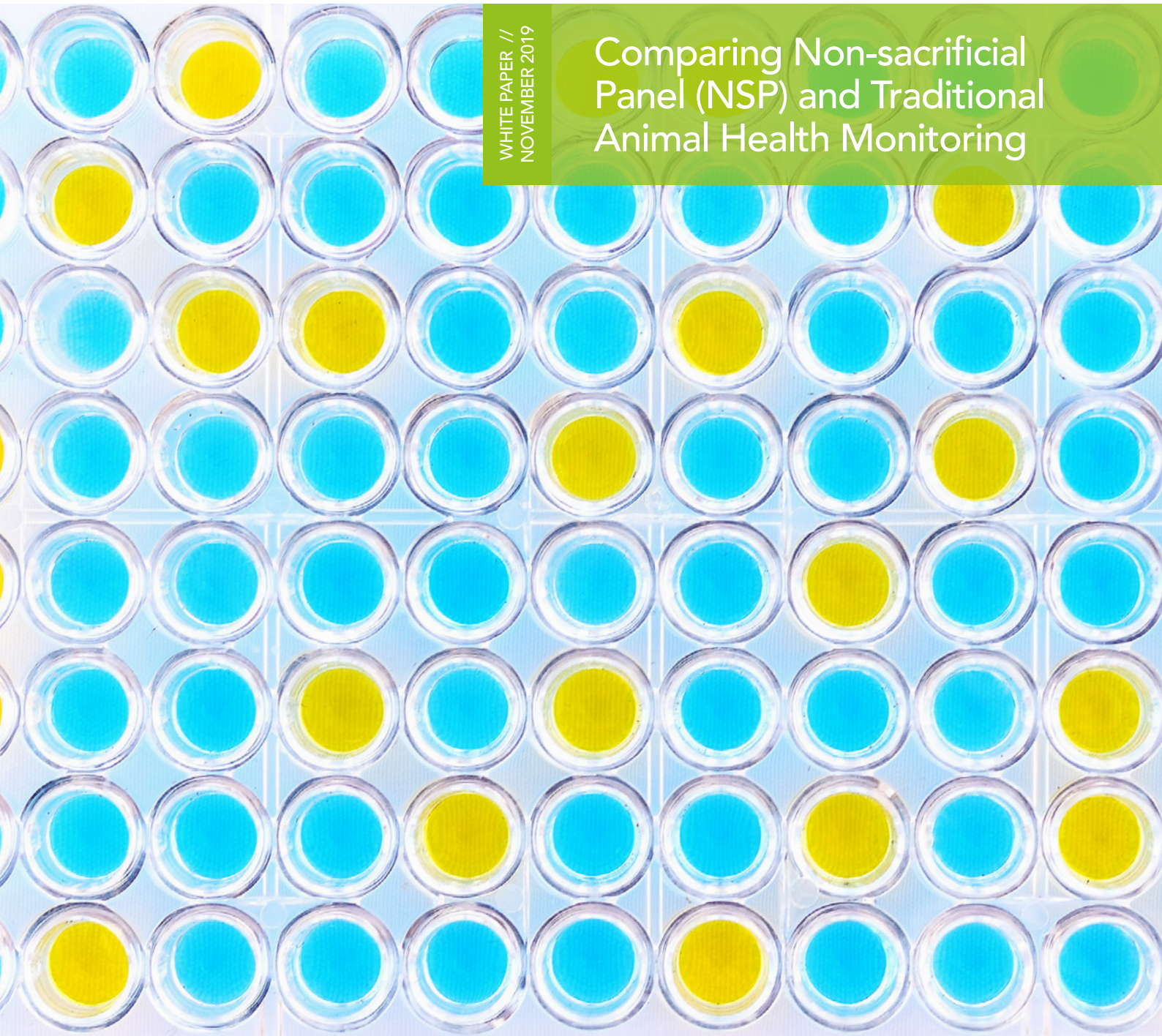


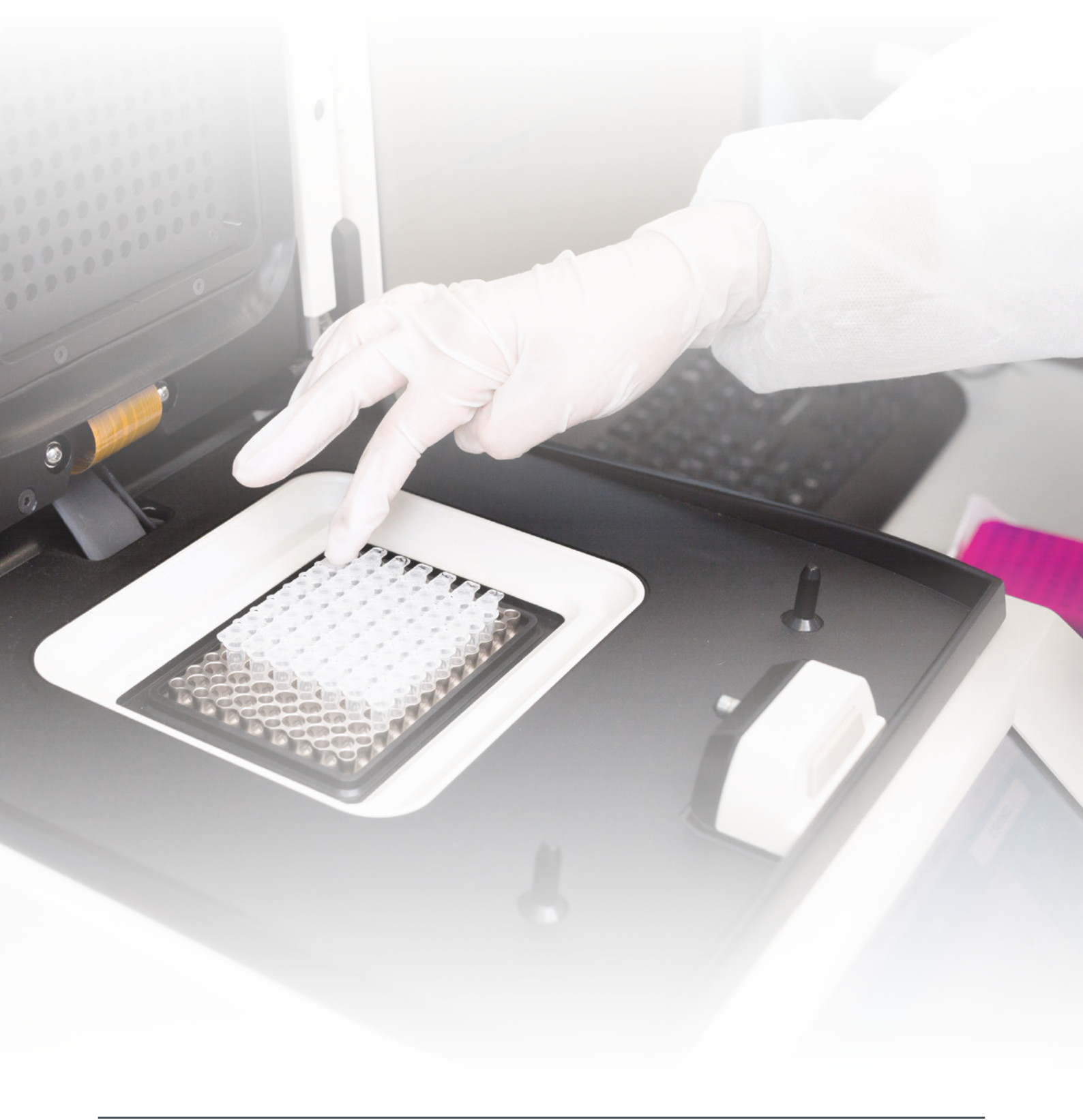
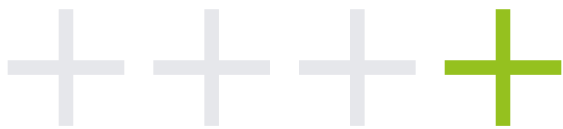


WHITE PAPER //  
NOVEMBER 2019

## Comparing Non-sacrificial Panel (NSP) and Traditional Animal Health Monitoring









## Introduction

Animal facility managers are responsible for implementing health monitoring programs that closely monitor and maintain the health status of research animals. When researchers use animals with a suboptimal health status, this can negatively impact research results and lead to unreliable data, financial loss, and damaged reputations.

