FLORIDA ATLANTIC UNIVERSITY

Division of Research
Comparative Medicine

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SOP # 114	**Title: Microbiological Monitoring Using ATP**

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 ATP

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 213 Workflow In and Between Vivaria
4. CM#050 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 115 (Microbiological Monitoring Using Microbial Agar) 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. Adenosine triphosphate (ATP) is the universal energy molecule found in all animal, plant, bacterial, yeast, and mold cells. Sanitation effectiveness can be measured by detecting ATP (i.e. organic matter) with a specific ATP test system like the Neogen AccuPoint. A relative light unit (RLU) is the measure of light produced from the enzymatic reaction of luciferase when it reacts with ATP in a sampling swab.
3. Quarterly testing of hard surfaces will be performed using both ATP and HYcheck™ (see SOP 115) testing in all five (5) CM managed centralized vivaria (i.e. 71, 35A, 35B, MC17 and MC19).
4. On average, the quarterly number of microbiological samples collected will be 131 ATP and. See Table 1 for quarterly distribution of ATP samples between the different vivaria.

IV. Procedure

1. General
   a. Testing will be organized using the Environmental Monitoring Sample Logs and the ATP Neogen AccuPoint Data Manager test plans. These logs/plans 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 ATP swabs 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 vendor 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 and expiration date before use.
   c. Equipment and room surfaces must be cool and dry prior to testing.
      i. In keeping with manufacturer's suggestions and consistency of product results, all ATP swabs must be read within 4 hours of sample collection.

2. Materials Needed

a. Sharpie marker
b. Pen/pencil
c. Environmental Monitoring Sample Log (See CM#50)
d. ATP test plan from Neogen AccuPoint Data Manager PC program
e. ATP swabs (quantity dependent on number of sample sites per Environmental Monitoring Sample Log)
f. Test tube holders (quantity dependent on number of ATP swabs needed)
g. Neogen AccuPoint Reader
h. Facility PPE

3. Sampling Procedures
   a. Review Environmental Monitoring Sample Log and Table 1 to ensure all sample sites are included; revise as needed.
   b. Update the test plan in the AccuPoint Data Manager PC program: R:\Comparative Medicine\Quality Assurance\ATP.
      i. Include all sample sites with site descriptions indicated on the Environmental Monitoring Sample Log; revise as needed
      ii. Include pass/marginal/fail parameters per test plan
         a. Parameters (i.e. Default Thresholds) are fixed in the program, so need to be changed for each testing site using Table 2
   c. Upload/sync final test plan into the AccuPoint Reader
      i. Turn on the AccuPoint Reader using the red power button.
         a. If not fully charged, plug in wall outlet using power cord.
      ii. The reader will perform a self-test and initialization routine; green checks should appear.
         a. If green checks do not appear (i.e. self-test and initialization fails), do not use; have reader serviced/replaced.
      iii. The reader will then self-calibrate.
         a. If self-calibration fails, do not use; have reader serviced/replaced.
      iv. Plug in the PC connector cord into the USB of the PC on one end and the reader on the other end.
      v. "Transmitting" icon on reader will appear.
      vi. Open the Neogen PC Data Manager: R:\Comparative Medicine\Quality Assurance\Neogen\AP DataManager\APDM.exe.
      vii. Then open the corresponding test that you want to use: R:\Comparative Medicine\Quality Assurance\ATP.
      viii. Go to "Test Plan Manager" tab.
      ix. Click "Test Plan" on menu bar.
         a. "Test Plan Manager" window will populate.
         b. Edit sampling sites as necessary (i.e. add/delete); including building, room #, description and test site.
         c. Before syncing or exiting program, click "Save As" to create a new test plan file name (i.e. after a fail and/or sanitation to include select sites only).
         d. Click "Sync to Reader" at bottom left corner of window.
         e. Green progress bar will appear next to "Sync to Reader" button.
         f. "Test Plan Synced" box will appear; click OK.
      x. Close "Test Plan Manager" window.

