Environmental Monitoring Methods to Determine the Effectiveness of Sterilization and Sanitation Procedures in an Animal Facility

Julius E. Thigpen, PhD
QAL, CMB, NIEHS
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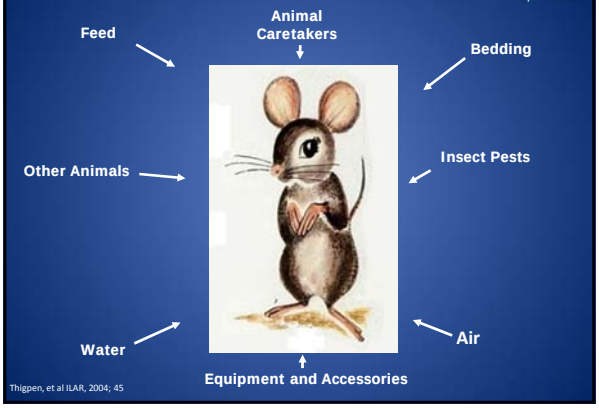
Presentation Outline

- Background: Why do EM Sanitation?
- Feed & Bedding: Autoclave or Irradiate
  - Methods to assess sterilization procedures
- Water: RO/DI , Tap or Hyper-chlorinated
  - Methods to assess water quality
- Methods to assess Sanitation Procedures:
  - Swab-rinse method, TPC's, RODACs, ATP?
  - Rooms, cubicles, floors
  - Supplies-cages, water bottles, sipper tubes
  - Cage/rack washers, etc.
- How to Determine Pass-Fail, Cut-off Values
- Advantages & Disadvantages of RODACs vs ATP Based Systems
- Pest Control Methods
- Conclusions

SOURCES OF MICROBIAL CONTAMINATION



Thigpen, et al ILAR, 2004; 45

ENVIRONMENTAL MONITORING RATIONALE

1. Recommended
   - AAALAC Accreditation
   - Guide for the Care and Use of Laboratory Animals
2. Provides Data to Support Research
3. Improves Quality of Research
   - detects/prevents potential disease outbreaks
   - prevents transmission/spread of disease agents
   - reduces number of animals used
4. Establishes Manageable Limits or Acceptable Levels
5. Detects Malfunctioning Equipment
6. Evaluates Effectiveness of Sterilizing and Sanitizing Procedures
7. Improves Sanitary Conditions

ENVIRONMENTAL MONITORING QUESTIONS

1. Responsible Person(s)
2. What to Monitor?
3. When to Monitor?
4. How Often (Frequency)?
5. Which Procedures to Use?
   - Routine procedures
   - Emergency procedures
6. Can You Use or Justify Results?

ENVIRONMENTAL MONITORING RESULTS

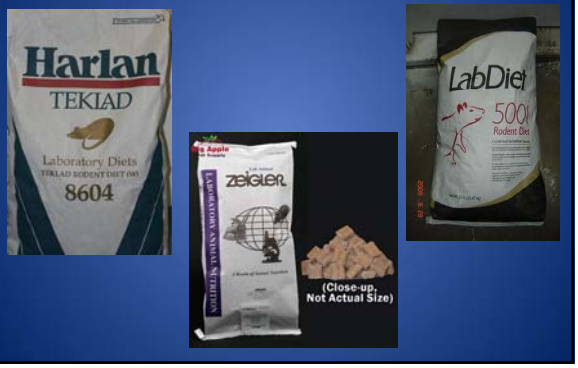
ARE THE RESULTS? :

1. Accurate? Confirmed?
2. "Within" Acceptable Limits?
3. "Outside" Acceptable Limits?

If "yes", activate corrective action plan(s).

