# NPB FINAL RESEARCH GRANT REPORT FORMAT

**Project Title and NPB project identification number.** Active environmental surveillance for swine pathogens using continous sampling; project #22-089

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**Industry Summary:** The long-term goal of this project is to develop a practical, cost-effective, active on-farm environmental surveillance system for swine pathogens based on continuous passive sampling. In brief, this would be achieved by placing "passive samplers" in swine production facilities and then periodically collecting and testing the samplers for targeted pathogens. Detection of targets would indicate their presence in the population without the need to collect samples from the animals themselves.

Our initial research focused on selecting the best material for use in the system. In Experiment 1, 9 candidate materials were evaluated in terms of absorbence and recovery of liquid. Sampling materials that could not absorb and then release liquid - a basic requirement for sampler processing in the laboratory - were eliminated from further evaluation. The results of Experiment 1 identified the best materials for liquid absorption/recovery as polyester filter paper (candidate 3), Swiffer® dry cloth (candidate 4), and Whatman filter paper (candidate 1). These three were selected for Experiment 2, but FTA® cards (candidate 2) were also included in Experiment 2 because FTA® cards are commonly used in diagnostics.

In Experiment 2, these 4 materials were inoculated with PRRSV and PEDV and then compared in terms of PRRSV and PEDV RNA recovery using different elution buffers (PCR-grade water, Tris EDTA (TE) buffer, and lysis buffer). Elution of PRRSV and PEDV RNA from the candidates was achieved by soaking the papers in 2 ml of PCR-grade water, TE buffer, or lysis buffer, and then testing the eluate by RT-qPCR. The results of Experiment 2 showed that RNA recovery depended both on the sampler material and the elution buffer, e.g., recovery for candidates (3, 4) was best with lysis buffer, whereas candidates (1, 2) had a higher RNA recovery with TE buffer. Overall, the findings in this study supported further research on the use of passive environmental samplers in an active surveillance system.

**Key Findings:**

- Based on results, polyester filter paper, Swiffer® dry cloth, and Whatman® filter paper grade 903 showed potential for further evaluation based on liquid and RNA recovery.
- PRRSV and PEDV RNA recovery was both material- and elution buffer-dependent.
- PEDV RNA recovery from some materials was as good or better than testing the inoculum itself -- which suggested that some materials may be protective of the virus.

**Keywords:** Passive sampling, environmental surveillance, PRRSV, PEDV

**Scientific Abstract:** Infected animals shed pathogens into the environment, making environmental sampling a potentially viable approach to surveillance in swine populations. In particular, continuous environmental sampling offers a practical alternative to traditional point-in-time environmental sampling methods because prolonged exposure provides for aggregating pathogens over time as pathogens dispersed by activity in the barn gradually settle out of the air onto the samplers.

This study evaluated 9 candidate materials for a low-cost, on-farm sampling system using porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) as targets. Two experiments were conducted: liquid recovery (Experiment 1), and RNA recovery (Experiment 2).

In Experiment 1, 9 candidate materials were assessed for liquid recovery: (1) Whatman® filter paper grade 903; (2) FTA® Cards; (3) Polyester filter paper (Optimice Cage Reemay Filter Media; (4) Swiffer®

dry cloth; (5) SmartSolve® water soluble paper; (6) Dry surface filter polyester; (7) Tacky surface filter polyester; (8) Water-soluble starch foam; (9) Sellars® blue shop towels. Candidate materials were evaluated by soaking them in increasing volumes of PCR-grade water, manually agitating, and then measuring the volume of liquid recovered. The 3 top-performing candidates from Experiment 1, i.e., materials (1), (2), and (4) were selected for evaluation in Experiment 2. FTA® cards (candidate 2) were also included in Experiment 2 in recognition of the fact that FTA® cards are commonly used in diagnostics.

In Experiment 2, the recovery of PRRSV and PEDV RNA was compared among candidate materials. Specifically, candidate papers were inoculated with fourfold dilutions of each virus, dried, vacuum-sealed, and stored at -80°C before being eluted with PCR grade water, Tris EDTA buffer, or general lysis buffer. Eluates from the candidates and the dilutions of virus inoculum (control) were tested by RT-qPCR. In Experiment 2, RNA recovery varied by material and elution buffer, with polyester filter paper/lysis buffer achieving superior RNA detection results. Notably some materials matched or exceeded RNA recovery from the controls. The findings in this study demonstrated the potential for continuous environmental sampling using specific materials.

