room #, room description and sample site description)
   c. Using the Environmental Monitoring Sample Log, ATP Neogen AccuPoint Data Manager test plans and Chain of Custody Form(s), arrange the environmental monitoring of the vivaria by campus, building and room; keeping in mind the work flow based on health status of animals and associated room/facility classifications (See SOP 213), and by obtaining the appropriate amount/number of testing material; in this case, Difco HYcheck™ plates.
   d. Verify all Difco HYcheck™ plates obtained for each quarterly testing:
      i. Have the same lot number, if possible, to eliminate the need to split buildings/testing sights and/or the need for additional controls.
         a. If Difco HYcheck™ plates do not have the same lot number, record all lot numbers used in the comments section of the Environmental Monitoring Sample Log.

ii. Each Difco HYcheck™ ID# comes as a set of 20; in 2 boxes with 10 plates in each box.
a. Ensure all ID# match and plates are numbered correctly (1-20).

e. Ensure that sufficient material is available to finish the sampling process within a one to two day window for all five vivaria; in this case, Difco HYcheck™ plates.
i. If samples cannot be shipped on the same day as collection, refrigerate until ready to ship.

f. Using the Environmental Monitoring Sample Log, ensure Difco HYcheck™ plates are labeled with a numerical sequence corresponding to the Chain of Custody Form(s) with Difco HYcheck™ ID# and plate # within the set (i.e. NP 15).
i. Difco HYcheck™ plates must acclimatize to room temperature for approximately 1 hour before use.
ii. Without opening the housing tube, if possible, inspect/examine all Difco HYcheck™ plates to ensure agar surface is not dehydrated or contaminated; if opening the housing tube is unavoidable, be careful not to touch the agar surface.
a. Discard if defective, touched other surface than that being tested or if dropped on the floor.
iii. Ensure Difco HYcheck™ plates are not expired.
a. Discard if expired.
iv. Arrange plates in order according to the Environmental Monitoring Sample Log and facility/room entry.

g. Following facility/room entry procedures, gown up with the facility specific PPE.
i. Disinfect gloves before each testing site and/or change gloves between different rooms or whenever soiled.

h. Start sampling in the cleanest areas (e.g. clean cage wash and surgical suite) and follow the workflow based on health status of animals and associated room/facility classifications (See SOP 213).
i. Sample cage wash room when it is operating.
a. Samples of sanitized items should be taken immediately after removed from the washer and have had time to dry.
ii. Sample a variety of sanitized items in the clean cage wash areas and sample points in animal holding rooms, procedure rooms, storage rooms, surgical suites, and soiled cage wash rooms.
a. Sample items stored on shelves.
iii. Rotate types of sanitized items and sample points in rooms each quarter.
iv. Collect samples from multiple sites on each surface or item.
v. Samples must be collected and shipped on the same day.
a. If samples cannot be shipped on the same day as collection, refrigerate until ready to ship.
vi. Method of sampling:
a. Remove the lid/top of the housing tube of the Difco HYcheck™ plate.
1. Agar plate attached to inside of lid.
b. While grasping the lid/top in one hand, grasp the farthest end of the white spike/plate with the other; ensuring not to touch the agar surface.
c. Place the agar of the plate flat against the surface of the sample site to be tested.
d. Turn the plate over and test an adjacent location to the first sample site.

e. Replace the lid/top of the housing tube of the Difco HYcheck™ plate; placing agar back into housing tube; securing the lid tightly.
f. Discard if Difco HYcheck™ plate is defective, touched other surface than that being tested or if dropped on the floor.
g. Return the housing tube to the appropriate plate box; matching the Difco HYcheck™ ID# on the tube and box; placing the tubes in numerical order (i.e. Box 1-10 or Box 11-20).
h. For each Lot # used for sampling, controls must be performed:
1. 1 Negative plate=untested/unused plate.
2. 1 Positive plate=bottom of shoe.

vii. After all sample sites tested/collected:
a. Place all plate boxes into plastic bags; keeping the same box ID #s together; using multiple bags if necessary.
b. If any hand-written changes made to Chain of Custody Form(s), scan the form(s); keeping the copy on the shared drive: R:\Comparative Medicine\Quality Assurance\Plates (HyCheck).
c. Place Chain of Custody Form(s) in a separate plastic bag; placing the bag in the shipping box; separate from the cooler.

viii. Shipping samples to commercial laboratory:
a. Place all plastic bags containing plate boxes into the shipping box with cooler.
b. Cooler must have ice packs lining the bottom of the cooler and on top if possible.
1. Due to overnight shipping costs, using 1 shipping box with cooler is preferable; prepare multiple shipping boxes with coolers if necessary.

ix. Ship samples per commercial laboratory overnight to be received by 8am the next day (i.e. FedEx First Overnight, UPS NextDay Air Early).