Thus, optimal health monitoring programs are important to ensure researchers obtain laboratory animals that are free from major pathogens and in excellent health. Such programs assure that contaminants are identified and eliminated as quickly as possible to minimize any consequences.

Animal facilities across the globe are striving to maintain a fixed and clean health status that is specific pathogen free (SPF) or specific and opportunistic pathogen free (SOPF). However, this can present a major challenge for facility managers, given the many evolving variables in a facility and the efforts to eliminate the use of live animals for health monitoring.

With an increasing number of available non-sacrificial panel (NSP) sampling techniques and testing methods, NSP testing provides facility managers with another option when it comes to optimizing their health monitoring program. NSP testing requires that samples—such as fecal, serum, and fur—are collected from living animals and/or their environments.

Prior to implementing an NSP health monitoring program, facility managers should be familiar with which pathogens are screened for and the detection techniques used.

The aim of this white paper is to present

- + Background information on NSP testing for health monitoring, and
- + Validation data demonstrating the reliability of several NSP tests compared to live animal testing (i.e., Traditional animal health monitoring).





## Background information on NSP Testing

There are broad challenges associated with traditional animal health monitoring methods, so facility managers have increasingly been seeking alternative approaches that minimize its use.

NSP methods offer an alternative approach to screening the health of animal colonies: samples are collected directly from the animals (without euthanasia) and/or their environments. The sampling and analytical techniques allow for rapidly and reliably detecting bacteria, viruses, and parasites in a manner that helps facility managers to replace the use of animals with alternative testing techniques, reduce the number of animals (i.e., reduce or eliminate the use of sentinels), and refine the processes of a health monitoring program.

NSP testing can also provide a cost benefit by reducing shipping costs and sampling and preparation time, while achieving faster results that are equally reliable or even more so.

### Commonly used NSP testing methods include the following:

- + Dried blood spot (DBS) sampling requires a drop of blood and is used to test for all FELASA-recommended infectious agents quarterly and annually, among other agents that are typically tested for via immunological methods.
- + Oral swabs are used to detect bacteria from the respiratory tract using real-time PCR or standard microbiology techniques.
- + Fur swabs are used to detect all ectoparasites and some bacteria, such as *Corynebacterium bovis*, using real-time PCR.
- + Fecal samples are used to detect bacteria, pinworms, protozoa, and gut bacteria using real-time PCR, fecal flotation, or standard microbiology techniques.
- + Environmental samples collected with filters and/or swabs are used to monitor the FELASA-recommended infectious agents quarterly and annually<sup>1</sup> via real-time PCR, which allows for evaluating cleaning procedures and basal bacterial loads in the environment.

With an increasing number of NSP tests marketed by commercial vendors, facility managers must ensure that these are reliable and validated, to avoid unnecessarily high levels of false positive or false negatives, both of which can have costly implications for the facility and researchers. NSPs also typically deliver a cost benefit, as they reduce the need to ship animals.

<sup>1</sup> FELASA working group on revision of guidelines for health monitoring of rodents and rabbits, Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim. 2014 Jul;48(3):178-192.



## Validation studies

Several studies were undertaken to validate the performance of NSP tests developed internally at Envigo. The following sections describe the purpose of these studies and their results.

