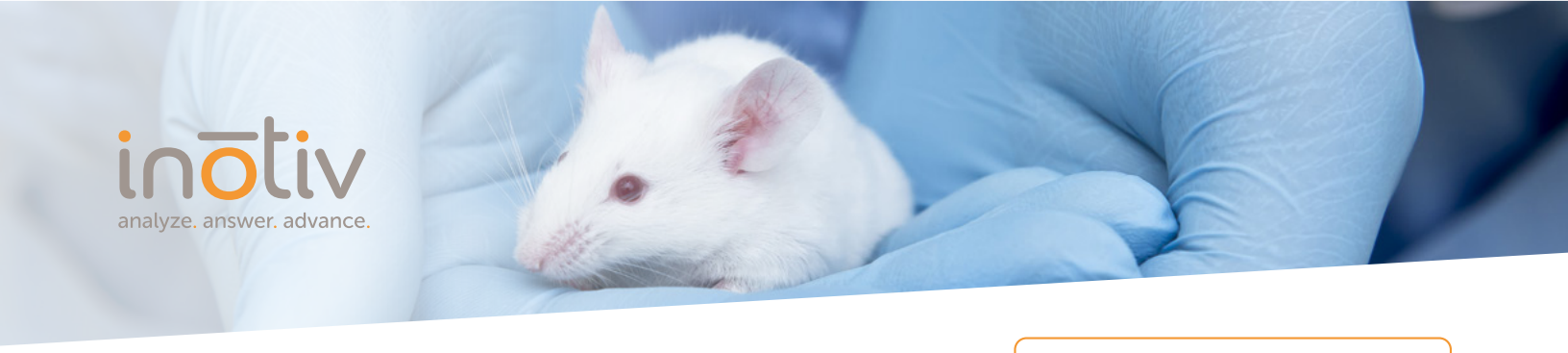

inotiv
analyze. answer. advance.

Research Models and Services
Health Monitoring

A CASE STUDY

Inotiv Sentinel Free Testing Solution (SiFT™)

Introduction

In laboratory animal facilities, monitoring the health of rodent colonies is essential for ensuring animal welfare and upholding the integrity of biomedical research. Traditionally, soiled bedding sentinel (SBS) mice have been employed as a surveillance tool, whereby mice of a known health status are exposed to soiled bedding from colony cages to detect the presence of infectious agents through subsequent serological or microbiological testing. However, the use of sentinel animals raises ethical concerns, increases animal use, and incurs significant labour and housing costs.

In response to these challenges, the use of environmental monitoring through filter materials, commonly referred to as sentinel free testing, has gained traction as an effective and ethical alternative. Two primary approaches have emerged: filters positioned within individually ventilated cage (IVC) air handling units (AHUs) which capture airborne contaminants; and in-cage filters which are used in a way that closely mirrors traditional soiled bedding sentinel protocols. In this method, a filter is placed into an empty cage and exposed to soiled bedding collected from multiple colony cages over time. This setup allows the filter to capture pathogens and biological particulates shed by the animals, enabling detection without the need for live sentinels.

At Inotiv, we have developed SiFT, our proprietary solution for your in-cage sentinel free monitoring system. SiFT was developed in collaboration with a leading biomedical research institution and validated in a facility where infectious agents are routinely identified using soiled bedding sentinels.

SiFT was evaluated in mouse rooms in tandem with soiled bedding sentinels. Both SiFT filters and dirty bedding sentinels were seeded with soiled bedding during the cleanout of occupied cages every week, for a period of up to 12 weeks.

PROTOCOL

On each rack, sentinel animals were seeded as per the unit's weekly seeding protocol.

Alongside each sentinel cage, a separate clean cage, designated as the soiled bedding cage, was set up to house the SiFT filter material.

SiFT filter material was placed into each clean mouse cage designated as the soiled bedding cage and seeded with 15ml of dirty bedding per occupied stock cage on each weekly cleanout.

The SiFT filters were left in contact with the dirty bedding and the cage was vigorously shaken once per week to promote particle capture. On each weekly cleaning cycle, the filters were transferred into freshly prepared soiled bedding cages and the seeding cycle repeated. This cycle continued for 12 weeks in total.

Filters were collected and analysed with Inotiv's FELASA Annual Specific and Opportunistic Pathogen-Free (SOPF) profile at intervals of 4, 8 and 12 weeks.

To confirm that the organisms detected by SiFT originated from the animals and not the surrounding environment, faecal samples were also collected from the dirty bedding cages and analysed in parallel.

Addendum 1 for our list of screening agents.