4. Test Results Evaluation
a. Test results will be provided by commercial laboratory; usually by email; including comments and highlighted areas for review and/or action plans.
b. Once all test results have been received, the CVT will prepare reports for review by the AV and FM.
c. Test results will be discussed by the AV, CVT and FM. Depending on results, a strategy will be developed for resolution of *Fail* ATP results and unacceptable HYcheck results. Further investigations might be initiated to find the reason for unacceptable test results. The FM is responsible for implementation of remedial actions in regards to sanitation. The veterinary staff is responsible for communicating concerns to research personnel, if necessary.
d. Resolution of unacceptable results can include but are not limited to:
i. Retesting of particular surfaces/items.
ii. Training of CM personnel regarding sanitation procedures.
iii. Adjustment of sanitation frequency/intervals.
iv. Changes in detergents/disinfectants.
v. Improvement of the cage washer function.
vi. Communication of proper procedures to research personnel.
e. Number and type of remedial actions will be decided on a case by case basis.

5. Record Keeping
   a. The Environmental Monitoring Sample Log will be prepared in advance for guidance during the sampling procedures including the date of sampling, reason for sampling (i.e. quarterly routine), name of person(s) sampling, type of sampling (i.e. ATP or Difco HYcheck™), facility, room number, sample site(s) description and sanitation status of the sampled surface/item.
   b. The Reading/Results column pertains to the RLU measured using the AccuPoint Reader or CFU measured after Difco HYcheck™ incubation time points at the commercial laboratory. Comments can be made as needed (i.e. pass/fail, changes made) and kept as "raw data".
   c. The HYcheck™ Microbial Sample Chain of Custody Form(s) will be prepared in advance in regards to facility being tested, room number, and contact person at this facility. The specific surface/item sampled, name of person taking samples and date and time of sampling will be completed during active sampling. If hand-written changes made to pre-printed form(s) it will be scanned; the original sent with the samples to the commercial laboratory for testing and the copy kept as "raw data".
   d. Final testing data will include the Microbiological Monitoring Log reporting the results by the commercial laboratory.
   e. All Environmental Monitoring Sample Logs, HYcheck™ Microbial Sample Chain of Custody Form(s) and HYcheck™ Microbiological Monitoring Log reports will be filed in a systematic manner as hard copies and in electronic format (if applicable) on the CM shared drive: R:\Comparative Medicine\Quality Assurance\Environmental Monitoring\Plates (HyCheck).

Table 1
Quarterly HYcheck™ Testing Distribution

Qty	Bldg Rm#
1	71 400B
1	71 400C
1	71 406/407A
1	71 408
1	71 409
1	41 410
1	71 412
1	71 413
1	71 414
1	71 415
1	71 416
1	71 417
1	71 418
2	71 419
1	71 419A
1	35A 100/100A
1	35A 100B
2	35A 113
1	35A 114
1	35A 107/108
1	35A 109
1	35A 104
1	35A 105
1	35A 106
1	35A 101
1	35A 110
1	35A 111
1	35A 112
1	35B 100A
1	35B 100B
1	35B 101B
1	35B 102B
1	35B 103B
1	35B 104B
1	35B 105B
1	35B 106B
1	35 TRAN VAN
1	MC17 121
1	MC17 121C/D
1	MC17 121F
1	MC17 121G
1	MC17 122
1	MC17 122A
2	MC17 122B
1	MC17 122C
1	MC17 122D
1	MC17 122E
1	MC17 122F
1	MC17 122G
1	MC17 122H
1	MC17 122J
1	MC17 122K
1	MC17 122L

1	MC17 122M
1	MC19 122
1	MC19 122A
1	MC19 122B

1	MC19 122C
1	MC19 122D
1	MC19 122F
1	MC19 122G

1	MC19 122H
1	MC19 122I
1	MC19 122J
1	MC19 121L

Review Date	Revision Date	Revision Number	Description of Revision