Detection of Prevalent Mouse Pathogens Using Sentinel Swabs as an Alternative to Sentinel Animals

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Introduction

Traditionally, Health Monitoring Programs have been performed by utilizing sentinel animals for the detection of pathogens that affect research. With the introduction of the 3Rs initiative, alternatives to the utilization of live animal as the main health monitoring detector has been investigated.

Different approaches like rack plenum filters, direct sampling from animals, and increases to the intervals between tests are the most utilized substitutes. Among those alternatives, plenum filters have become the standard. However, not all facilities have racks containing plenum filters or the financial resource to change their facilities to use plenum filter containing racks.

As an alternative for plenum filter testing, we have investigated the use of swabs incubated with soiled bedding. A panel of the most prevalent pathogens found in mice were tested utilizing pet shop mice as source of the agents. The data presented in this poster indicates that swabs incubated with soiled bedding are an effective alternative to the use of live animals in animal health monitoring.

Virus

Viruses-1

	EDIM	MAD-1	MAD-2	MHV	MNV	MPV	MMV	TMEV
Positive Cages	0/4	0/4	3/4	1/4	4/4	1/4	0/4	3/4
Fecal Pellet Pool								
1 Month	-	-	+	+	+	-	-	+
2 Month	-	-	+	-	+	-	-	+
3 Month	-	-	+	-	+	-	-	+
Sentinel Swab								
1 Month	-	-	+	+	+	+	-	+
2 Month	-	-	+	+	+	+	-	+
3 Month	-	-	+	+	+	+	-	+

Viruses- 2

	EDIM	MAD-1	MAD-2	MHV	MNV	MPV	MMV	TMEV
Positive Cages	0/3	0/3	3/3	3/3	1/3	3/3	0/3	0/3
Fecal Pellets Pool								
Month 1	-	-	+	+	+	+	-	-
Month 2	-	-	+	-	+	+	-	-
Month 3	-	-	+	+	+	+	-	-
Sentinel Swab								
Month 1	-	-	+	-	+	+	-	-
Month 2	-	-	+	+	+	+	-	-
Month 3	-	-	+	+	+	+	-	-

Bacteria

Bacteria- 1

	Helico spp.	B-Streps	S.aureus	K.oxytoca	K.pneumoniae	R.heylii	R. pneumotropica
Positive Cages	4/4	0/4	0/4	0/4	0/4	0/4	3/4
Fecal Pellet Pool							
1 Month	+	-	-	-	-	-	-
2 Month	+	-	-	-	-	-	+
3 Month	+	-	-	-	-	-	+
Sentinel Swab							
1 Month	+	-	-	-	-	-	+
2 Month	+	-	-	-	-	-	-
3 Month	+	-	-	-	-	-	+

Bacteria- 2

	Helico spp.	B-Streps	S.aureus	K.oxytoca	K.pneumoniae	R.heylii	R. pneumotropica
Positive Cages	3/3	0/3	0/3	0/3	0/3	0/3	2/3
Fecal Pellets Pool							
Month 1	+	-	-	-	-	-	+
Month 2	+	-	-	-	-	-	+
Month 3	+	-	-	-	-	-	+
Sentinel Swab							
Month 1	+	-	-	-	-	-	+
Month 2	+	-	-	-	-	-	+
Month 3	+	-	-	-	-	-	+

Methods

Pet Shop mice were obtained from commercial vendors and separated into cages in cohorts of 3-4 animals per cage. Cage bedding changes were performed weekly. 8-10 tablespoons from the soiled bedding were collected from each cage and added to a clean cage containing several swabs. After a week, the soiled bedding from the sentinel swab cage was disposed of and new soiled bedding was added. This process was performed weekly during cage changes.

The swabs were tested at monthly intervals for up to 3 months via PCR. The swabs were tested for MMV, MPV, MAD-1, MAD-2, MHV, EDIM, TMEV, MNV, Pinworms, Fur mites, *Helicobacter* and *Rodentibacter,* among others.