4. Reports To:
   - Attending veterinarian
   - Facility Manager/Supervisors
   - Investigators
   - File

Rodent Feed



Bulk Shipments of Rodent Diets

Feed Grinder

QUALITY ASSURANCE OF Feed (#1)

Each Shipment Assayed For	Acceptable Concentration(TPC/g)	
1. Microbial Quality:		
Non-autoclaved Feed	Salmonella sp.	0
	TPC	<50,000
	Coliforms	< 5,000
	Fungi/Yeasts	<10,000
Autoclaved Feed	TPC	0
	Coliforms	0
Autoclaved Feed (Dispenser Bins)	Salmonella sp.	0
	TPC	<200
	Coliforms	< 30
	Fungi/Yeasts	<200
2. Nutritional Quality	Protein, Fat, Vitamins, etc.	C.S.*
	Moisture Content	11.0 ± 1.0%
3. Physical Properties	Pellet Hardness	<70.0 k.g.

*C.S. = Contract Specifications

QUALITY ASSURANCE OF ANIMAL Feed (#2)
Each Shipment Monitored For

I. Chemical Contaminants:		Permissible Levels
A. Pesticide Screens I, II, & III		
1. PCB's - Total Aroclors		< 0.2 ppm
2. Organo-phosphates - Diazinon, Malation, etc.		< 1.0 ppm
3. Chlorinated hydrocarbons - Aldin, Dieldrin Endrin, BHC's, Lindane, Chlordane, etc.		< 1.0 ppm
B. Nitrosamines - NDMA, NDEA, NDPA, etc.		<15.0 ppb
C. Mycotoxins	- Aflatoxins	<10.0 ppb
	- Fumonisins	< 2.0 ppm
D. Heavy Metals	- Arsenic	< 0.5 ppm
	- Mercury	< 0.05 ppm
	- Lead	< 1.0 ppm
	- Cadmium	< 0.l ppm

NOTE: Results Must Be Completed Before USE

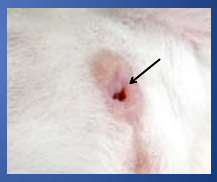
QUALITY ASSURANCE OF ANIMAL Feed (#3)

Estrogenic Assays	Maximum Concentrations
1. Total Estrogenic activity	
- Mouse uterotrophic bioassay	
- Mouse vaginal opening bioassay	
2. Phytoestrogen chemical assays-1	
- Daidzein & Genistein (NIH-31 diet)	<100µg/g diet
- Soy-alfalfa free diets	< 10µg/g diet
- AIN-76A purified (casein diet)	< 5µg/g diet
3. Estrogenic mycotoxins – chemical assays	<100ppb
- Zearalenone	

Phytoestrogens in my lab animal diet?

Vaginal Opening

Closed



Open

Thigpen et al,

Why is the VO Endpoint Important?

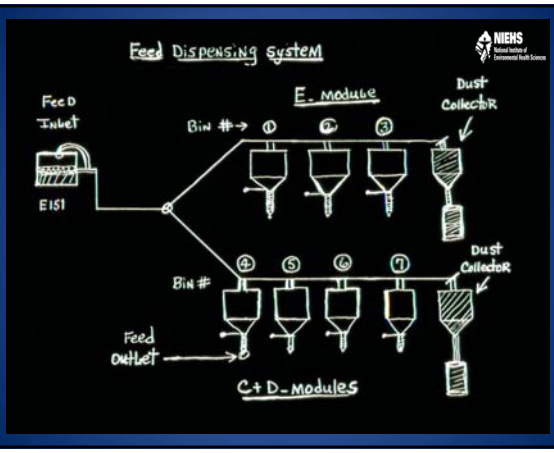
1. The vaginal canal is not open prior to the onset of puberty.
2. The bioassay is conducted in pre-pubertal female mice/rats.
3. The VO endpoint is an estrogen-sensitive biomarker of sexual maturation in rodents.
4. The time of VO can be significantly accelerated by:
   - Endogenous estrogens – Normal growth
   - High total metabolizable energy (ME)
   - Exogenous estrogens
     - Dietary estrogens/phytoestrogens
     - Zearalenone – Corn-cob bedding
     - Bisphenol A – Caging and water bottles??
5. VO determination does not require that animals be euthanized.

