RUTGERS UNIVERSITY Office for Research	RUAC STANDARD OPERATING PROCEDURE	
TITLE: Sanitization Effectiveness Testing		
SOP Category: Husbandry	**RUAC SOP #: 2.02**	**Page: 1 of 9**
Effective Date: 11/10/2021	**Approval:** Lisa Antonucci	
Revisions: 5/27/2022, 5/18/2023, 2/4/2025, 4/1/25		

SCOPE:

This document describes the procedures to be followed when testing for evaluation of the efficacy of disinfection of surfaces and equipment that come into contact with live animals. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities. ACS, ACSS, and VS Staff compromise Rutgers University Animal Care (RUAC) staff.

OBJECTIVE:

The objective is to verify microbial reduction after sanitation of surfaces that animals contact (e.g., surgical, restraint, behavior, housing, devices and/or equipment) within the vivaria and investigator laboratories. The approved verification methods are detection of live organic matter through the presence of ATP (Adenosine Triphosphate) and/or growth of microorganisms on RODAC plates (Replicate Organism Detection and Counting).

NOTE: Either method (ATP or RODAC) may be used to verify results, or used as a backup, as needed.

PROCEDURES:

Frequency/Responsibility/Reporting results:
Responsibility for testing lies with RUAC Staff.

Location	Frequency	Fail/Pass Result	Data stored
PI laboratory animal testing equipment	~3 years	Email lab: Sanitize/re-test, retrain staff until pass result	share drive
ATS/BSC	Quarterly 1 hood per facility	Sanitize/re-test, retrain staff until pass result	share drive

Cage Wash	~quarterly 1 item per facility	Sanitize/re-test, retrain staff until pass result	share drive
NHP equipment	~1 year	Sanitize/re-test, retrain staff until pass result	Husbandry files

ATP Sanitization Monitoring

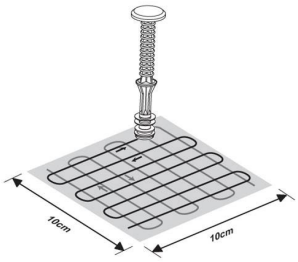
The ATP system consists of a handheld device, surface testing swabs, and computer software.

Surface swabs and associated software will be used as per the manufacturer's recommendation.

Surface Sample Collection:

- A positive control must be collected before sampling surface of interest. The positive control sample is collected from a known contaminated surface (e.g., sole of shoe) to confirm that the ATP unit is functioning properly. Then, subsequent samples can be taken from sanitized items.

- Wearing clean gloves, collect a test sample from the cleaned surface of interest by rubbing the swab tip over the surface in a grid pattern (maximum surface area 10cm x 10cm square). Make sure the sampler swab tip only contacts the designated test surface to avoid contaminating swab tip.

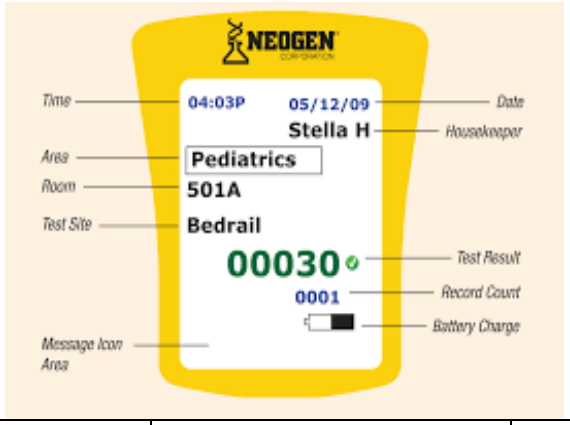


Sample activation and reading:

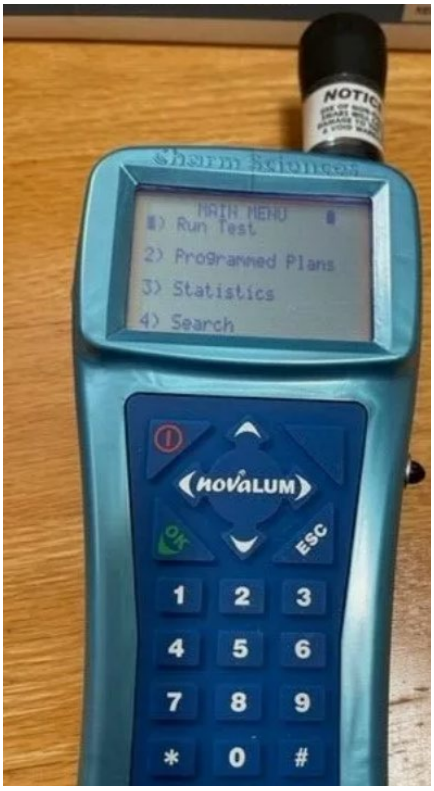
After the sample is collected, insert the sampler swab completely into the cartridge to initiate activation of ATP. Once the sampler swab tip is inserted completely, gently swirl for 2 seconds in the upright/vertical position. The cartridge must be kept/held in a vertical position and the result is read immediately or within 1 minute of activation. If you do not want to read the swab immediately after swabbing then insert the swab in the protective cartridge and do not puncture the foil until you are ready to insert it in the machine. Be sure to label the swab. Once ready to read the swab, push down on the plunger to break the seal (neogen device) or twist the cap and break the seal (Novolum-Charms device). Insert in device and press "OK" on Novolum device to run test. On the Neogen device you only need to insert the swab and close the door to initiate the reading.

- Insert the cartridge into the preprogrammed handheld ATP monitoring device, making sure that the correct collection site/surface is selected as applicable. Once the cartridge is inserted, close the door to initiate reading.

