



United States
Department of
Agriculture

Veterinary Services

March 2021



National Animal Health Monitoring System (NAHMS)

**NAHMS Swine 2021 Large Enterprise
Study**

BIOLOGICS MANUAL

Contents

Biologics Overview	4
NAHMS BIOLOGICS CONTACTS AND SITES.....	4
COMPONENTS OF BIOLOGICS	5
BIOLOGICS BENEFITS	6
Kit Orders and Collection Schedule	7
KIT ORDERS.....	7
COLLECTION SCHEDULE.....	7
Enteric Microbe Collection Design and Procedures	9
ENTERIC MICROBE OVERVIEW	9
SAMPLE COLLECTION.....	10
FARM ID AND SAMPLE LABELS.....	12
Enteric Microbe Instructions and Fecal Collection Record	13
Fecal Collection Record Example.....	15
Enteric Microbe Producer Report Example	17
Oral Fluids Collection Design and Procedures	21
ORAL FLUIDS OVERVIEW	21
SAMPLE COLLECTION.....	22
FARM ID AND SAMPLE LABELS	24
ORAL FLUIDS STEP BY STEP CHECKLIST.....	25
Oral Fluids Instructions and Collection Record	27
Oral Fluids Collection Record Example	29
Oral Fluids Producer Report Example	31

Biologics Overview

NAHMS BIOLOGICS CONTACTS AND SITES

NAHMS Biologics Contacts

Name	Role	Phone Number	E-Mail
Dr. Alyson Wiedenheft	Biologics Coordinator	Office: (970) 494-7290 Cell: (970) 494-7238	alyson.m.wiedenheft@usda.gov
Dr. Charles Haley	Study Lead	(970) 494-7216	charles.a.haley@usda.gov
Ms. Abby Zehr	Field Liaison	(970) 494-7252	abigail.c.zehr@usda.gov

Lab Contacts

Name	Role	Address
Diane Beck David Hampe	NVSL Shipping and Receiving	National Veterinary Services Laboratories 1920 Dayton Avenue Office B24-1911 Ames, IA 50010-9602
Dr. Megan Jacobs	Primary Enteric Microbe Testing	North Carolina State University College of Veterinary Medicine 1060 William Moore Dr. Raleigh, NC 27607
Dr. Roger Main	Oral Fluid Testing	Iowa State University Veterinary Diagnostic Laboratory 1850 Christensen Drive Ames, IA 50011-1134

NAHMS Swine 2021 Website

Collection instructions, collection record forms, training videos, and Tableau tracking dashboards for biologics can be found at the NAHMS website address:

[HTTPS://WWW.APHIS.USDA.GOV/APHIS/OURFOCUS/ANIMALHEALTH/MONITORING-AND-SURVEILLANCE/NAHMS/SWINE QUESTIONNAIRES](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/NAHMS/SWINE_QUESTIONNAIRES)

NAHMS Mailing Address

USDA:APHIS:VS:NAHMS

2150 Centre Avenue Bldg. B., Mail Stop 2E7

Fort Collins, CO 80526

Biologics Overview

COMPONENTS OF BIOLOGICS

Enteric Microbe Testing

VS-collected fecal samples, 30 per site, from swine 20 weeks and older, will be cultured for *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter*. *Salmonella* isolates will be serogrouped and serotyped. Selected *Enterococcus* and *Campylobacter* isolates will be speciated. *E. coli* will be evaluated for the presence of shigatoxin genes and other shiga-toxin *E. coli* (STEC) virulence factors. Isolates confirmed to be STEC will be serogrouped. *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter* isolates will be evaluated for susceptibility to antimicrobials commonly used in veterinary medicine. Reports summarizing individual operation prevalence and antimicrobial susceptibility results will be generated, and mailed in sealed envelopes to VS Coordinators for distribution to respective operations. *E. coli* STEC results will not be reported to individual operations. Producers should expect to receive reports within 4 months of sample collection.

Oral Fluid Pathogen Testing

VS-collected oral fluid samples, via 8 ropes per site, from pens of swine 20 weeks and older will be tested for the nucleic acid of Seneca Valley virus A (SVA). Producer reports summarizing oral fluid pathogen testing for an individual producer's operation will be generated, and sealed reports will be sent to Coordinators for distribution within 4 months of sample collection.



Safeguarding the U.S. Swine Industry

Collectively, swine producers like you will play an important role in safeguarding the U.S. swine industry. Information provided in the Swine 2021 study will:

- Provide transparent, credible information on U.S. swine industry practices to help counter misinformation and ultimately protect U.S. swine production.
- Aid in preparedness strategies for foreign animal diseases such as African Swine Fever.
- Facilitate trade negotiations by providing trading partners with a summary of the structure and health status of the U.S. swine industry.
- Assist policymakers and industry stakeholders in making more informed decisions affecting the swine industry.

Free Enteric Microbe Tests a \$3,225 Value*

- Detection, serotyping and susceptibility testing of *Salmonella*
- Detection and susceptibility testing of *E. coli*
- Detection, speciation and susceptibility testing of *Enterococcus*



Oral Fluids Virus Tests a \$200 Value*

- Detection of Nucleic acid presence of Seneca Valley virus



■ Biological testing costs include:

- ▶ Diagnostic testing
- ▶ Confidential, descriptive report of results

* Values based on estimated average cost at diagnostic laboratories for testing 8 ropes for oral fluids and 30 fecal samples per farm

Kit Orders and Collection Schedule

KIT ORDERS

NAHMS will place 2 rounds kit orders based on State turnover numbers. The first round of kits will be sent to the Area Offices or State Coordinators for distribution before the study starts. The second round of kits will be sent to State Coordinators for distribution soon after the study begins. Additional kits can be requested by emailing Alyson Wiedenheft at Alyson.M.Wiedenheft@usda.gov.

