CM SOP #113 – Microbiological Monitoring of Sanitation

Original release date: 06/28/2017 Version: 5 Date last revised: 02/10/2026

I. Purpose & Scope

To verify the effectiveness of sanitation and disinfection practices in Comparative Medicine–managed vivaria through routine, risk-based environmental monitoring, primarily using ATP testing, with limited use of culture-based methods when indicated. This SOP applies to all Comparative Medicine–managed vivaria and to Comparative Medicine personnel involved in sanitation, environmental monitoring, data review, and implementation of corrective actions. This SOP replaces previous ATP- and agar-specific monitoring SOPs.

II. Roles & Responsibilities

Comparative Medicine Staff

- Conducts or coordinates monitoring activities for which they are trained
- Follow this SOP and associated safety requirements
- Maintains monitoring records

Research Staff, Students, Visitors

- Not applicable

CM Assistant and Associate Directors

- Ensures sanitation procedures are implemented as directed
- Reviews results and trends
- Initiates corrective actions and follow-up testing related to sanitation failures

CM Director and AV

- Oversees the Environmental Monitoring Program (EMP)
- Establishes acceptance criteria and reviews results
- Determines when culture-based testing is warranted

III. General Notes & Definitions

- Environmental monitoring is a quality assurance activity, not a diagnostic test.
- ATP testing is the primary routine method for assessing sanitation effectiveness.
- Culture-based agar testing is used sparingly and only when justified by risk or repeated failures.
- Monitoring locations, frequency, and methods are risk-based and may change following process modifications.
- Surfaces and equipment must be clean, dry, and at ambient temperature prior to sampling to ensure valid results, particularly for ATP testing.
- Results are evaluated by trained Comparative Medicine personnel and reviewed by veterinary staff. Unacceptable results trigger corrective actions, which may include retraining, process modification, re-sanitization, or follow-up testing.

- **ATP Testing**: Rapid detection of residual organic material using relative light units (RLUs).
- **RLU**: Quantitative measure of ATP detected on a surface.
- **Culture-Based (Agar) Testing**: Detection of viable microorganisms via surface contact agar.
- **Corrective Action**: Actions taken to address sanitation failures (e.g., retraining, re-sanitization, equipment repair).

IV. Materials & Equipment

- ATP Monitoring
  - ATP swabs (single lot when possible)
  - ATP reader (e.g., Neogen AccuPoint)
  - Environmental Monitoring Sample Log
  - Facility PPE
- Culture-Based Monitoring (When Indicated Only)
  - D/E neutralizing contact agar slides (e.g., HyCheck)
  - Chain-of-custody forms
  - Cooler and ice packs for shipment
  - Facility PPE

V. Procedure

A. General Sampling Principles (All Methods)

- Surfaces must be cleaned, dry, and cool prior to sampling.
- Follow facility workflow from clean to dirty areas.
- Wear appropriate PPE and change or disinfect gloves between rooms.
- Sample sites are selected based on risk assessment and include:
  - Cage wash output
  - Cage-changing stations / biosafety cabinets
  - Surgical and procedure surfaces
  - High-touch or difficult-to-clean areas

B. Routine Monitoring — ATP Testing (Primary Method)

1. Preparation

a) Verify ATP swabs have been refrigerated, are intact, and are not expired prior to use.

b) Allow swabs to equilibrate to room temperature per manufacturer instructions (~1 hour, out of direct sunlight).

c) Ensure ATP reader passes self-test and calibration by confirming green checks appear after initialization.

i. If green checks do not appear, do not use.

ii. Inform management to have reader serviced or replaced.

2. Sampling Method

a) Place swab flat against the surface.

b) Apply firm pressure so the tip is slightly compressed and swab a defined area (~4” × 4”) using a cross-hatch pattern (see figure below).

c) Avoid contact with unintended surfaces.

3. Reading Samples

a) Place the sampler swab back in to the housing compartment and activate the swab by fully depressing it into the housing compartment, keeping the sampler vertical and ensuring the pad breaks through the aluminum foil at the tip.
b) Gently mix the sampler for 2 seconds/2 turns; keeping the sampler vertical.
c) Open the sampler compartment door on the reader by depressing the black button on the left side of the reader.
d) Place the entire sampler into the reader in this compartment; close the compartment door firmly. Reading will automatically begin; a progress screen will appear on the test screen display.
e) Read samples within the manufacturer's specified time window.
f) Record RLU values in the monitoring log.

4. Interpretation

a) Results are classified as **Pass, Marginal, or Fail** using surface-specific thresholds outlined in the Table below.
b) Trends are reviewed routinely.
c) All ATP AccuPoint Test Results Summary Reports will be filed in a systemic manner in electronic format on the CM Shared Drive:

R:\Comparative Medicine\Quality Assurance\Environmental Monitoring\ATP.