d. Click "CheckList" on menu bar.
   i. "CheckList" window will populate.
   ii. All rooms/sites populate on the list to the right unless you click the boxes to the left, then only those sites will appear on the list to the right.
   iii. Print the "CheckList" using the print icon at the top of the window.
e. Close PC program or leave up until all samples collected and read in AccuPoint Reader.
f. Using the Environmental Monitoring Sample Log and AccuPoint Reader/Data Manager test plan, 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, ATP swabs.
g. Verify all ATP swabs 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 ATP swabs do not have the same lot number, record all lot numbers used in the comments section of the Environmental Monitoring Sample Log.
h. 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, ATP swabs.
   i. Using the Environmental Monitoring Sample Log, label ATP swabs with a numerical sequence corresponding to the AccuPoint Reader/Data Manager test plan.
   ii. ATP swabs must acclimatize to room temperature for at least 1 hour before use (out of direct sunlight); per manufacturer's instructions, swabs can remain at room temperature for up to 2 weeks in the original silver pouch (out of direct sunlight).
   iii. Ensure ATP swabs are not expired.
      a. Discard if expired.
   iv. Arrange swabs in order in test tube holders according to the Environmental Monitoring Sample Log and facility/room entry.
i. 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.
j. 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. 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.
   iii. Rotate types of sanitized items and sample points in rooms each quarter.
   iv. Collect samples from multiple sites on each surface or item.
k. Collecting and reading samples using ATP swabs and Neogen AccuPoint Reader:
   i. 2 Methods:
      a. Read as you swab.
      b. Accumulate swabs and read in batches; must be read within 4 hours of sample collection.

ii. Turn on the AccuPoint Reader using the red power button.
   a. If not fully charged, plug in wall outlet using power cord.
iii. The reader will perform a self-test and initialization routine; green checks should appear.
   a. If green checks do not appear or reader stops during initialization routine, check to see if a used swab was left in the sampler compartment.
   b. If green checks do not appear (i.e. self-test and initialization fails), do not use; have reader serviced/replaced.
iv. The reader will then self-calibrate.
   a. If self-calibration fails, do not use; have reader serviced/replaced.
v. If accumulating swabs and reading in batches, the reader can be turned off until swabs are ready to read.
   a. Reader will self-test, initialize and self-calibrate again when you turn it back on.
vi. If reading swabs as samples taken, the user screen will appear.
   a. Toggle up and down to choose appropriate user using the black joystick/toggle button; press toggle button in to select.
vii. Test screen display will appear.
   a. Toggle side to side or up and down between fields using the black joystick/toggle button.
viii. If you need to change information in a field.
   a. Press toggle button in to select field; blue box will turn red.
   b. Toggle up and down to the desired information in the field.
   c. Press toggle button in to select field; red box will turn blue.
ix. The number below the time/date, to the left, is the sample site that corresponds to the test plan and Environmental Monitoring Sample Log.
   a. Confirm the number on the swab and reader are the correct site before swabbing.
x. Remove the sampler swab from the housing compartment by grasping and pulling straight out.
   a. Discard if sampler swab is defective, touched other surface than that being tested or if dropped on the floor.
xi. With the white pad placed flat on the sample site, apply enough pressure so that the sponge tip is slightly compressed, swab your site in a cross hatch pattern; meaning "draw" a 4 inch x 4 inch square by swabbing in back-and-forth motion, moving through the "drawn" square and then in the opposite 90 degrees direction by swabbing in an up-and-down motion moving through the "drawn" square (See Diagram 1).
xii. Place the sampler swab back into the housing compartment and activate sampler by fully depressing it into the housing compartment; keeping the sampler vertical and ensuring the pad breaks through the aluminum foil at the tip.
xiii. Gently mix the sampler for 2 seconds/2 turns; keeping the sampler vertical.
xiv. Open the sampler compartment door on the reader by depressing the black button on the left side of the reader.
xv. Place the entire sampler into the reader in this compartment; close the compartment door firmly.
xvi. Reading will automatically begin; a progress screen will appear on the test screen display.
xvii. Test results measured in RLUs will appear in 15-20 seconds:
   a. Pass: will display with a green check mark
   b. Marginal: will display with a yellow question mark

c. Fail: will display with a red X

xviii. Once reading complete, open the sampler compartment door on the reader by depressing the black button on the left side of the reader.

xix. Dispose of the used/read sampler swab.

xx. All ATP swabs must be read within 4 hours of sample collection.

xxi. Results will automatically be stored in the Neogen AccuPoint Reader to the associated/uploaded test plan and can be synced/downloaded to the PC Data Manager to be saved digitally.