COLLECTION SCHEDULE

VS Collection.....September 2021 – January 2022

Collection and Shipping days

Samples	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Fecal	Collect*	Collect	Collect	Collect**			
	Ship	Ship	Ship	Ship			
Oral Fluids	Collect*	Collect	Collect	Collect	Collect	Collect**	
	Ship	Ship	Ship	Ship	Ship	Ship	

*Samples collected on Sunday can be shipped on Sunday if shipping is available. If Sunday shipping is not an option, then samples must be kept refrigerated until they can be shipped on the following Monday. Samples must be shipped within 24 hours of collection.

Samples collected on this day must be shipped on the **same day.

Please note that collection and submission days for fecal samples and oral fluids differ. Do not collect fecal samples on Thursday or Friday. You may collect oral fluid samples on Thursday and Friday if the operation is not submitting fecal samples.

Do not ship the day before a holiday because the sample that arrive at the lab on the holiday will not be processed. Blackout dates for shipping include:

BLACKOUT SHIPPING DATES	OBSERVED HOLIDAY
September 5	Labor Day (9/6/21)
October 10	Columbus Day (10/11/21)
November 10	Veteran's Day (11/11/21)
November 21-27	Thanksgiving (11/25/21)
December 19-January 1	Christmas (12/25/21)/New Year's (1/1/22)

Enteric Microbe Collection Design and Procedures

ENTERIC MICROBE OVERVIEW

Fecal testing for microbes and detection of antimicrobial resistance (AMR)

- VS Fecal Collection:** Up to 30 samples from up to 6 pens containing pigs 20 weeks and over
1. Determine how many pens on the site contain late finisher pigs that are 20 weeks and older. The pens with the pigs of the right age may be scattered among different rooms and in different barns.
 2. If **less** than 6 pens, collect roughly equal numbers of samples from all pens for a maximum of 30 samples.
 3. If **more** than 6 pens, attempt to geographically space the pens sampled (collect from pens roughly equidistant from each other).
 4. Using a clean glove, collect about a palm full (20 grams) of fresh feces from the pen floor and place in a single Whirl-Pak® bag.
 5. Express the air out of the bag and fold over the top twice before securing closure tabs (Do not fold top more than twice). Copy the 6 digit Farm ID (operation/site) from the first page of the VS Questionnaire onto each sample label. Also, write in the Facility and Pen IDs on each sample label, then peel off and place securely on the bag. Repeat.
 6. Place all samples from one pen into a gallon sized Ziploc® bags and seal the ends of the large bags. Repeat for the rest of the sampled pens.
 7. Place the filled Ziploc® bags inside the liner bag with 3 frozen ice packs and the absorbent sheet. Tie the liner bag shut and close the cooler lid.
 8. Complete the Fecal Collection Record. Copy the 6 digit Farm ID (operation/site) from the first page of the VS Questionnaire onto the Collection Record. Make sure the sample numbers on the form and bag match. Place the white copy on top of the closed insulated cooler. Leave the yellow copy of the collection record with the producer.
 9. Keep samples cool, on ice, and ship with 24 hours of collection. Replace ice packs before shipping to insure the icepacks are completely frozen.
 10. Use the supplied shipping airbill to ship overnight to NCSU.



NCSU Receives Samples

- Samples tested for presence of *Salmonella*, *E. coli*, *Campylobacter* and *Enterococcus* (subset).
- *Campylobacter* and *Enterococcus* isolates speciated and tested for antimicrobial sensitivity.
- *Salmonella* isolates serogrouped and tested for antimicrobial sensitivity. Culture slants sent to NVSL for serotyping.
- *E. coli* isolates tested for Shiga toxin (STEC).
- *E. coli* STEC isolates genotyped (e.g., 0157, etc.).
- *E. coli* antimicrobial sensitivity panel run on all cultures positive for growth.



Results returned to NAHMS

- Results entered, validated/edited.
- Results returned to producer via NAHMS Coordinator (not STEC and STEC genotyping).

