Eradicating sentinel dependency: animal free enhanced detection in rodent health monitoring

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Introduction

What is ghost sentinel®?

Ghost sentinel® is a specialised environmental monitoring system developed by Fera Science Ltd, for detecting rodent pathogens without the need for sentinel animals. Based on the sentinel free soiled bedding (SFSB) method, the ghost sentinel® uses soiled-bedding media sampling combined with proprietary real-time PCR analysis to optimise the detection of infectious agents in rodent colonies.

Recent studies have demonstrated that SFSB is an effective strategy for monitoring rodents maintained in individually ventilated cage systems and other cage types.[1,2,3] However a potential pitfall of shake cages was described by O'Connell *et al.*, 2021.[2] They reported that in several cases for MHV (Mouse Hepatitis Virus), MNV (Mouse Norovirus), TMEV (Theiler's Murine Encephalomyelitis Virus) and in one case *Aspiculuris tetraptera,* in-cage filters were positive after one month of exposure and negative after two months of exposure to soiled bedding. Either nucleic acids had degraded over time, or the collection media had not had sufficient contact with soiled bedding because of the reduction in size of the filter sampled at month two. To overcome these pitfalls, we increased the capture surface area of SFSB media by using multiple collection media in a single cage and freezing at time points, stabilising nucleic acids to provide improved detection.

Method

Exposure of the ghost sentinel®

Soiled bedding was transferred to a ghost sentinel® cage containing three SFSB media according to each facilities own soiled bedding transfer protocol (Figure 1). In our initial trial, cages were shaken for 30 seconds once per week. For subsequent trials the cage shake was increased to twice weekly for two minutes. After transfer of soiled bedding and shaking for two minutes, the cages were placed back into the racks until the next cage change and cage shake. For all trials this process was repeated for 13 weeks.

At weeks five and nine, one of the collection media was removed from the cage and frozen at -20°C in a 50 ml conical tube or sealed sterile bag. At 13 weeks all three SFSB media were returned to the laboratory for analysis. For the initial trial the individual media was analysed. In the later trials all three media were pooled for nucleic acid extraction and real-time PCR analysis.

Figure 1. Soiled bedding shake cage.

Laboratory trials

SFSB media trial

A comparison of five collection media including commercial exhaust dust test (EDT) filters was carried out to determine the most effective in-cage media. EDT filters have been previously established as a suitable material for the detection of micro-organisms and this was used as gold standard. Ten unused collection media from each batch of material were analysed to determine the presence of any residual nucleic acid using a broad range 16S ribosomal RNA (rRNA) assay. Collection media types with high nucleic acid background were excluded from the study to prevent potential interference with our down-stream analysis. All six-collection media were placed into the same cage and soiled bedding was transferred weekly. The cage was shaken twice weekly and the copy number was determined after four weeks using a 16S rRNA assay to determine total bacterial adherence. We conducted three replicates and used the mean copy number as the result. The results of the collection media trial are presented in Figure 2. All five of the collection media outperformed the existing commercial EDT collection media.

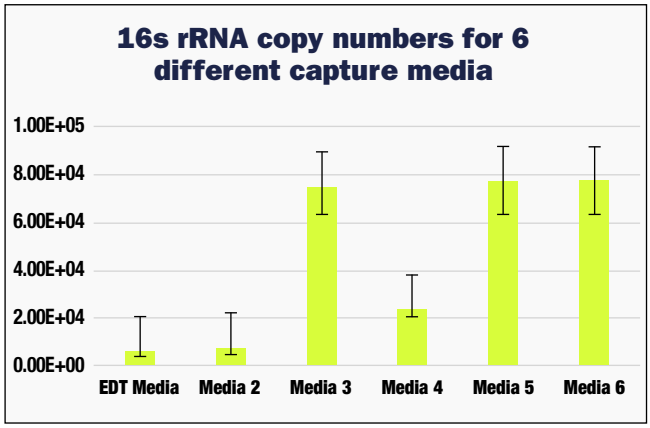

Figure 2. 16S rRNA copy number of five different capture media and EDT media.

Extraction efficiency

To assess potential inhibition by the collection media, a novel plasmid was added to the PCR lysis buffer prior to extraction and confirmation of no loss of plasmid during downstream PCR analysis was confirmed.

Different volumes of lysis buffer were analysed to determine the optimal volume to add to the shake cage collection media for the pre-analytical phase of testing by determining the total nucleic acid recovery. The pre-analytical phase of testing was conducted similarly to our EDT collection media screening process, whereby lysis buffer was added directly to the collection media and homogenised on a bullet blender® 5E pro (Thistle Scientific).

Facility trials

Initial trials were performed in collaboration with the University of Aberdeen. During the first trial, the ghost sentinel® cage was shaken once per week for 30 seconds and the collection media were individually extracted for analysis in the laboratory. In the second trial shaking was increased to twice a week for two minutes and the three-collection media were pooled for analysis in the laboratory.