C. Response to ATP Results

1. Pass: no action required
2. Marginal: Evaluate trends; consider retraining or process review if recurring.
3. Fail: Immediate corrective action (e.g., re-sanitization, retraining, equipment assessment); Repeat ATP testing after corrective action

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D. Decision Tree for Culture-Based (Agar) Testing

Culture-based testing is **NOT** routine and is performed only if one or more of the following occur:

→ Proceed to Agar Testing ONLY if:

- Repeated ATP failures persist after corrective actions, OR
- A significant sanitation process change occurs (e.g., new disinfectant, cage washer modification), OR
- Directed by the AV as part of an investigation or risk assessment

→ Do NOT perform Agar Testing if:

- ATP failures are isolated and resolved with corrective action
- There is no evidence of systemic sanitation failure

When used, agar testing follows validated contact plate methods with appropriate controls and commercial laboratory analysis.

E. Culture-Based (Agar) Testing Procedure (When Indicated)

1. Preparation

a) Verify agar plates are **not expired** and stored refrigerated.
b) Prepare chain-of-custody documentation:
   - Sample date and time
   - Printed name and signature of person sampling

- Difco HYcheckTM ID # (letters)
- Expiration date /Lot #
- Campus, building, room numbers, sample site
- Customer (FAU)
- Contact name, phone number and email address for report (VT)

c) Confirm that agar is not dehydrated nor contaminated.
d) Room order for testing should be planned from clean to dirty.

2. **Sampling Method**
   a) Don appropriate PPE and disinfect gloves prior to sampling and change gloves between rooms.
   b) Remove lid without touching agar surface. While grasping the lid/top in one hand, grasp the farthest end of the white spike/plate with the other.
   c) Press agar firmly and evenly onto the test surface. Surfaces to be tested should be based on risk analysis and in consultation with the AV.
   d) Turn the plate over and test an adjacent location to the first sample site.
   e) Replace lid securely and label appropriately: match the Difco HYcheck ID# on the tube and box; place the tubes in numerical order.
   f) Include required **positive and negative control plates** (bottom of shoe and unused plate, respectively).
3. **Handling and Shipment**
   a) Refrigerate plates immediately after sampling.
   b) After all sample sites have been tested/collected, place all plate boxes into plastic bags and place all bags into a shipping container with ice packs.
   c) Ship overnight to a commercial laboratory.
4. **Interpretation**
   a) Results are reported as **CFUs**.
   b) Results are reviewed by the VT, AV, and FM.
   c) Findings are interpreted in context of surface type, risk level, and ATP trends. All reports will be filed in a systematic manner in electronic format on the CM shared drive:

R:\Comparative Medicine\Quality Assurance\Environmental Monitoring\Plates (HyCheck)

F. Corrective Actions

Corrective actions may include:

- Re-sanitization
- Personnel retraining
- Adjustment of sanitation frequency or methods
- Equipment maintenance or repair
- Limited follow-up agar testing (if indicated)

All actions must be documented.

VI. Health & Safety

- Wear facility-required PPE during sampling.
- Treat all environmental samples as potentially biohazardous.
- Dispose of used materials according to institutional waste procedures.
- Follow EH&S and biosafety guidance for handling and shipment.

VII. References & Attachments

- *Guide for the Care and Use of Laboratory Animals*
- *Biosafety in Microbiological and Biomedical Laboratories* (CDC/NIH)
- Manufacturer instructions for ATP and agar testing systems
- Retired SOPs 113, 114, and 115

VIII. Revision History

Revision Date	Revision Number	Summary of Changes
08/09/2017	2	Updated general procedures and quantities in Table 1; changed verbiage; changed formatting
02/10/2021	3	Inclusion of risk analysis, revision of sample points
04/04/2024	4	Update to include what is actually done in facilities as this has changed.
03/01/2026	5	Major revisions: consolidated SOPs 113, 114, 115 into the current version 113.5; deemphasized culture based methods for routine use; adapted to ADA compliant formatting

Approved by: Nicole Compo, DVM, DVSc, DACLAM, Attending Veterinarian

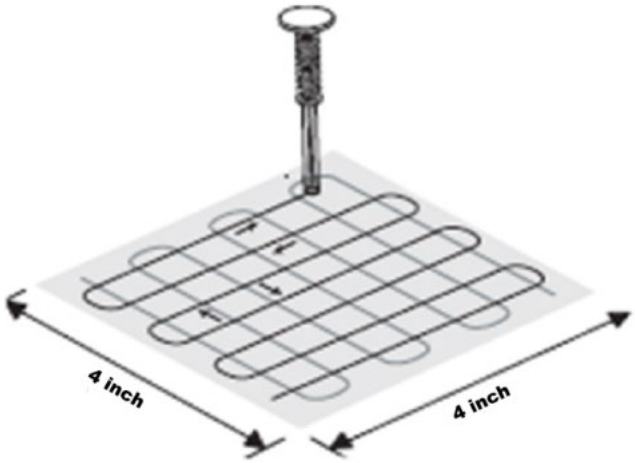

Figure 1: Cross hatch pattern used for ATP swabbing

Table 1 : 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