**Introduction:** In general, infected animals shed pathogens in secretions and excretions, thereby contaminating the environment. It follows that exposure of susceptible animals to contaminated environments is a common route of infection (Pirtle and Beran, 1991). Although pathogens are in the environment, sampling of the environment (air, water, surfaces, feedstuffs) is not practical for routine point-in-time sampling. The core problem is that the concentration of micro-organisms varies widely and unpredictably over time in any defined space (O'Connor et al., 2005). As a consequence, we do not know where to sample, how many samples to collect, or how to interpret a negative result.

A potential solution to this problem is continuous environmental sampling, i.e., placing samplers in the environment to allow for the accumulation of analyte(s) over time (Kot et al, 2000). That is,, aggregation of target over time addresses the problems of low concentration and heterogeneous distribution that compromise the effectiveness of point-in-time environmental sampling (Górecki and Namieśnik, 2002). Passive environmental sampling has been used successfully in laboratory animal surveillance (Dubelko et al, 2018; O'Connel et al, 2021; Winn et al, 2022), and food production areas (Bobal et al, 2019). For instance, O'Connel et al. (2021) tested paper from SPF laboratory animal environments by PCR and found that: "*Filter papers detected all pathogens (mouse hepatitis virus, m urine norovirus, minute virus of mice, mouse parvovirus, Theiler murine encephalomyelitis virus, Helicobacter spp., Sypahcia obvelata, and Aspicularis tetraptera) as effectively or more effectively than sentinel mice …*"

The long-term objective of this research is to develop a practical, low-tech, low-cost, on-farm continuous environmental sampling system. In brief, such a system would be implemented by placing sampling devices in a production facility, e.g., a barn, collecting them periodically ((weekly or monthly), and testing them for the pathogen(s) of interest. This project is divided into two components: a laboratory component (this report) and a field component (to be conducted in the future). Herein we address the laboratory component.

**Objectives:** The goal of the laboratory component was to select the best material to be used in field collection devices. Initially, potential materials were evaluated in terms of volume recovery and then in terms of target RNA recovery. In this research, we used porcine respiratory and reproductive syndrome virus (PRRSV; respiratory) and porcine epidemic diarrhea virus (PEDV; enteric) as targets.

**Materials & Methods:** Volume and target (PRRSV RNA and PEDV RNA) recovery was evaluated for 9 candidate materials (Table 1) in two experiments: experiment 1, recovery of liquid; experiment 2, recovery of target.

**Table 1. Candidate materials and their composition**

| | Candidate | Composition | Source |
|---|---|---|---|
| 1 | Whatman® filter paper (903) | Cellulose | Cytiva, Malborough, MA |
| 2 | FTA® Cards | Cellulose | Cytiva, Malborough, MA |
| 3 | Polyester filter paper (Optimice Cage Reemay Filter Media) | Polyester | Animal Care Systems; Centennial, CO |
| 4 | Swiffer® dry cloth | Polyester | Procter & Gamble Co.; Cincinnatti, OH |
| 5 | SmartSolve® water soluble paper | Cellulose | SmartSolve Industries, Bowling Green, OH |
| 6 | Dry surface filter polyester | Polyester | McMaster-Carr, Elmhurst, IL |
| 7 | Tacky surface filter polyester | Polyester | McMaster-Carr, Elmhurst, IL |
| 8 | Water-soluble starch foam | Potato starch | ULINE, Pleasant Prairie, WI |
| 9 | Sellars® blue shop towels | Cellulose | Sellars, Milwaukee, WI |

**Experiment 1 - Recovery of liquid**

In order to evaluate the candidates' absorptive/desorptive behavior, sections of material (1" × 1.5" in size) were soaked in increasing volumes of PCR-grade water and the liquid recovered was measured and compared across candidates. In brief, candidate papers were placed in 50 ml conical tubes (Corning Life Sciences, Corning, NY), and increasing volumes of PCR-grade water were added, starting with 1000 ul and up to 3000 ul, in 500 ul increments. Candidates were manually agitated, the liquid released was poured off into a different tube, and the volume measured.