Parasites

Parasites- 1

	Furmites	A. tetraptera	Syphacia spp.	Crypto spp.
Positive Cages	0/4	0/4	0/4	0/4
Fecal Pellets Pool				
Month 1	-	-	-	+
Month 2	-	-	+	+
Month 3	-	-	+	-
Sentinel Swab				
Month 1	-	-	-	+
Month 2	-	-	+	+
Month 3	-	-	-	+

Parasites-2

	Furmites	A. tetraptera	Syphacia spp.	Crypto spp.
Positive Cages	0/3	1/3	0/3	3/3
Fecal Pellet Pool				
1 Month	-	+	+	+
2 Month	-	-	+	+
3 Month	-	+	-	+
Sentinel Swab				
1 Month	-	+	+	+
2 Month	-	+	+	+
3 Month	-	-	+	+

Summary

- **Based on the data presented in this poster, we can conclude that incubation of swabs with soiled bedding is a viable alternative to the use of sentinel mice.**
- **This method allows compliance with the 3Rs initiative.**
- **The cost savings from purchasing sentinel mice, new racks that accommodated plenum filters and the filters, will allow the facilities to utilize their financial resources more efficiently.**
- **Another advantage of this system is the ease of sample collection and technical staff training.**

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SENTINEL SWAB

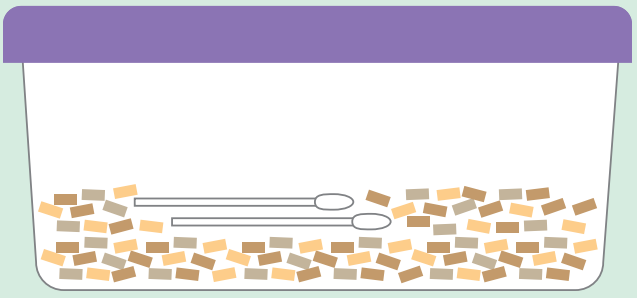
A better alternative for plenum filter testing

PCR has become one of the most useful tools in pathogen detection over the last 20 years. The advent of PCR has allowed for a more sensitive detection of pathogens in animal colonies, while decreasing the number of sentinel animals used in health surveillance.

In this document we explain how to use swabs as a replacement for sentinel animals. This alternative does not require special systems and it can be used with any type of rack and cage. **The process is similar to the traditional sentinel program using live animals, but instead of using animals, our solution uses swabs.**

Materials Used:

- Gloves
- Sterile Swabs
- Measuring Tablespoon
- Sentinel Cage
- 50 ml Conical Tube
- Permanent Markers
- Shipping Box or Envelope



(Instructions on reverse side)

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SENTINEL SWAB

VRL ANIMAL HEALTH DIAGNOSTICS

PROCEDURE

(Hang this in your lab for quick reference)

1. **Put gloves on**
   Note: Change gloves when collecting dirty bedding from different racks.
2. **Remove lid from the designated sentinel cage**
3. **Place two sentinel swabs in a designated sentinel cage**
   Note: Two swabs are used in the event a retest is necessary due to unexpected results.
4. **When changing cage bedding, collect approximately 1 Tbsp of dirty bedding and place in designated sentinel cage**
   Note: The dirty bedding should be collected from the area where the animal defecated and urinated.
5. **Replace lid on the designated sentinel cage**
6. **Shake the cage, swabs should remain in the dirty bedding until the next cage change**
7. **In a week or two, remove the dirty bedding from the cage (keep the swabs in the cage)**
   Note: The timing of dirty bedding changes is dependent on your husbandry program.
8. **Repeat steps 4-7 through the end of your monitoring cycle**
9. **At the end of the cycle, collect the swabs and place in a collection tube**
10. **Label the collection tubes and submit swabs to VRL for PCR testing**
    Note: Two swabs are used in the event a retest is necessary due to unexpected results.

REFERENCE: Detection of Prevalent Mouse Pathogens Using Sentinel Swabs as an Alternative to Sentinel Animals. Leon, A; Lankasky, J; Walters, R; Pitts, D; Berger, R; Zao, C. ALAAS 2021