### Study #1: DBS Sample Versus Serum Sample

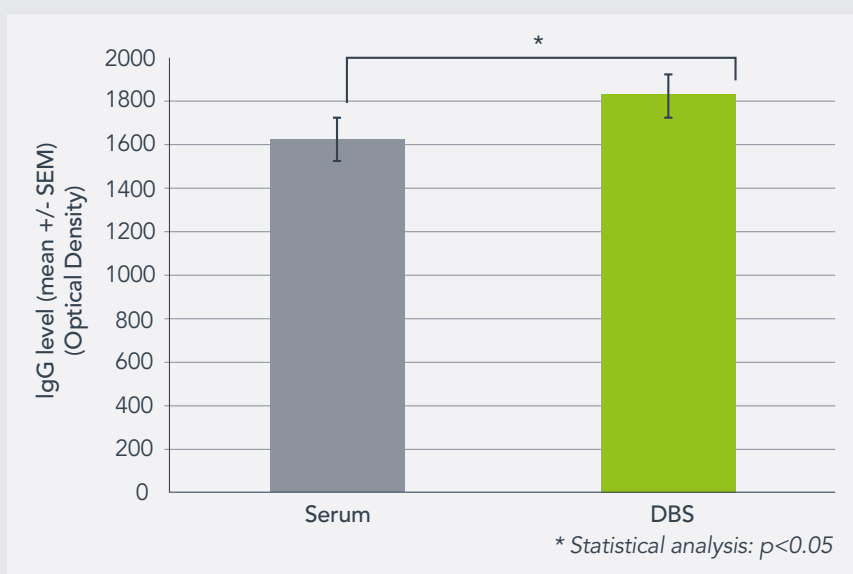
The purpose of this study was to evaluate the specificity, sensitivity, repeatability, and reproducibility of DBS samples compared to serum samples—the gold standard—in rats and mice (only mouse data shown) using multiplexed fluorometric immunoassay (MFIA), immunofluorescence assay (IFA), and ELISA to detect antibodies against specific antigens.

All FELASA-recommended infectious agents (quarterly and annually) were tested, in addition to several bacterial strains, including CAR bacillus,

*Mycoplasma pulmonis*, *Encephalitozoon cuniculi*, and *Clostridium piliforme*. First, paired serum and DBS samples were collected from each animal (n = 100; 50 historically negative samples, 40 historically positive samples for several antigens, and 10 unknown samples).

IgG extraction from a pooled mouse sample demonstrated that the IgG level from the DBS samples was statistically significantly higher than that of the serum samples (Figure 1).

Figure 1: Statistically significant difference in IgG levels extracted from DBS and serum samples





The historically negative and positive samples were separately tested using MFIA, ELISA, and IFA. Notably, a subset of the historically positive samples was retested to confirm positivity (most prevalent antigens only) by ELISA and IFA.

Overall, the results obtained from the three assays (MFIA, ELISA, and IFA) showed no statistically significant differences between the DBS and serum samples in the majority

of the agents that were tested for. Further, internal data show that the DBS samples provide more accurate data than serum samples do, because there were fewer borderline results.

This study validates the use of DBS samples to detect FELASA-recommended infectious agents, among others including specific bacterial strains, using MFIA, ELISA, and IFA.

## Study #2: Bacteriology Testing Validation

The purpose of this study was to conduct a bacteriology NSP validation.

First, to ensure that common bacterial strains were viable over multiple days and at different temperatures (i.e., to simulate typical conditions when samples are shipped from an animal facility to the Envigo testing facility), six different bacterial strains were stored for five days at each of the following temperatures: 37°C, 25°C, 4°C, or -20°C. This analysis found that all six bacteria types had high survival rates after the five days at each of the temperatures. In addition, bacterial load and bacterial type were evaluated in wet oral swabs stored at room temperature for 10 days.

Notably, for the wet oral swab samples, after five days at room temperature, detecting exigent bacteria became increasingly difficult due to an overgrowth of enteric bacteria.