AUTOCLAVE FUNCTION

Items Monitored	Assayed For	Frequency
Charts	Temperature	Each Load
Tapes	Color Change	Each Load
Biological Indicators - Chemspor* - Kilits - Verify	Sterility	Weekly
Feed	Sterility	Weekly
Bedding	Sterility	Weekly
DART**	Color Change	As Needed (problems)

*Lowest Shelf on Rack, Middle of Bag, Above Drain
**Direct Air Removal Test

Dumping Rodent Diets in Automatic Feed Dispensing System



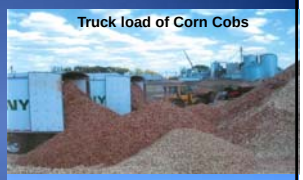
Feed Pellet Hardness Tester

Thigpen et al,

Rodent Bedding

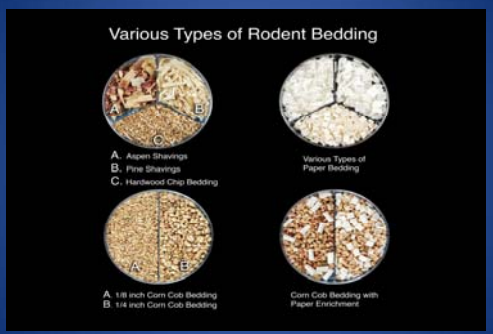
Variables Affecting Endotoxin Content in Rodent Bedding

- Type of bedding
- Bedding source
- Seasonal variability
- Storage conditions (outdoor and indoor)
- Bacterial and fungal loads
- Processing procedures
- Batch to batch variability
- Dust content
- Shipping conditions



Bulk Shipment of Hardwood Bedding

Various Types of Rodent Bedding



QUALITY ASSURANCE OF ANIMAL BEDDING (#1)

Microbial Quality	Assayed For	Acceptable (TPC/g)
I. Non-Autoclaved	Salmonella sp.	0
	TPC	5,000
	Coliforms	200
	Fungi/Yeasts	200
II. Autoclaved	Sterility	0
III. Autoclaved (Dispensers)	Salmonella sp.	0
	TPC	<100
	Coliforms	0
	Fungi/Yeasts	<100

QUALITY ASSURANCE OF ANIMAL Bedding (#2)
Each Shipment Monitored For

	Permissible Levels
1. Pesticide Screens I, II, and III	
I - PCB's - Aroclors	< 0.2 ppm
II - Organo-phosphates - Diazinon, Malathion	< 1.0 ppm
III - Chlorinated Pesticides - Aldin, BHC's, Endrin, Lindane, Etc.	< 1.0 ppm
2. Pentachlorophenol (PCP)	< 0.2 ppm
3. Physical Characteristics; Dust Particle Size, Etc.*	< 0.1%
4. Microbial Sterility*	

*See other slide.

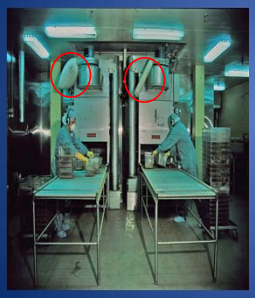
Rotating Tapping Sieve Shaker for Measuring Dust Content and Particle Size

AUTOCLAVE FUNCTION

Items Monitored	Assayed For	Frequency
Charts	Temperature	Each Load
Tapes	Color Change	Each Load
Biological Indicators - Chemspor* - Kilits - Verify	Sterility	Weekly
Feed	Sterility	Weekly
Bedding	Sterility	Weekly
DART**	Color Change	As Needed (problems)

*Lowest Shelf on Rack, Middle of Bag, Above Drain
**Direct Air Removal Test

Automated Bedding Dispensing System



Wear Appropriate PPE

- Face masks
- Hair bonnets
- Coveralls
- Respirators
- Gloves

RO/DI Water System

QUALITY ASSURANCE OF ANIMAL SUPPLIES WATER MONITORED FOR /FREQUENCY

1. **Microbial Quality**

   - Coliforms / weekly
   - TPC / weekly
   - P. aeruginosa / weekly
   - Endotoxins / weekly

2. **Chemical Quality**

   - Conductivity / weekly
   - Total Trihalomethanes / 6 months
   - Total Carbon / 6 months
   - Total Organic Halides / 6 months
   - Heavy Metals*/ 6 months
   - Individual Elements (40) / 6 months

   *Lead, Mercury, Arsenic, Barium,Cadmium, Silver, Selenium, etc.