SUMMARY OF RESULTS

13 Pathogens (covering viruses, bacteria and parasites (Appendix 1) that were known to be present in the facility either from historical results since February 2022 and current sentinel screens (performed prior to and during the trial)

VIRUSES	
1	*Mouse Hepatitis Virus*
2	*Mouse Norovirus*
BACTERIA	
3	*Helicobacter ganmani*
4	*Helicobacter hepaticus*
5	*Helicobacter mastomyrinus*
6	*Helicobacter typhlonius*
7	*Rodentibacter heylii*
PARASITES	
8	*Aspiculuris tetraptera*
9	*Chilomastix spp*
10	*Entamoeba spp*
11	*Spironucleus muris*
12	*Syphacia obvelata*
13	*Tritrichomonas spp*

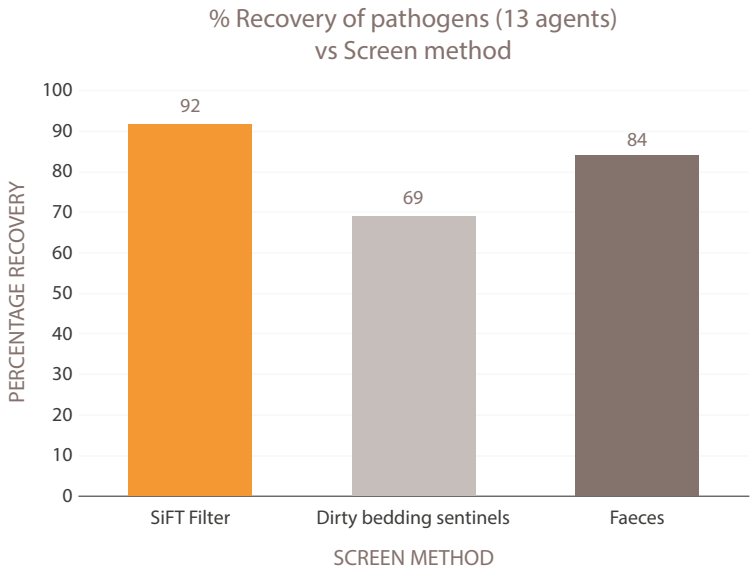

Fig.1 Collated data for % Recovery of Pathogens by Method showed that SiFT achieved a markedly higher detection rate of infectious agents compared to traditional soiled bedding sentinels. Faecal samples collected from the dirty bedding cages showed strong correlation with the pathogens identified by SiFT, supporting the validity of the results.

CONCLUSIONS

SiFT demonstrated a clear improvement in pathogen detection rates in laboratory mouse colonies compared to conventional sentinel methods. While not every pathogen was detected at every time point, the overall performance of the system showed more than a 33% increase in recovery of infectious agents.

It is important to note that some variability in detection was observed during the study. This was primarily attributed to inconsistencies in the seeding protocol and cage-shaking procedure and specifically, variations in the volume of soiled bedding collected per stock cage and differences in shaking duration or technique. These factors have since been addressed through protocol refinements aimed at enhancing the consistency and sensitivity of SiFT. Adherence to Inotiv's standardised protocol is essential, as consistent application is critical to ensuring reliable and reproducible results.

Additionally, intermittent shedding, clearance or degradation of agents can impact detection. This is a critical consideration and supports the idea that alternative or complementary screening methods may still prove valuable to ensure robust and comprehensive colony surveillance.

Further to this, no unexpected infectious agents were detected by SiFT (i.e. not previously detected in the unit by traditional sentinels), indicating that the amplification of background nucleic acid from sources such as diet, may not be readily amplified using this method.

In summary, SiFT represents a significant advancement in sentinel-free health monitoring, offering enhanced sensitivity, reduced animal use, and greater operational efficiency. With robust validation, refined protocols, and a commitment to scientific integrity, SiFT provides a reliable and ethical alternative to traditional methods, setting a new standard for pathogen surveillance in laboratory animal facilities.

ADVANTAGES OF USING A SENTINEL FREE TESTING SYSTEM

Direct Monitoring of Colony Animals:
This provides greater sensitivity, especially for pathogens not shed in high amounts in bedding or for agents that are inconsistently transmitted.

Non-Invasiveness and Animal Welfare:
Sentinel-free methods reduce or eliminate the need for purpose-bred sentinel animals, aligning with the 3Rs (Replacement, Reduction, Refinement) by minimizing animal use.

Increased Sensitivity and Specificity:
Molecular methods such as PCR enable the detection of low levels of pathogen DNA/RNA that might not lead to detectable infections in sentinels.