l. Download to the PC Data Manager:

i. Open the corresponding test plan in the Neogen PC Data Manager that just tested/read from the AccuPoint Reader.

ii. Turn on the AccuPoint Reader using the red power button.

iii. Reader will self-test, initialize and self-calibrate again when you turn it back on.

iv. Plug in the PC connector cord into the USB of the PC on one end and the reader on the other end.

v. "Transmitting" icon on reader will appear.

vi. Screen on PC will populate "### New Samples Found"; click OK.

vii. Results will then be downloaded from the reader to the PC Data Manager for the specified test plan.

viii. Before printing reports or closing the program, click "Save As" to create a test plan results file name.

ix. Results can be populated into reports.
a. Click on "Reports" tab.
b. Click on either "Details," "Summary" or "Trends" button.

x. Print or save reports to the CM shared drive as needed: R:\Comparative Medicine\Quality Assurance.

4. Test Results Evaluation

a. ATP results measured in RLUs will be classified as *Pass*, *Marginal*, and *Fail* according to ranges established based on prior testing as well as on type of surface or item sampled. See Table 2.

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. 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. Comments can be made as needed (i.e. pass/fail, changes made) and kept as "raw data".
   c. Final testing data will include the Test Results Summary Report printed from the Neogen AccuPoint Data Manager PC program.
   d. All Environmental Monitoring Sample Logs and ATP AccuPoint Test Results Summary 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\ATP.

Table 1
Quarterly ATP Testing Distribution

Qty	Bldg Rm#
2	71 400B
2	71 400C
2	71 406/407A
2	71 408
2	71 409
2	41 410
2	71 412
2	71 413
2	71 414
2	71 415
2	71 416
2	71 417
2	71 418
3	71 419
2	71 419A
2	35A 100/100A
2	35A 100B
3	35A 113
2	35A 114
2	35A 107/108
2	35A 109
2	35A 104
2	35A 105
2	35A 106
2	35A 101
2	35A 110
2	35A 111
1	35A 112
2	35B 100A
2	35B 100B
2	35B 101B
2	35B 102B
2	35B 103B
2	35B 104B
2	35B 105B
2	35B 106B
1	35 TRAN VAN
2	MC17 121
2	MC17 121C/D
2	MC17 121F
2	MC17 121G
2	MC17 122
2	MC17 122A
3	MC17 122B
2	MC17 122C
2	MC17 122D
2	MC17 122E
2	MC17 122F
2	MC17 122G
2	MC17 122H
2	MC17 122J
2	MC17 122K
2	MC17 122L
2	MC17 122M
2	MC19 122
2	MC19 122A
2	MC19 122B
2	MC19 122C
2	MC19 122D
2	MC19 122E
2	MC19 122G
2	MC19 122H
2	MC19 122I
2	MC19 122J
2	MC19 121L

Diagram 1

Cross Hatch Pattern Used for Swabbing

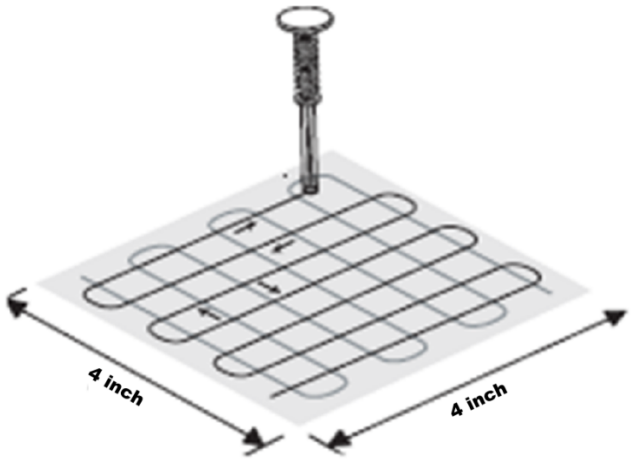

Table 2
RLU Ranges (*Pass*, *Marginal*, and *Fail*)

Test Site	**Pass**	**Marginal**	**Fail**
Floors	0-150	151-300	>300
Walls	0-25	26-50	>50
Doors	0-50	51-100	>100
Caging/Equipment	0-25	26-50	>50
Hood/Work Surfaces	0-50	51-100	>100

Review Date	Revision Date	Revision Number	Description of Revision