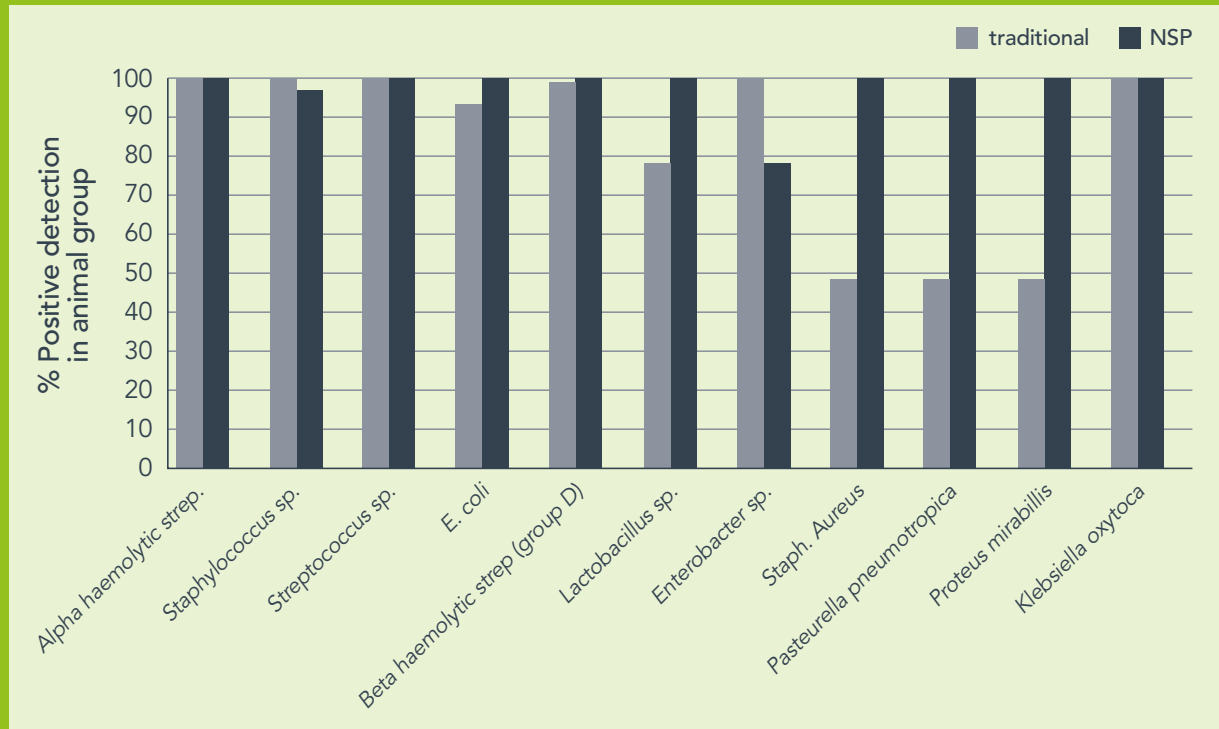
Next, an Envigo facility located in the UK collected samples from animals using two different methods: 1) traditional screening (nasopharyngeal swab and caecum swab) and 2) NSP screening (oral swab and fecal pellets). The UK facility tested the specimens to identify the spectrum of pathogens in the different samples. The samples were packaged and shipped to the Envigo Health Monitoring Laboratory in Bresso, Italy. Upon arrival, the samples were processed and incubated on agar plates, and pathogen detection was carried out with a Vitek 2 Compact automated instrument.

The results from the Bresso laboratory are shown in Figure 2. The NSP method (black bars) was found to outperform the traditional bacteriology screening method (grey bars). The graph shows the percentage of animals that tested positive for a specific bacterial strain. For instance, from a sample of four animals, *S. Aureus* was identified in all four animals using the NSP method (100%), and in only two animals using the traditional method (50%).





Figure 2: Bacteriology NSP validation: Traditional screening and NSP screening results



Overall, the results from the first part of this study found that under simulated shipping conditions, all bacterial strains had high rates of survival. These data were corroborated in the second part of the study, which assessed samples shipped from a UK-based Envigo facility to the Envigo Health Monitoring Laboratory in Bresso. The NSP screening method was found to be superior at detecting bacterial strains relative to the traditional screening method.



## Study #3: Environmental Samples

The purpose of this study was to compare the health status of animals obtained using a traditional screening method (i.e., live animal monitoring), which necessitates the sacrifice of the animal, versus an NSP screening method based on real-time PCR using nucleic acids extracted from Interceptor filters from individual ventilated cages (IVC) rack system. This study was conducted under real-world conditions.

Real-time PCR test conditions were first developed and validated for use on environmental samples (specifically, air dust monitoring using Interceptor filters from IVCs). The assay development process involved an assessment phase, where a target was identified (i.e., organism, protein, sequence) and confirmed with secondary sources, followed by a resource phase, where the design of probes and primers was established. Finally, in the actuation phase, the sensitivity, specificity, repeatability, and reproducibility of the test were assessed and refined, to ensure total nucleic acid extraction from filters was acceptable, establish acceptance thresholds, and determine the time course of pathogen identification, among other activities.

### Real-time PCR offers several advantages:

- + It allows for pooling sample types (i.e., feces, swabs, and/or IVC filters).
- + It is extremely sensitive and specific.
- + It is rapid, providing results within two days (typically).