Room and Cage/Rack Washer Sanitation

Methods of Microbial Monitoring to Determine the Effectiveness of Room and Cage/Rack Washer Sanitation Procedures

1. Replicate organism detection and counting (RODAC) or contact plates
   - Direct agar impression technique developed by the US Public Health Service and recommended by American Public Health Association for surface sampling to determine the effectiveness of cleaning procedures
2. Swab - Rinse Method (2"x4" areal for flat surfaces
   - Rinse in liquid agar (56°C)
   - Incubate-24-48 hrs, determine TPC (<50 per swab)
3. Swabs - BG broth-durham tube: culture for coliforms;
   - Water bottles/sipper tubes
4. Adenosine Triphosphate (ATP) Bioluminescence
   - Accupoint ATP - Neogen, Inc
   - NovaLUM ATP – Charm Sciences, Inc
   - System Sure Plus ATP - Hygiena

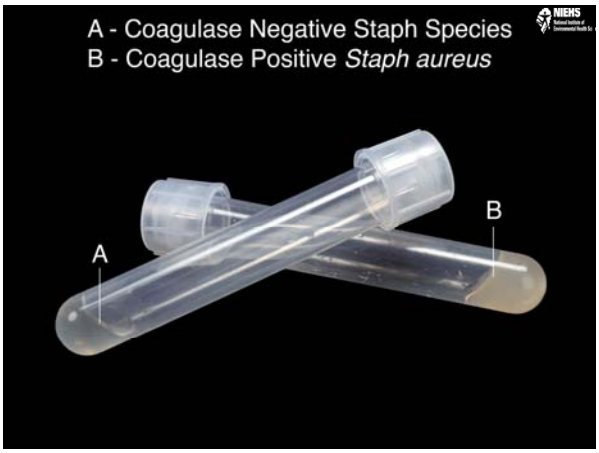
APHA Guidelines for Interpreting RODAC Plate Counts

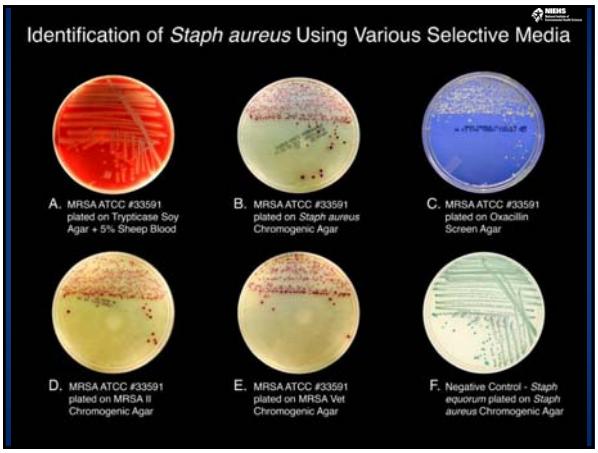
	Colonies per RODAC Plate		
Critical Areas	**Good**	**Fair**	**Poor**
Floors O.R., O.B., Isolation (terminal clean-up)	0-5	6-15	16 and up
Nursery Table Tops	0-5	6-15	16 and up
Patient rooms Floors Table Tops	0-25 0-5	26-50 6-15	51 and up 16 and up
Bathrooms Floors Sinks & Tubs Toilet seat	0-25 0-15 0-5	26-50 16-25 6-15	51 and up 26 and up 16 and up
All other floors	0-25	26-50	51 and up
All other horizontal non-porous surfaces	0-5	6-15	16 and up