**Experiment 2 - Target recovery**

Four candidate materials, i.e., (1) Whatman® filter paper grade 903, (2) FTA® Cards, (3) Polyester filter paper, (4) Swiffer dry cloth were evaluated in Experiment 2. To evaluate the effect of elution conditions on target recovery, 3 elution buffers (PCR-grade water, Tris EDTA (TE) buffer (Fisher Scientific), and lysis buffer (Buffer G2, Qiagen, Hilden, Germany)) were compared. In brief, 300 ul of fourfold dilutions ($4^{-2}$ to $4^{-5}$) of a PRRSV isolate (PRRSV MN 184) and a PEDV isolate (PEDV USA/NC32140/2013) were used to inoculate each candidate paper (1 × 1.5 in) in triplicates. Inoculated papers were allowed to dry for a minimum of 3 h at 25°C inside a water-jacketed $CO_2$ incubator (NAPCO 6301; Thermo Fisher). Once dried, papers were vacuum sealed using a chamber vacuum packaging machine (VacMaster VP215C) and stored at -80°C until eluted. Prior to elution, candidate papers were thawed overnight at 4°C. Thereafter, candidate papers were placed in 50 ml conical tubes (Corning Life Sciences) and 2 ml of PCR-grade water, TE buffer, or lysis buffer were added. Manual agitation was performed to ensure that the whole surface of the paper was covered by the elution buffer. Then, the eluate was poured off into a 5 ml snap cap tube (Corning Life Sciences) and stored at -80°C until tested. For testing, total nucleic acid extraction was performed following the manufacturer's instruction (MagMAX™ Pathogen RNA/DNA Kit; Applied Biosystems by Thermo Fisher Scientific, Waltham, MA) and RT-qPCR assays for the detection of PRRSV (EZ-PRRSV™ MPX 4.0; Tetracore Inc, Rockville, MD) and PEDV (EZ-PED/TGE/PDCoV MPX 1.1; Tetracore Inc) were used to amplify the extracts following the manufacturer's instructions. As controls, 300 ul of each inoculum dilution were mixed with 2 ml of each elution buffer and tested.

**Results:**

In Experiment 1, the volume of liquid recovered relative to the volume of liquid inoculated was used to calculate the percent of liquid recovered. The results of Experiment 1 identified the best materials for liquid absorption/recovery as polyester filter paper (candidate 3), Swiffer® dry cloth (candidate 4), and Whatman filter paper (candidate 1). FTA cards were included in Experiment 2 because of their common usuage.

For Experiment 2, raw Cqs were re-expressed as efficiency standardized Cqs (ECqs) as a function of amplification efficiency (Armenta-Leyva, 2024). ECqs express the concentration of target in the sample relative to the concentration of target in the reference standards included in the RT-qPCR run. These were used to calculate percent recovery of target relative to the control.

**Table 2. Recovery of target RNA (%) by dilution and elution buffer relative to the control [1]**

| Candidates[2] | Elution buffer | Dilution of virus inoculated | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $4^{-2}$ | | $4^{-3}$ | | $4^{-4}$ | | $4^{-5}$ | |
| | | Recovered RNA (%) | | Recovered RNA (%) | | Recovered RNA (%) | | Recovered RNA (%) | |
| | | PRRSV | PEDV | PRRSV | PEDV | PRRSV | PEDV | PRRSV | PEDV |
| (1) | Water | 31.7 | 47.3 | 13.4 | 72.3 | 8.2 | 9.4 | 4.1 | 16.5 |
| | TE | 9.1 | 62.9 | 12.7 | 144.9 | 15.1 | 65.3 | 3.0 | 23.8 |
| | Lysis | 18.9 | 31.0 | 13.5 | 27.7 | 10.6 | 25.3 | 7.8 | 25.6 |
| (2) | Water | 19.1 | 27.1 | 3.0 | 23.1 | 3.7 | 4.6 | 0.7 | 8.2 |
| | TE | 50.0 | 80.2 | 2.9 | 272.6 | 17.2 | 19.0 | 5.7 | 9.6 |
| | Lysis | 0.3 | 0.7 | 0.1 | 0.4 | 0.1 | 0.5 | 0.7 | 1.6 |
| (3) | Water | 70.3 | 24.9 | 89.4 | 79.6 | 33.9 | 3.3 | 10.1 | 9.3 |
| | TE | 30.3 | 189.2 | 12.3 | 85.2 | 15.2 | 32.8 | 9.0 | 34.1 |
| | Lysis | 191.6 | 126.1 | 42.7 | 143.7 | 39.6 | 63.4 | 41.6 | 151.7 |
| (4) | Water | 16.7 | 86.8 | 48.4 | 92.6 | 7.5 | 10.5 | 0.4 | 18.5 |
| | TE | 11.4 | 182.8 | 10.6 | 95.7 | 6.2 | 24.8 | 0.5 | 20.5 |
| | Lysis | 68.5 | 129.2 | 54.7 | 94.0 | 7.5 | 70.8 | 10.1 | 102.3 |