There are also some recognized challenges, including extreme sensitivity and specificity, which can lead to false positives and false negatives (i.e., if the agent mutates or is not in the sample type tested), respectively.

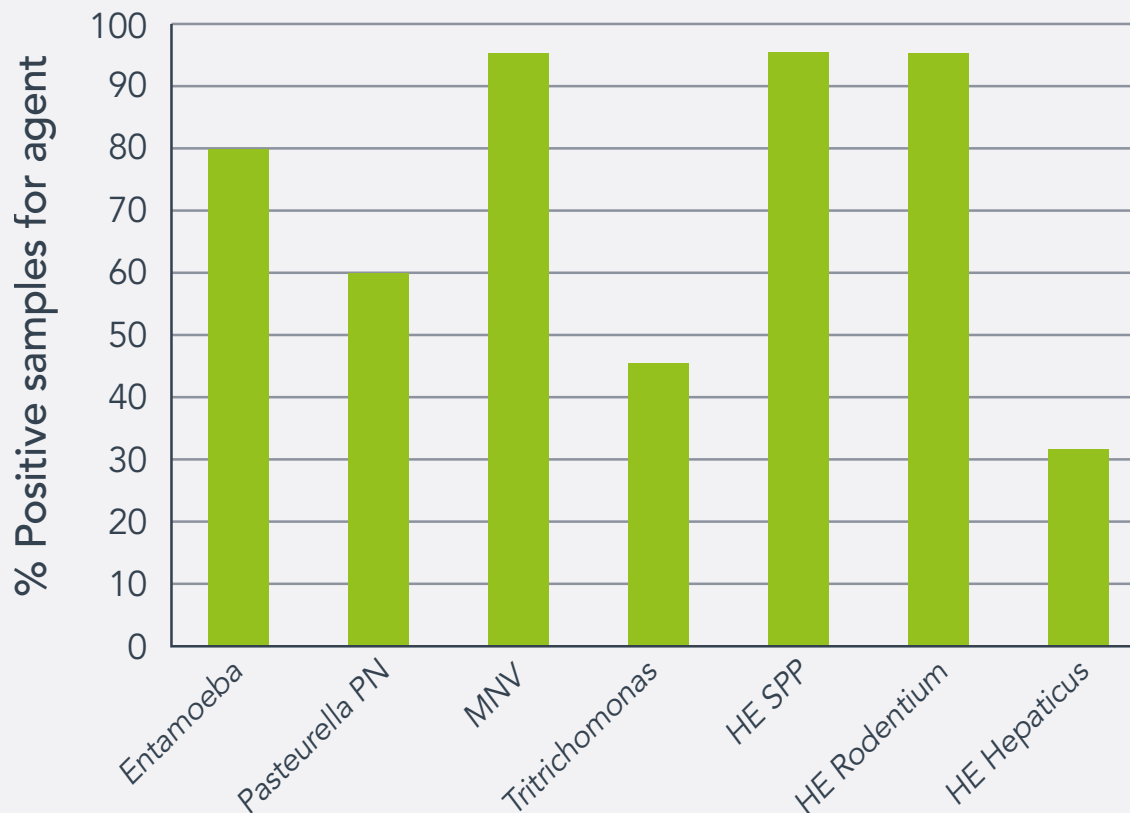
To first confirm that the established real-time PCR assay was working as anticipated, pooled fecal samples were tested from a historical cohort of animals that had also been assessed via live animal testing and had been issued a Health Monitoring Report. It was determined that the data from the real-time PCR (Figure 3) successfully matched the earlier Health Monitoring Report.

Thus, relative to the traditional screening method, which necessitates animal sacrifice, real-time PCR of nucleic acids extracted from Interceptor filters successfully detected the same pathogens.