The second trial resulted in a marked increase in copy number because of the increasing shaking frequency and pooling of the shake cage media measured by 16S rRNA analysis presented Table 1. Up to 1.2 million DNA copies could be detected on the surface of the pooled collection media in the second trial, in contrast to 1073 DNA copies in the initial trial.

The results of the initial trial can be found in Table 2. Detections were inconsistent in the initial trial over time. Five racks were negative at month three but positive at either month one or two.

Individual Initial Trial Collection media		Pooled Second Trial Collection media
1.30E+02	5.29E+02	6.01E+05
1.30E+02	2.62E+02	1.47E+05
1.30E+02	2.62E+02	2.98E+05
2.62E+02	1.30E+02	1.47E+05
1.30E+02	6.41E+01	6.01E+05
1.30E+02	6.41E+01	6.01E+05
6.41E+02	6.41E+02	2.98E+05
1.07E+03	1.07E+03	1.22E+06
2.62E+02	6.41E+01	2.98E+05
5.29E+02	5.29E+02	7.28E+05
2.62E+02	1.30E+02	1.22E+06
6.41E+01	6.41E+01	1.47E+05
2.62E+02	1.30E+02	7.28E+04
1.30E+02	2.62E+02	8.83E+03
5.29E+02	5.29E+02	

Table 1. 16S rRNA copy number from individual collection media in the initial pilot trial versus second trial copy number.

		Initial Individual Collection Media Results		
		Filter 1 1 month exposure	Filter 2 2 months exposure	Filter 3 3 months exposure
Rack 1	*Rodentibacter pneumotropicus* *Trictrichomonas species*	+ +	– +	– +
Rack 2	*Trictrichomonas species*	+	+	+
Rack 3	*Chilomastix species* *Trictrichomonas species*	– –	+ +	– +
Sample 4	*Rodentibacter heylii* *Chilomastix species* *Trictrichomonas species*	– – +	+ + +	– + –
Sample 5	*Trictrichomonas species*	–	+	+
Sample 6	*Chilomastix species* *Trictrichomonas species*	– +	+ +	– +
Sample 7	*Trictrichomonas species*	–	–	+
Sample 8	*Proteus mirabilis*	–	–	+
Sample 9	*Rodentibacter heylii* *Trictrichomonas species*	+ –	– –	+ +
Sample 10	*Rodentibacter pneumotropicus* *Chilomastix species* *Trictrichomonas species*	– – –	– – +	+ + +
Sample 11	*Chilomastix species* *Trictrichomonas species*	+ +	– –	+ +
Sample 12	*Trictrichomonas species*	+	–	+

Table 2. Initial Aberdeen trial results of individual media exposed for 1 month, 2 months and 3 months.

Ghost sentinel® SFSB collection media versus sentinel animals second trial

During the Aberdeen University trials, the ghost sentinel® was compared to traditional screening of sentinel animals. Sentinel animals were screened by an alternative laboratory via necropsy, serology, culture, microscopy and PCR. The results shown in Table 3 demonstrated improved detection for all agents prevalent in the facility.

	SBS Result Positives/total	GSS Result Positives/total	History
Chilomastix species	14/30	13/15	Yes
Entamoeba muris	2/30	11/15	Yes
Helicobacter ganmani	Not tested	10/15	Yes
Helicobacter rodentium	Not tested	6/15	Yes
Helicobacter species	Not tested	14/15	Yes
Helicobacter typhlonius	Not tested	12/15	Yes
Klebsiella pneumoniae	0/30	8/15	Yes
Mouse Norovirus	5/30	12/15	Yes
Rodentibacter heylii	0/30	14/15	Yes
Rodentibacter pneumotropicus	0/30	8/15	Yes
Staphylococcus aureus	0/30	1/15	Yes
Tritrichomonas species	4/30	15/15	Yes
Enteromonas species	6/30	Not tested	Yes
Pasteurellacae	2/30	Not tested	Yes

Table 3. Comparison of soiled bedding sentinel screening results versus the ghost sentinel®.