Enteric Microbe Collection Design and Procedures

SAMPLE COLLECTION

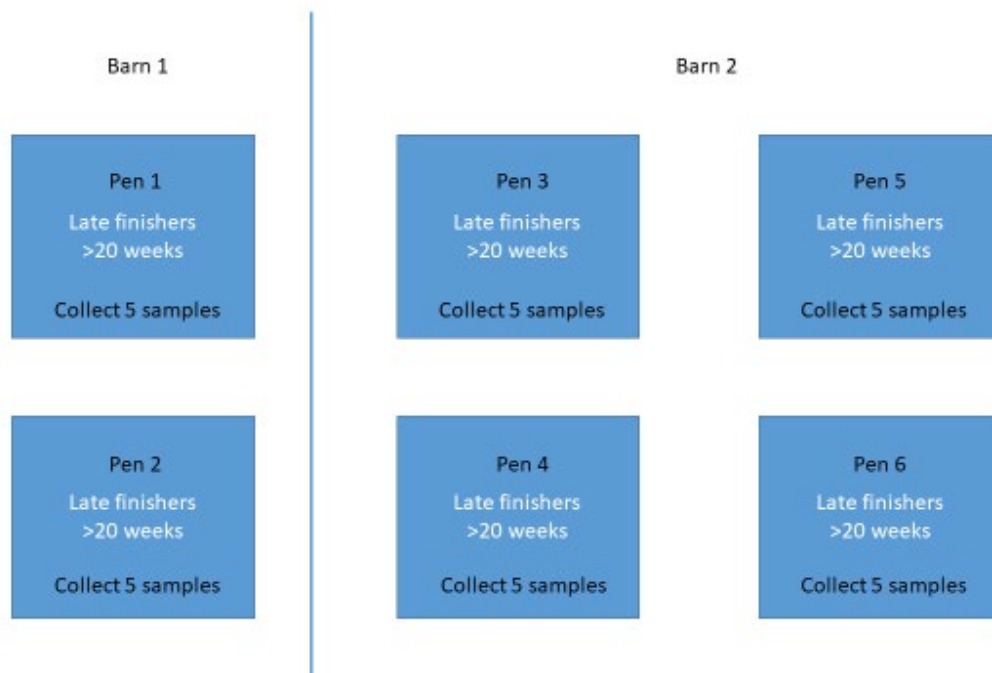
Collect up to **30 samples from up to 6 pens** (5 samples/pen) containing pigs 20 weeks and over. The pens with the pigs of the right age may be scattered among different rooms and in different barns.

If **less** than 6 pens, collect roughly equal numbers of samples from all pens containing pigs 20 weeks and over for a maximum of 30 samples. For example, if there are 4 pens take 8 samples from each of 2 pens and 7 samples from each of 2 pens.

If **more** than 6 pens, attempt to geographically space the pens sampled (collect from pens roughly equidistant from each other).

The following schematics demonstrate 3 sampling scenarios for a site with 2 barns of finisher pigs.

Sampling Scenario 1: 2 Barns, 6 Pens of Finisher Pigs



Enteric Microbe Collection Design and Procedures

Sampling Scenario 2: 2 Barns, 8 Pens of Finisher Pigs



Sampling Scenario 3: 2 Barns, 4 Pens of Finisher Pigs



Enteric Microbe Collection Design and Procedures

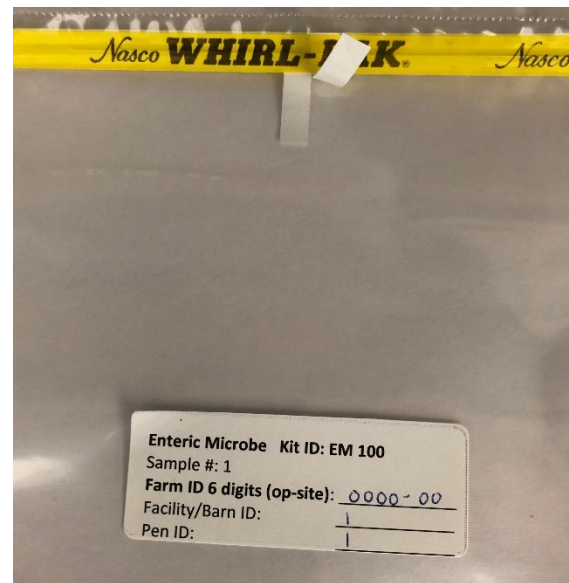
FARM ID AND SAMPLE LABELS

- The ***Farm ID** can be found on the first page of the VS questionnaire. It consists of the **Operation number and the Site number**. We recommend pre-recording the Farm ID on the Collection Record and the sample labels.

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES NATIONAL ANIMAL HEALTH MONITORING SYSTEM 2150 CENTRE AVE, BLDG B FORT COLLINS, CO 80526			2021 NAHMS Swine Large Enterprise VS Visit		
State FIPS: _____ 2 digits	Operation #: _____ 4 digits	Site #: _____ 2 digits	Interviewer: _____ Initials	Date: ____/____/____ mm/dd/yy	
Arrival time at site: _____			Start time of questionnaire: _____		

- On the sample label, record:
 - *Farm ID (6 digits):**
Operation (4 digits), Site (2 digits)

The Farm ID must match the ID on the Collection Record.
 - Facility or Barn ID:**
This is a generic number. Any designation will do whether text or numeric.
 - Pen ID:**
This is a generic number. Any designation will do whether text or numeric.



*Please note, we are not identifying sampling using the FIPS ID due to confidentiality. The Farm ID will only include the Operation number and the Site number. We will determine the FIPS ID based on the Kit ID and the Coordinator's name written on the Collection Form. **Please be sure to write the State Coordinator's name on the Collection Form.**

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0315. The time required to complete this information collection is estimated to average 2 hours 45 minutes per response, including the time to review instructions, search existing data resources, gather the data needed, and complete and review the information collected.

OMB Approved
0579-0315
EXP: 01/2023

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
NATIONAL ANIMAL HEALTH MONITORING SYSTEM
2150 CENTRE AVE, BLDG B
FORT COLLINS, CO 80526

2021 NAHMS Swine Large Enterprise Fecal Collection Record

Kit Contents

- 30 Whirl-Pak® bags
- 2 Extra Whirl-Pak® bags
- 6 pairs of gloves
- 6, 1 Gallon-size Ziploc® bags
- Insulated cooler and shipping box
- 3 Ice packs (freeze prior to farm visit)
- 1 Absorbent sheet
- 2 Liner bags (one used as trash bag, one used to line the cooler)
- Paperwork packet: Fecal Collection Record, 30 sample labels, shipping airbill

Collection Instructions

Collect samples on a **Sunday-Wednesday** and ship within 24 hours. Sample from pens populated by late finisher pigs (20 weeks and older). **Ideally, take 30 samples from the floors of a maximum of 6 pens (5 samples per pen).**

Determine how many pens on the site contain late finisher pigs that are 20 weeks and older. These pens may be scattered among different rooms and in different barns. If **less** than 6 pens, collect samples from all pens for a maximum of 30 samples. For example, if there are 4 pens take 8 samples from each of 2 pens and 7 samples from each of 2 pens. If **more** than 6 pens, attempt to geographically space the pens sampled. That is, from pens roughly equidistant from each other (especially if there are multiple pens in a room).