- The Neogen monitor will present a color-coded numeric value: see table below.



Test Result	Numeric Value	Color
Pass	<150	Green
Marginal	150-300	Yellow
Fail	>300	Red



Test Result	Numeric Value in RLu's
Pass	Water=0 Stainless steel≤1000 Plastic≤2500 Rubber≤3500 environmental surfaces≤3500 Epoxy≤5000 Polycarbonate≤1000

- Once surface testing is complete, download data to a computer with the Neogen® ATP software or other appropriate software installed. Data can be converted to an Excel, Word, or PDF document, and archived to RUAC share drive.

- Dispose of used ATP cartridges in the regular trash.

RODAC TESTING

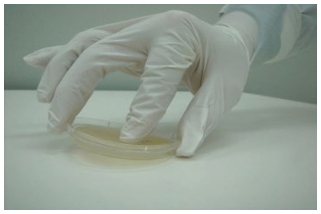
General Information

- Store RODAC plates in a refrigerator.
- Do not freeze and minimize exposure to light.
- Store plates with agar surface up and lid down to minimize the potential of contamination from condensation i.e., upside down.
- Always wear gloves when performing sampling.
- Do not use expired plates.

Sample Collection

- When taking samples using RODAC plates from walls & floors or other surfaces in the room, test only surfaces that are dry and have been sanitized within twelve hours of being sampled. Sample items such as cages and sipper tubes within five minutes of them being disinfected or as soon as the surface to be tested is dry.
- Prior to sample collection, warm the plates to room temperature for approximately 15-20 minutes with the agar up and the lid down.
- Use one of the following techniques, depending on the site to be sampled.

<u>Flat Surfaces:</u>



Gently press the rounded agar surface of the plate to the sample surface. Use a rolling motion with a light uniform pressure to ensure that the entire surface of the agar will contact the sample surface. Avoid pulling or sweeping the agar surface over the sample area as this will destroy the agar surface, thereby rendering the plate unusable.

<u>Irregular Surfaces:</u>

A sterile, cotton-tipped swab is moistened with sterile water and swabbed over such surfaces and into the corners of the equipment.

For sipper tubes, pass the swab into and out of the tube three times. For other irregular surfaces, rub the swab over a surface area roughly the same size as the base of the RODAC plate.

Following sampling, rotate the swab head gently over the surface of the agar.

- After each sample is transferred to the RODAC plate, replace the lid, tape it shut, and using a waterproof marking pen label the bottom of the plate with a description of the location the sample was obtained from (ex: Nelson Room 142, back wall)

- Store plates upside down to prevent any condensation from dripping onto the agar.

Incubation and Counting

- Place the RODAC plates upside down in the incubator located in the lab. The incubator should always be set to 36.7 degrees Celsius.
- After 24 hours, observe the plates for growth.
- After 48 hours, remove the plates from the incubator and count the colonies.
- After the results are recorded, place the plates in a bio-hazard bag for disposal.

Results

Use the following system to rate the results:

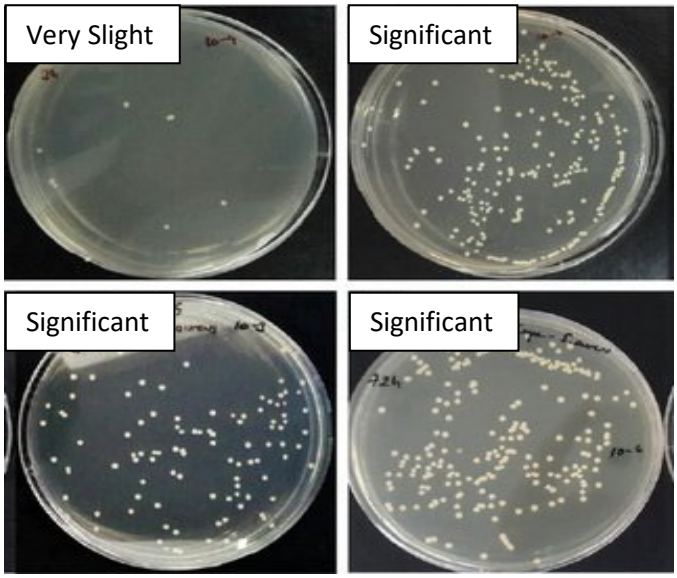
• 0-5 colonies: **None** or **Very slight** colonies (considered excellent)

• 6-15 colonies: **Slight** (considered good)

• 16-30 colonies: **Moderate** (borderline acceptable)

• 31-50 colonies: **Significant** (poor)

If significant results are noted, the area in question will be re-sanitized and new RODAC plates collected from the same area.



REFERENCE:
IACUC Policy G7 Sanitation and Monitoring of Research Equipment

Microbial Monitoring Report

Date Samples were collected
Samples obtained by:

Sample	Location/lab	Equipment	Pre RLU	Post RLU
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

RODAC Plate Report

Surface Samples obtained by:_____ Date:_____

Specimen #	Location of tested surface	Time of sampling	A-B*	Colony count per plate		Comments
				24hrs	48hrs	

* A= after cleaning, B= before cleaning

Time plates placed in incubator: _____

Incubation time (hours)	24hrs	48hrs
Plates read by		
Date		

Use the following system to rate the results:
• 0-5 colonies: **None/Very slight** (considered excellent)
• 6-15 colonies: **Slight** (considered good)
• 16-30 colonies: **Moderate** (borderline acceptable
• 31-50 colonies: **Significant** (poor)

Approved: _____