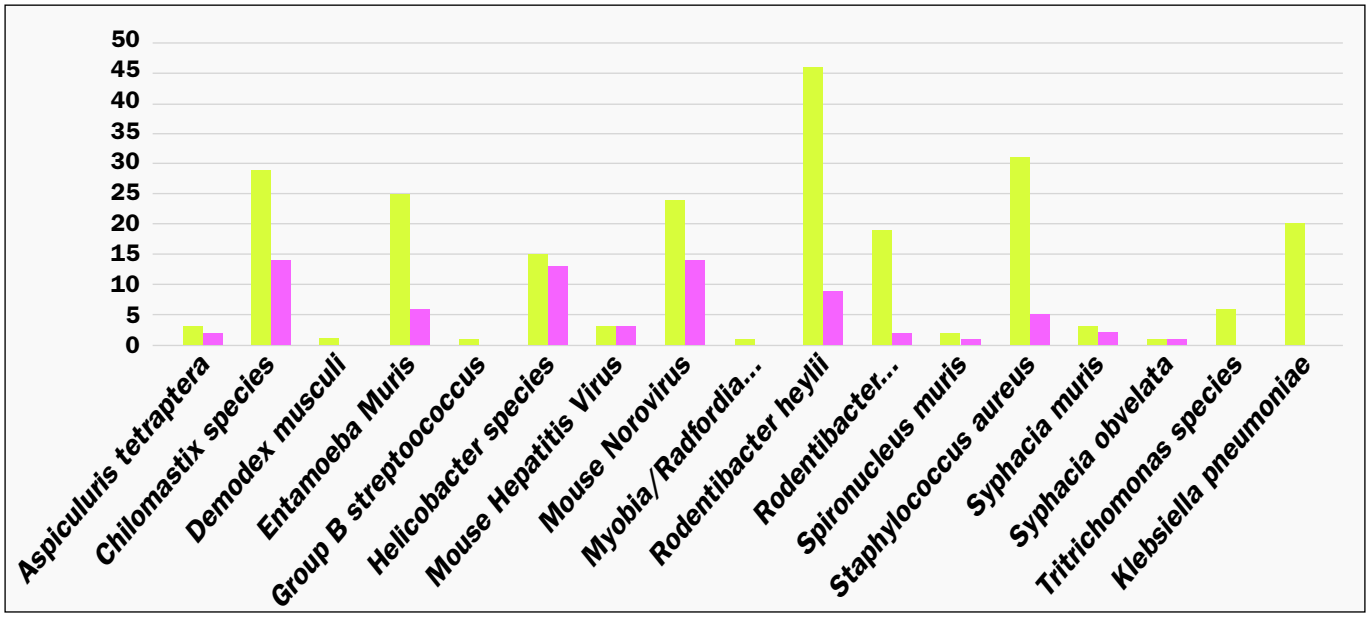

Figure 3. Alternative screening methods versus ghost sentinel® detection.

Following the Aberdeen trials, multiple trials were conducted over a two-year period in 2023 and 2024 resulting in 178 screen comparisons. This included soiled bedding sentinels, direct screening of biological materials from sentinels using non-sacrificial methods and exhaust dust screening. The results of the trials demonstrated significantly improved detection using the ghost sentinel® versus other methods of detection (Figure 3).

Cage numbers trial

The volume of soiled bedding and numbers of cages that could be reliably transferred to the ghost sentinel® cage was determined. A comparison was undertaken with ghost sentinel® and direct sampling of faeces from sentinels and found that for 204 cages the ghost sentinel® detected MNV, *R. heylii*, Trictrichomonas species, E. muris, S. aureus, H. ganmani, *H. typhlonius* and *H. hepaticus*. For the same racks during the same exposure period, MNV, *H. hepaticus* and E. muris were detected in faeces from sentinel animals.

Residual nucleic acid trial

Facility trials were conducted to determine if the ghost sentinel® could provide a method of screening racks where residual nucleic acid presents a challenge. One drawback of EDT screening is that residual nucleic acid after a rack has been depopulated and cleaned, can result in persistent positive results during routine screening. The facility for this trial had a history of pinworm positives and the facility had made efforts to eradicate it from the facility. We found that the EDT exhaust filter media remained positive for several months after cleaning and depopulation of the racks.

Summary

Fera's ghost sentinel® provides a more sensitive method of detecting micro-organisms than soiled bedding sentinels and direct sampling of sentinels for any cage type. The incorporation of three collection media increases the surface area of the in-cage media and may allow detection of organisms that may otherwise degrade over time by freezing at one month time points. A significant constituent of our final media incorporates cellulose. Cellulose forms a fibrous structure with a large surface area, which may enhance its ability to capture and bind pathogens. In addition to increased surface binding capacity, the hydroxyl groups in the shake cage collection media make it highly hydrophilic, allowing it to readily interact with urine and faeces where large numbers of organisms are shed. Cellulose is biocompatible and non-toxic, making it safe for use for pathogen detection in rodent facilities.

References

1 **Dubelko, A.R., Zuwannin, M., McIntee, S.C., Livingston, R.S., Foley, P.L.** (2018). PCR testing of filter Material from ICV lids for microbial monitoring of mouse colonies. J Am Assoc Lab Anim Sci 57:477–482. 10.30802/AALAS-JAALAS-18-000008.

2 **O'Connell, K.A., Tigyi, G.J., Livingstone, R.S., Johnson, D.L., Hamilton, D.J.** (2021). Evaluation of in cage filter paper as a replacement for sentinel mice in the detection of murine pathogens.J Am Assoc Lab Anim Sci. Mar:60(2) 160-167 10.30802%2FAALAS-JAALAS- 20-000086

3 www.fera.co.uk/animal-health-monitoring