Using clean gloves, collect about a palm full (20 grams) of fresh feces from the pen floor and place in a single Whirl-Pak® bag. Express the air out of the bag and fold over the top twice before securing closure tabs (Do not fold top more than twice).

Write the **6-digit Farm ID** (operation-site), **Facility, and Pen IDs on a sample label**, peel off and place securely on the bag (Do not use the sample label to secure bags closed). Repeat. Place all samples from one pen into a gallon sized Ziploc® bags and seal the ends of the large bags. Repeat for the rest of the sampled pens.

Place the Ziploc® bags inside the liner bag with 3 frozen ice packs and the absorbent sheet. Place the liner bag inside the insulated shipment container and close the shipping container lid.

Collection Record Instructions

Complete the Fecal Collection Record Form. **Place the original (white) collection record on top of the insulated cooler box lid.** Leave the yellow copy of the collection record with the producer. Secure the shipping box.

Shipping and Results

Keep samples cool in a refrigerator, attach a shipping airbill (addressed to NCSU) to the shipping box, and ship **within 24 hours of collection on Sunday-Wednesday**. The samples will be tested for *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter*. All results will be returned to the Producer approximately 90 days after collection.

Swine 2021: Fecal Collection Record

State Coordinator Name: _____

Farm ID	# of People Involved		Key Collector Initials	Collection Date	Hours to Take and Prep Samples	Hours of Overall Travel Time	Kit Number
	<input type="checkbox"/> Fed VMO <input type="checkbox"/> Fed AHT <input type="checkbox"/> Producer <input type="checkbox"/> State VMO <input type="checkbox"/> St AHT <input type="checkbox"/> Priv Vet <input type="checkbox"/> Others-specify: _____						
6-digits: Op, Site	Enter number for each category		MM/DD/YY	In quarter hours	In quarter hours	Must match labels	

First, answer the following three questions regarding this site:

1. On this site, how many buildings house finisher pigs? _____ finisher buildings
2. What is the average number of rooms in the finisher buildings? _____ rooms per building
3. What is the average number of pens in each room? _____ pens per room

Pen Index	# of bags	LIST ALL BAG numbers taken for this pen	Facility/Barn ID ¹	Pen ID ¹	Facility Type T=Total Confinement O=Open building ²	Approximate # of pigs in pen	Average age of pigs in pen (weeks)	Approximate # of pigs share air space ³	Gender of pigs in pen G=Gilts, B=Barrows M=Mixed	Evidence of diarrhea in pen? Y=Yes N=No
1										
2										
3										
4										
5										
6										

¹Any designation will do whether text or numeric so long as it differentiates between row entries.

²Total confinement means solid exterior walls and mechanical ventilation. An open building generally has open sides or the potential for sides to be opened weather permitting.

³This would be the number of pigs in the barn or room within a barn usually.

Fecal Collection Record Example

Swine 2021: Fecal Collection Record

State Coordinator Name: Dr. Bob Smith

Farm ID <u>0999-12</u>	# of People Involved <input checked="" type="checkbox"/> Fed VMO <input type="checkbox"/> Fed AHT <input checked="" type="checkbox"/> Producer <input type="checkbox"/> State VMO <input type="checkbox"/> St AHT <input type="checkbox"/> Priv Vet <input type="checkbox"/> Others-specify: _____	Key Collector Initials <u>AW</u>	Collection Date <u>10/16/21</u>	Hours to Take and Prep Samples <u>1.75</u>	Hours of Overall Travel Time <u>1.25</u>	Kit Number <u>100</u>
6-digits: Op, Site	Enter number for each category		MM/DD/YY	In quarter hours	In quarter hours	Must match labels

First, answer the following three questions regarding this site:

1. On this site, how many buildings house finisher pigs? 2 finisher buildings
2. What is the average number of rooms in the finisher buildings? 4 rooms per building
3. What is the average number of pens in each room? 16 pens per room

Pen Index	# of bags	LIST ALL BAG numbers taken for this pen	Facility/Barn ID ¹	Pen ID ¹	Facility Type T=Total Confinement O=Open building ²	Approximate # of pigs in pen	Average age of pigs in pen (weeks)	Approximate # of pigs share air space ³	Gender of pigs in pen G=Gilts, B=Barrows M=Mixed	Evidence of diarrhea in pen? Y=Yes N=No
1	5	1, 2, 3, 4, 5	1A	8	T	6	21w	200	G	N
2	5	6, 7, 8, 9, 10	1A	2	T	7	23w	200	G	N
3	5	21, 22, 23, 24, 25	1A	4	T	7	23w	200	G	N
4	5	11, 12, 13, 14, 15	1A	10	T	6	21w	200	G	N
5	5	26, 27, 28, 29, 30	2B	5	O	8	24w	100	B	Y
6	5	16, 17, 18, 19, 20	2B	7	O	8	21w	100	B	N

¹Any designation will do whether text or numeric so long as it differentiates between row entries.