[1]Control: PRRSV (PRRSV MN 184) and PEDV (PEDV USA/NC32140/2013) were fourfold diluted and 300 ul of each dilution was further mixed with 2 ml water, TE buffer, or lysis buffer, and tested.
[2]Candidates: (1) Whatman® filter paper grade 903; (2) FTA® Cards; (3) Polyester filter paper (Optimice Cage Reemay Filter Media); (4) Swiffer® dry cloth.

The results of Experiment 2 are given in Table 2 by candidate, elution buffer, and virus dilution. Results are expressed as the percent RNA recovered relative to the control. Values >100% indicate that RNA recovery was higher from the candidate material than from the control. Thus, values >100% imply that the material was protective of the virus. In the aggregate, i.e., across all dilutions, the highest mean recovery was 79% and 121% from candidate 3 × lysis buffer for PRRSV and PEDV. Candidate 4 × lysis buffer and candidate 2 × TE also provided high recovery rates. Thus, recovery of RNA was shown to be a function of both candidate material and elution buffer.

**Discussion:** Surveillance based on by point-in-time environmental sampling is unpredictable because pathogens are not predictably and uniformly distributed in the environment (López-Lorenzo, et al., 2021; O'Connor et al., 2006). For that reason, results of point-in-time environmental sampling are difficult to interpret, i.e., does a negative result mean the environment is free or that the wrong location was selected for sampling?

The long-term goal of this project is to develop an active surveillance system based on the use of continuous passive samplers. That is, passive sampling devices would be placed in a defined environment (barn or room) thereby allowing targets to accumulate on samplers over time. Periodically, samplers would be tested for designated targets. If successful, this approach could provide a cheaper and easier alternative to surveillance based on collecting samples from animals (Schang et al., 2021).

In this study, 9 candidate papers were evaluated for their potential use in a passive environmental sampling system. In this study, two qualities were evaluated: liquid recovery/release (Experiment 1), and target (RNA) recovery (Experiment 2).

Experiment 1 evaluated the quality of liquid release by the candidate materials. While this feature is not often described in the literature for materials used in diagnostics (Cardona-Ospina et al., 2019;

Smit et al., 2014), recovery of liquid is a pre-requisite for passive sampling because a material with low liquid recovery is unlikely to yield the sample volume required for extraction. In this experiment, remarkable differences in liquid recovered were shown among candidate papers screened, regardless of their composition.

The recover of target from candidate materials is a second pre-requisite. Experiment 2 showed that PRRSV and PEDV RNA recovery was possible from various types of materials following desiccation (25°C for 3 h), but recovery depended both on material and elution buffer. Reports in the literature have described the detection of swine pathogens from FTA® cards (candidate 2) (Cardona-Ospina, et al., 2019; Elnagar et al., 2021; Stringer et al., 2021); Swiffer® wipes (candidate 4) (Edwards et al., 2014; Zewde et al, 2009); and some grades of Whatman® (candidate 1) (Randriamparany et al, 2023). However, none of these reports were made in the context of continuous passive environmental sampling.

Overall, these findings are very promising. Specifically, viral RNA recovery from FTA® Cards, Swiffer® dry cloth, Whatman® filter paper and especially polyester filter paper (Optimice Cage Reemay Filter Media) justify further exploration into the characteristics of these materials and their application in an active suveillance system based on continuous environmental sampling.

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