Figure 3: Agents detected using real-time PCR on pooled fecal samples confirmed the health status in the preceding Health Monitoring Report (which used live animal testing)

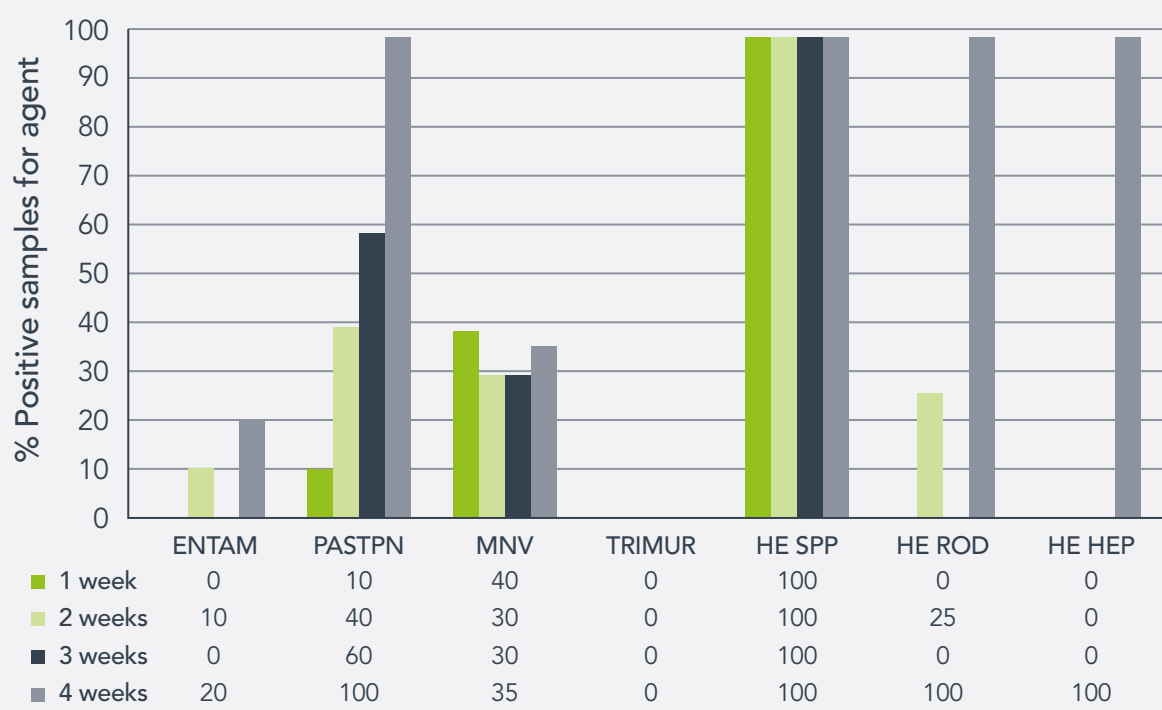


Subsequently, real-time PCR was used to test the Interceptor IVC filters at different time points (one, two, three, and four weeks).

At the one-week time point, *murine norovirus* (MNV) and *Helicobacter Spp.* were successfully identified (Figure 4). At two and three weeks, the majority of the agents had been identified (Figure 4). Finally, after four weeks of exposure, all pathogens except for *Tritrichomonas muris* (TRIMUR) were identified (Figure 4). Notably, this real-time PCR assay was recently used to test customers' environmental filters, and successfully detected the presence of *Tritrichomonas muris*.



Figure 4: Agents detected using real-time PCR on Interceptor filters



The results of this study validate the use of an NSP screening method that relies on real-time PCR to detect pathogens from environmental filters.<sup>2</sup>

<sup>2</sup>Please note that the species *Pasteurella pneumotropica* has been reclassified under the new genus *Rodentibacter* (namely *R. pneumotropicus* and *R. heylii*). [Adhikary, S. et al. 2017 Int J. Syst. Evol. Microbiol. 67:1793-1806; Benga L. et al. 2018 Vet Microbiol 217: 121-134].



## Summary

NSP testing provides facility managers with another option when it comes to optimizing their health monitoring program. There are an increasing number of available NSP sampling techniques and testing methods available, and facility managers should be aware of advantages and challenges associated with the available tests, and the underlying studies that have led to the validation of these tests.

NSP methods are allowing for rapid and reliable detection of various agents that help facility managers to better adhere to the principles of the 3Rs (Replacement, Refinement, and Reduction).

Please visit the Envigo website for more information and recommendation on sampling and pooling samples on our *"Full Spectrum Health Monitoring services"*.



Envigo provides the broadest range of standard research models and related services to the pharmaceutical and biotechnology industries, government, academia and other life science organizations.

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