²Total confinement means solid exterior walls and mechanical ventilation. An open building generally has open sides or the potential for sides to be opened weather permitting.

³This would be the number of pigs in the barn or room within a barn usually.

National Animal Health Monitoring System (NAHMS) Enteric Microbe Report

Dear participant,

Thank you for participating in the enteric microbe testing portion of the NAHMS Swine 2021 Study. This report contains testing results for *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter* performed on samples from swine pens at your site.

If you have questions about your results, please contact Dr. Alyson Wiedenheft, the NAHMS biologics coordinator at (970) 494-7290 or alyson.m.wiedenheft@usda.gov.

Background on *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter*:

The bacteria *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter* can inhabit the intestinal tract of swine and can be shed in their feces. Swine that are shedding these enteric microbes can have clinical signs such as diarrhea or fever, or can appear totally healthy. Many *E. coli* and *Enterococcus* are normal (commensal) flora of the intestines of humans and animals, and while most subtypes are harmless, others, like *E. coli* O157:H7, can cause disease in humans and swine by producing a toxin called Shiga toxin. We expect most animals to shed *E. coli* normally.

Overview of Enteric Microbe Testing Performed and Results Reported:

Fecal samples collected from finishing pens on your site were tested for the presence of *Salmonella*, *E. coli*, *Enterococcus*, and *Campylobacter*.

The presence ("Positive") or absence ("Negative") of the microbes in the samples are reported for each sample. For pen samples, there may not be enough fecal samples to complete all the testing. If an insufficient amount of fecal sample was submitted, the column will read "NA."

Date of report: **11/1/2021**

Enteric Microbe test results for NAHMS ID: **99-9999-99**

Date of sample collection: **10/1/2021**

Enteric Microbe RESULTS:

Individual Sample Level Results:

Sample #	<i>Salmonella</i>	Generic <i>E. coli</i>	<i>Enterococcus</i>	<i>Campylobacter</i>
1	Negative	Positive	Negative	Negative
2	Negative	Positive	Positive	Negative
3	Negative	Positive	Positive	Positive
4	Negative	Positive	Positive	Positive
5	Negative	Positive	Positive	Positive
6	Negative	Positive	Positive	Positive
7	Negative	Positive	Positive	Positive
8	NA	NA	NA	NA
9	Positive	Positive	Positive	Negative
10	Negative	Positive	Negative	Negative
11	Negative	Positive	Negative	Negative
12	Negative	Positive	Positive	Negative
13	Negative	Positive	Positive	Negative
14	Negative	Positive	Positive	Positive
15	Negative	Positive	Positive	Positive
16	Negative	Positive	Positive	Positive
17	Negative	Positive	Positive	Positive
18	Negative	Positive	Positive	Positive
19	Negative	Positive	Positive	Negative
20	Negative	Positive	Positive	Negative
21	Negative	Positive	Positive	Negative
22	Negative	Positive	Positive	Negative
23	Negative	Positive	Positive	Negative
24	Negative	Positive	Positive	Negative
25	Negative	Positive	Positive	Negative
26	Positive	Positive	Positive	Positive
27	Positive	Positive	Positive	Negative
28	Negative	Positive	Positive	Negative
29	Positive	Positive	Positive	Positive
30	Negative	Positive	Positive	Negative

Percentage of Salmonella Isolates by *Salmonella* Serotypes Identified and percentage of *Enterococcus* and *Campylobacter* Isolates by Species Identified:

<i>Salmonella</i> Serotype	Percent Isolates	<i>Enterococcus</i> Species	Percent Isolates	<i>Campylobacter</i> Species	Percent Isolates
<i>Derby</i>	29.6	<i>E. hirae</i>	29.6	<i>C. coli</i>	89.1
<i>Typhimurium Copenhagen</i>	22.6	<i>E. faecalis</i>	27.4	<i>C. jejuni</i>	9.0
<i>Agona</i>	20.8	<i>E. spp*</i>	16.0	Other <i>C. spp.</i>	0.9
<i>Anatum</i>	10.5	<i>E. faecium</i>	10.9		
<i>Typhimurium</i>	6.5	<i>E. mundtii</i>	7.7		
<i>Worthington</i>	4.5	<i>E. casseliflavus</i>	3.9		
<i>Johannesburg</i>	4.0	<i>E. gallinarum</i>	2.5		
<i>Muenchen</i>	1.5	<i>E. avium</i>	2.0		
Total	100.0	Total	100.0	Total	100.0

Antimicrobial Resistance among *Salmonella*, *Enterococcus*, *Campylobacter* and *E. coli* Identified:

<i>Salmonella</i>		<i>E. coli</i>		<i>Enterococcus</i>		<i>Campylobacter</i>	
Antimicrobial	Percent Isolates	Antimicrobial	Percent Isolates	Antimicrobial	Percent Isolates	Antimicrobial	Percent Isolates
Streptomycin	60.8	Azithromycin	86.4	Tetracycline	96.4	Ampicillin	96.4
Ampicillin	54.3	Tetracycline	85.4	Tylosin tartrate	80.4	Tetracycline	80.4
Chloramphenicol	29.3	Trimethoprim / sulfamethoxazole	80.5	Chloramphenicol	79.5	Amoxicillin/ clavulanic acid	79.5
Tetracycline	14.5	Gentamicin	64.4	Nitrofurantoin	60.4	Streptomycin	60.4
Gentamicin	8.2	Amoxicillin/ clavulanic acid	58.8	Streptomycin	58.8	Sulfisoxazole	58.8
Amoxicillin/ clavulanic acid	7.6	Streptomycin	35.4	Linezolid	35.4	Chloramphenicol	35.4
Ceftriaxone	7.6	Ceftriaxone	28.6	Erythromycin	28.6	Nalidixic acid	28.6
Ceftiofur	7.4	Sulfisoxazole	12.0	Quinupristin/ Dalfopristin	12.0	Trimethoprim/ sulfamethoxazole	12.0
Cefoxitin	7.2	Ceftiofur	10.7	Ciprofloxacin	10.7	Ciprofloxacin	10.7
Azithromycin	4.2	Chloramphenicol	10.5	Vancomycin	10.5	Ceftiofur	10.5
Nalidixic acid	1.3	Ciprofloxacin	9.8	Kanamycin	9.8	Cefoxitin	9.8
Ciprofloxacin	1.1	Cefoxitin	4.8	Penicillin	4.8	Gentamicin	4.8
Sulfisoxazole	1.0	Ampicillin	3.2	Daptomycin	3.2	Ceftriaxone	3.2
Trimethoprim/ sulfamethoxazole	0.8	Nalidixic acid	1.0	Tigecycline	0.4	Azithromycin	0.4
				Lincomycin	0.2		
				Gentamicin	0.1		

Oral Fluids Collection Design and Procedures

ORAL FLUIDS OVERVIEW

Oral fluids testing for viral exposure to Seneca virus A (Seneca Valley virus, SVA).

View: <https://www.securepork.org/training-materials/disease-monitoring-sample/>
prior to collection visit.

VS Oral Fluid Collection-Collect 8 samples from 8 pens from pigs 20 weeks and older.

1. Hang ropes in **8 pens** populated by pigs 20 weeks and older. If one barn or room contains the site's oldest finishing pigs, pick that one. You may sample from more than one barn.
2. Cut 8 pieces of rope (approximately 4 foot sections). Depending on where you hang the ropes, trim the ropes so the end is approximately shoulder height of the pigs in the pen.
3. The ropes should be hung in pens that are roughly equidistant from one another. Take one end of the cotton rope (5/8" 3 Strand Cotton) and tie to the top bar of each pen or the gate.
4. Tie the rope into a knot on the gate and cut the end in the pen so that that this end hangs approximately at shoulder height for most of the animals in the pen.
5. Unravel the part of the rope that hangs in the pen rope into its strands. Shake the end of the rope in the pen so that pigs are attracted to it.
6. Wait 30-45 minutes while they chew on the ropes.



7. Insert the lower, wet portion of each rope into a quart sized Ziploc® bag and cut the part that's inside the bag from the remainder of the rope still hanging. Manually squeeze the rope pieces while inside the Ziploc® bag to release the oral fluid.
8. Cut the corner of the Ziploc® bag and let the fluid drip into a 50 ml centrifuge tube. Put the lid on each centrifuge tube, and check that the lid is straight and tight.
9. Place the used Ziploc® bags and the rope ends into the trash bag. Untie and place excess hanging rope in small trash bag along with small plastic bags containing the rope pieces.
10. Copy the 6 digit Farm ID (operation/site) from the first page of the VS Questionnaire onto each sample label. Also, write in the Facility and Pen IDs on each sample label, then peel off and place securely onto a centrifuge tube. Repeat for the other 7 ropes.
11. Place centrifuge tubes in the centrifuge box. Place boxed centrifuge tubes in the gallon sized Ziploc® plastic bag and seal. Place the Ziploc® bag inside the liner bag with 3 frozen ice packs and the absorbent sheet. Place the liner bag inside the insulated shipment container and close the shipping container lid.
12. Complete the Oral Fluids Collection Record Form. Copy the 6 digit Farm ID (operation/site) from the first page of the VS Questionnaire onto the Collection Record. **Place the original (white) collection record on top of the insulated cooler box lid.** Leave the yellow copy of the collection record with the producer. Secure the shipping box.
13. Keep samples cool, on ice, and shipped with 24 hours of collection. Replace ice packs before shipping to insure the icepacks are completely frozen.



ISU receives samples and tests for SVA



Results returned to NAHMS

- Results entered, validated/edited.
- Results returned to producer via NAHMS Coordinator.