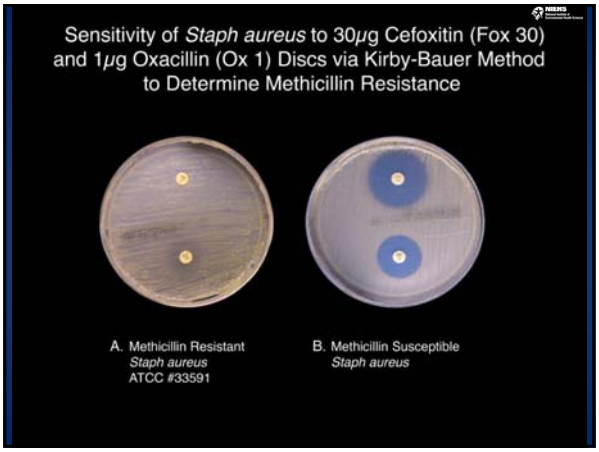
APHA, 1970.

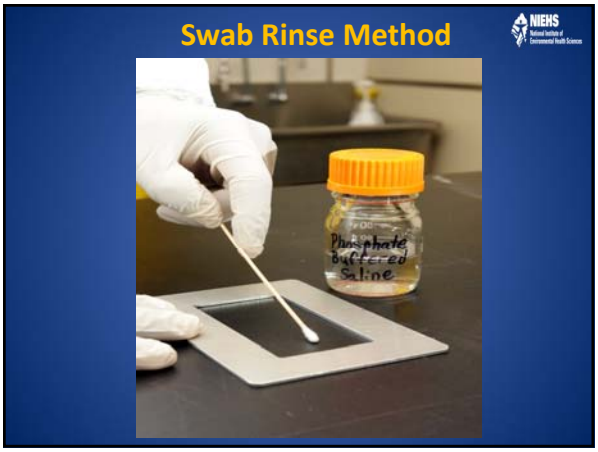
RODAC Plates

Trypticase Soy Agar with 5% Sheep Blood Showing Double Zone *Staph aureus* with (A) Complete Hemolysis (B) Partial Hemolysis & (C) Non-Hemolytic Staph









EM-SANITIZED ROOMS-Swab-Rinse Method

To Assess Sanitizing Procedures

Sampling Area*	No. Samples	Before	Microbial TPC After**
Work Station	2	___	<50
Sink	2	___	<50
Counter Top	2	___	<50
Floor	4	___	<50
Vent(s)	2	___	<50
Balance	2	___	<50

*2" x 4" Area
**>50 TPC = Room Must Be Re-sanitized

Detection of Coliforms

Temperature Tapes

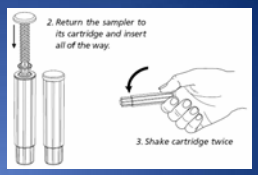
Different ATP Systems for Determining the Effectiveness of Sanitation Procedures

1. **Accupoint ATP** – Neogen, Inc
   (distributed by Quip Laboratories)

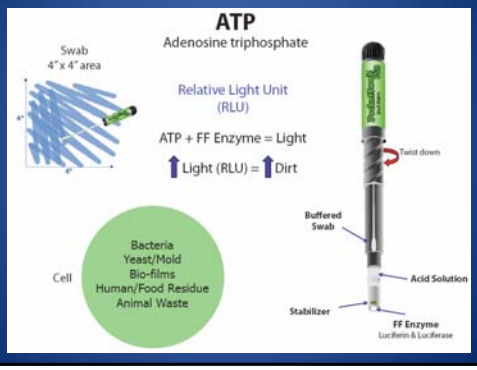
2. **NovaLUM ATP** - Charm Sciences, Inc
   (distributed by Pharmacal Research)

3. **System Sure Plus ATP** - Hygiena
   (distributed by Sanitation Solutions)

AccuPoint®



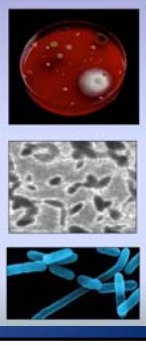
NovaLUM ATP



Dirt = Bio-load = ATP

"Dirt" is biological. It contains the potential for microbiological contaminants such as:

- Pathogenic bacteria
- Mold
- Yeast
- Viruses



Comparison of RODAC vs ATP Guidelines for Establishing Pass/Fail Cut-Off Values

Items Monitored	RODAC Readings (CFUs)			ATP Readings (RLUs)		
	Pass		Fail	Pass		Fail
Animal Rooms:						
Floor (Epoxy)	<	25	>		Must	
Walls	<	25	>		Establish	
Counter Tops	<	25	>		Own Values	
Work Station	<	25	>			
Water		N/A			Must	
Water Bottles		N/A			Establish	
Sipper Tubes		N/A			Own Values	
*Mouse Cages	<	10	>	<	2.6×10^4	>
Floors:						
-Single Mopping	<	200	>	<	120×10^4	>
-Double Mopping	<	10	>	<	40×10^4	>

**Ednie et al. 1998 vol 37(6): 71-74 Contemporary Topics* -Readings=quite variable

RODAC Plates

Advantages	Disadvantages
• Detects live bacteria, fungi and yeasts • TPC & identification • Detects beta hemolysis- *Staph aureus* • Established guidelines for interpretation of RODAC plate counts	• Results in 24-48 hours • Corrective action taken after 24-48 hours

ATP Bioluminescence Assays

Advantages	Disadvantages
• Computerized results within minutes • Allows for immediate action • Detects organic matter, food residue, biofilm, microbial agents • Measure of the actual cleanliness	• Initial cost may be expensive • Does not identify pathogenic bacteria • Must establish RLU values for each area or equipment monitored

NIEHS' Wild Life

NIEHS' Wild Life Helicobacter Profile

Animal Species	# Samples/ # Positive	Helicobacter speciation
Wild Mice	38/51 (75%)	*Helicobacter rodentium, *hepaticus, *typhlonius, *anmani, apodemus* and *species*
Flying Squirrel	4/4 (100%)	*Helicobacter bilis* and *species*
Mole	2/2 (100%)	*Helicobacter species and Helicobacter sp.* ('pancake tortoise' 16S ribosomal RNA gene)
Vole	0/4 (0%)	-
Opossum	1/1 (100%)	*Helicobacter species* and *ganmani*
Norway Rat	1/1 (100%)	*Helicobacter rodentium, ganmani* and *species*
Snake	0/2 (0%)	-
Canada Geese	3/7 (43%)	*Helicobacter species*
Five-lined Skink	0/2 (0%)	-
Box Turtles	0/3 (0%)	-
Deer	0/3 (0%)	-

*One mouse tested positive for 3 different *Helicobacter species*

Captured Wild Mice in Lunar Top with Napa Nectar and Peanut Butter

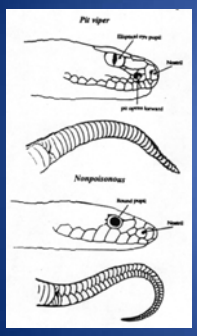
Psocids – "Booklice" – "Bark lice"

- Small, harmless, soft-bodied insects, 6 legs, segmented antennae
- Gray or light brown – winged (outdoors) or wingless (indoors)
- Large abdomen
- Narrow thorax
  - Two or three distinct thoracic segments
- Large head
  - Two, 4 or 7 faceted eyes
- Biting mouth parts (don't bite humans or animals)
- Cannot climb on smooth vertical surfaces
- Harmless, except they contaminate food

Psocids in Excessively Damp Locations

- Psocids
- Cockroaches (Oriental)
- Silverfish
- Earwigs
- Springtails
- Sow bugs
- Millipedes
- Fungus beetles

Snake!!! Is It Poisonous?



Three key differences:

Pit Viper (poisonous)

- Deep pit between eye and nostril
- Eyes = elliptical pupil
- One row of scales under tail

Non-poisonous

- Does not have a pit
- Eyes = round pupil
- Two rows of scales

Conclusions

- Both RODAC and ATP systems can be used to assess effectiveness of sanitation procedures
- Animal research facilities differ and may use different sanitizing procedures, thus no one method fits all EM programs
- What to monitor, frequency of monitoring, and methods used will be determined by ones budget and the goals of the animal care program

Quality Assurance Lab

Questions?