Faster Turnaround Time:
Environmental sampling and PCR-based diagnostics can yield results quickly, allowing for more responsive colony management.

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ADDENDUM 1

SiFT or Sentinel Free Filter Testing Health Monitoring for Mouse

Inotiv's SiFT or Sentinel Free Filter Testing Health Monitoring service offers an animal-welfare conscious approach to colony health surveillance. Instead of relying on traditional sentinel animals, this service utilizes whole-rack-level health monitoring using filters placed within sentinel free soiled bedding cages. These samples are then analyzed using highly sensitive qPCR technology to detect the presence of pathogens. Contact us and let us help you design a program for your facility.

VIRUSES	QUARTERLY* (SiFT-MQ)	QUARTERLY SOPF* (SiFT-MQS)	ANNUAL* (SiFT-MA)	ANNUAL SOPF* (SiFT-MAS)	COMPREHENSIVE (SiFT-MC)
Mouse Hepatitis Virus	X	X	X	X	X
Mouse Minute Virus	X	X	X	X	X
Mouse Parvovirus	X	X	X	X	X
Mouse Rotavirus (EDIM)	X	X	X	X	X
Murine Norovirus	X	X	X	X	X
Theiler's Mouse Encephalomyelitis Virus	X	X	X	X	X
Ectromelia Virus			X	X	X
Lymphocytic Choriomenigitis Virus			X	X	X
Mouse Adenovirus (MAV-1 and MAV-2)			X	X	X
Mouse Kidney Parvovirus			X	X	X
Pneumonia Virus of Mice			X	X	X
Respiratory Enteric Virus (REO3)			X	X	X
Sendai Virus			X	X	X
Hantaan Virus+					X
K Virus (Mouse Polyoma Virus)					X
Lactate Dehydrogenase Elevating Virus					X
Mouse Thymic Virus					X
Murine Cytomegalovirus					X
Polyoma Virus					X

*All agents meet FELASA guidelines
+Testing includes Seoul and Sin Nombre subtypes

BACTERIA AND FUNGI	QUARTERLY* (SiFT-MQ)	QUARTERLY SOPF* (SiFT-MQS)	ANNUAL* (SiFT-MA)	ANNUAL SOPF* (SiFT-MAS)	COMPREHENSIVE (SiFT-MC)
Helicobacter spp+	X	X	X	X	X
Rodentibacter heylii	X	X	X	X	X
Rodentibacter pneumotropicus	X	X	X	X	X
Beta-hemolytic Streptococcus (Non-Group D)	X	X	X	X	X
Streptococcus pneumoniae	X	X	X	X	X
Klebsiella oxytoca		X		X	X
Klebsiella pneumoniae		X		X	X
Proteus spp.		X		X	X
Pseudomonas aeruginosa		X		X	X
Staphlyococcus aureus		X		X	X
Citrobacter rodentium			X	X	X
Clostridium piliforme			X	X	X
Corynebacterium kutscheri			X	X	X
Mycoplasma pulmonis			X	X	X
Salmonella spp.			X	X	X
Streptobacillus monoliformis			X	X	X
Bordatella bronchiseptica					X
Corynebacterium bovis					X
Encephalitozoon cuniculi					X
Filobacterium Rodentium (CAR Bacillus)					X
Pneumocystis murina					X

*All agents meet FELASA guidelines

+All positive results for Helicobacter will be speciated through additional testing.

PARASITES	QUARTERLY* (SiFT-MQ)	QUARTERLY SOPF* (SiFT-MQS)	ANNUAL* (SiFT-MA)	ANNUAL SOPF* (SiFT-MAS)	COMPREHENSIVE (SiFT-MC)
Ectoparasites					
Myobia musculi	X	X	X	X	X
Myocoptes musculinus	X	X	X	X	X
Radfordia affinis	X	X	X	X	X
Endoparasites					
Aspiculuris tetraptera	X	X	X	X	X
Chilomastix spp.	X	X	X	X	X
Eimeria spp.	X	X	X	X	X
Entamoeba muris	X	X	X	X	X
Giardia muris	X	X	X	X	X
Spironucleus muris	X	X	X	X	X
Syphacia muris	X	X	X	X	X
Syphacia obvelata	X	X	X	X	X
Tritrichomonas spp.	X	X	X	X	X

*All agents meet FELASA guidelines

For more information on requesting sampling kits or testing for other samples or species, contact us at **healthmonitorlab@inotiv.com**

Please contact us if you have any questions.

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