Oral Fluids Collection Design and Procedures

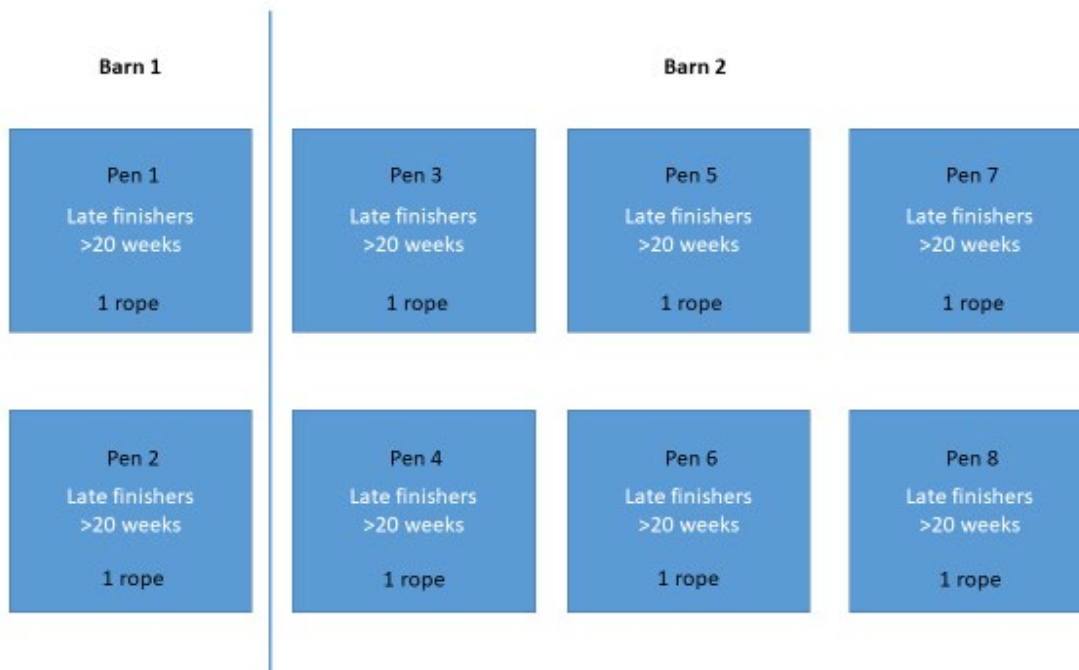
SAMPLE COLLECTION

Collect **8 samples** from **8 pens** containing pigs 20 weeks and over. The ropes should be hung in pens that are roughly equidistant from one another, one rope hung in each pen. You may sample from more than one barn as long as the pigs are 20 weeks and over.

If **more** than 8 pens, attempt to geographically space the pens sampled (collect from pens roughly equidistant from each other).

The following schematics demonstrate 2 sampling scenarios for a site with 2 barns of finisher pigs.

Sampling Scenario 1: 8 Pens of Finisher Pigs in 2 Barns



Oral Fluids Collection Design and Procedures

Sampling Scenario 2: 10 Pens of Finisher Pigs in 2 Barns

Barn 1	Barn 2			
Pen 1 Late finishers >20 weeks 1 rope	Pen 3 Late finishers >20 weeks 1 rope	Pen 5 Late finishers >20 weeks 1 rope	Pen 7 Late finishers >20 weeks No samples	Pen 9 Late finishers >20 weeks 1 rope
Pen 2 Late finishers >20 weeks 1 rope	Pen 4 Late finishers >20 weeks No samples	Pen 6 Late finishers >20 weeks 1 rope	Pen 8 Late finishers >20 weeks 1 rope	Pen 10 Late finishers >20 weeks 1 rope

Oral Fluids Collection Design and Procedures

FARM ID AND SAMPLE LABELS

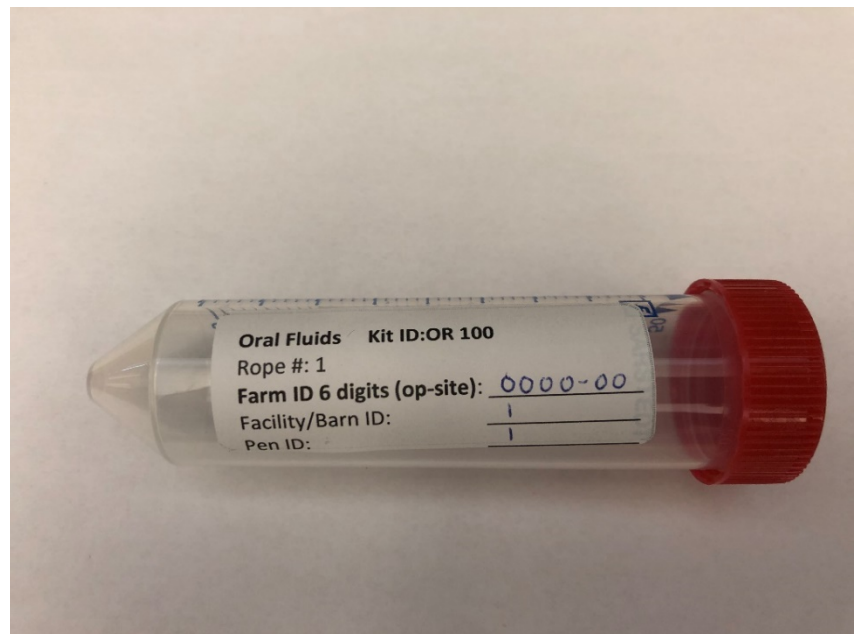
- The ***Farm ID** can be found on the first page of the VS questionnaire. It consists of the **Operation number and the Site number**. We recommend pre-recording the Farm ID on the Collection Record and the sample labels.

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES NATIONAL ANIMAL HEALTH MONITORING SYSTEM 2150 CENTRE AVE, BLDG B FORT COLLINS, CO 80526			2021 NAHMS Swine Large Enterprise VS Visit		
State FIPS: _____ 2 digits	Operation #: _____ 4 digits	Site #: _____ 2 digits	Interviewer: _____ Initials	Date: ____/____/____ mm/dd/yy	
Arrival time at site: _____			Start time of questionnaire: _____		

- On the **sample label**, record:
 - *Farm ID (6 digits):**
Operation (4 digits), Site (2 digits)

The Farm ID must match the ID on the Collection Record.

- Facility or Barn ID:**
This is a generic number. Any designation will do whether text or numeric.
- Pen ID:**
This is a generic number. Any designation will do whether text or numeric.



*Please note, we are not identifying sampling using the FIPS ID due to confidentiality. The Farm ID will only include the Operation number and the Site number. We will determine the FIPS ID based on the Kit ID and the Coordinator's name written on the Collection Form. **Please be sure to write the State Coordinator's name on the Collection Form.**

Oral Fluids Collection Design and Procedures

Oral Fluids Step by Step Checklist

1. We recommend reading the NAHMS Swine 2021 Oral Fluids Instructions and watching the following video:
<https://www.securepork.org/training-materials/disease-monitoring-sample/>

2. **You are required to use the provided kit and paperwork for sampling and submission.**

Prepare for sampling:

- ☐ Remove the 3 ice packs from the kit and freeze them completely (24 hours) before shipping.
- ☐ Select up to **8 pens** of finisher pigs that are roughly equidistant from one another to sample.
- ☐ Cut the rope so that each rope segment will hang at shoulder length for most of the animals in the pen for each of the 8 pens.

3. At the time of sampling:

- ☐ Unravel the strands at the end of the rope in the pen.
- ☐ Wait 30-45 minutes while the pigs chew on the ropes.
- ☐ For each pen, insert the chewed end of each rope into a quart sized Ziploc® bag and cut the part that's inside the bag from the remainder of the rope still hanging.
- ☐ Manually squeeze the rope pieces while inside the Ziploc® bag to release the oral fluid. Place the rope piece in one of the liner trash bags.
- ☐ Cut the corner of the Ziploc® bag and let the fluid drip into a 50 ml centrifuge tube until the tube is half full. Put the lid on each centrifuge tube, and check that the lid is straight and tight. Place the quart sized bag remnant in the same liner trash bag as the rope piece.
- ☐ **Label the tubes:** Write the 6-digit Farm ID (operation-site), Facility, and Pen IDs on a centrifuge tube label and attach the labels onto a centrifuge tube. Place the tube back in its box.
- ☐ Repeat for the other 7 pens.

4. Fill out the Collection Form

- ☐ Include the **6-digit Farm ID, Kit ID**, and the other information asked in the header.
- ☐ Fill out the chart with rows pertaining to each rope sample.
- ☐ Keep yellow copy of the collection record for you records.

5. Prepare the samples for shipping

- ☐ Place boxed centrifuge tubes in the **gallon** sized Ziploc® plastic bag and seal.
- ☐ Place the Ziploc® bag inside the second liner bag with **3 frozen ice packs** and the absorbent sheet.
- ☐ Place the liner bag inside the insulated shipment container and close the shipping container lid.
- ☐ **Place the original (white) collection record on top of the insulated cooler box lid.**

6. Shipping

- ☐ **Keep samples cool, on ice, and shipped with 24 hours of collection.**
- ☐ Be sure the ice packs are completely frozen before shipping.
- ☐ Secure the shipping box.
- ☐ Fill out and attach the pre-paid airbill pre-addressed to ISU and attach it to the shipping box.
- ☐ Drop off the shipping box at a local Fed-Ex shipping site.

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0315. The time required to complete this information collection is estimated to average 2 hours per response, including the time to review instructions, search existing data resources, gather the data needed, and complete and review the information collected.

OMB Approved
0579-0315
EXP: 01/2023

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
NATIONAL ANIMAL HEALTH MONITORING SYSTEM
2150 CENTRE AVE, BLDG B
FORT COLLINS, CO 80526

Swine 2021 Large Enterprise Saliva Collection Record

Kit Contents

- 32 Feet of 5/8" 3 strand cotton rope
- 10 Quart Ziploc® bags
- 8 pairs of gloves
- 1 Gallon Ziploc® bag
- 8, 50 ml Centrifuge tubes with a box
- Insulated cooler and shipping box
- Rope Cutters
- 3 Ice packs (freeze prior to farm visit)
- 1 Absorbent sheet
- 2 Linear bags (one used as trash bag, one used to line the cooler)
- Paperwork packet: Oral Fluids Collection Record, 10 centrifuge tubes labels, shipping airbill

Collection Instructions

Collect samples on a **Sunday-Friday**. The site must house **1 or more finishing pigs (20 weeks or older) housed in a barn or enclosure (i.e. a hoop barn) in pens**. Hang ropes in a maximum of **8 pens in a barn(s) or room within a barn** in pens populated by these pigs. If one barn or room contains the site's oldest finishing pigs, pick that one. The ropes should be hung in pens that are roughly equidistant from one another.

Ropes are hung by taking one end of the cotton rope (5/8" 3 Strand Cotton) and tying one end to the top bar of each pen or the gate. Tie the rope knot on the gate and cut the end in the pen so that that this end hangs approximately at shoulder height for most of the animals in the pen. Unravel the part of the rope that hangs in the pen rope into its strands. Shake the end of the rope in the pen so that pigs are attracted to it.

Wait 30-45 minutes while they chew on the ropes. While the rope is still hanging, insert the lower, wet portion of each rope into a quart sized Ziploc® bag and cut the part that's inside the bag from the remainder of the hanging rope. Manually squeeze the rope pieces while inside the Ziploc® bag to release the oral fluid. Cut the corner of the Ziploc® bag and let the fluid drip into a 50 ml centrifuge tube. **You only need to collect 25ml of fluid, so only fill the tube half full.** Close each centrifuge tube, checking that the lid is tight. Place the used Ziploc® bags and the rope ends in the trash bag. Untie and place excess hanging rope in the trash bag. Write the **6-digit Farm ID** (operation-site), **Facility, and Pen IDs on a sample label**, peel off and place securely on the appropriate centrifuge tubes. Place the centrifuge tubes in a centrifuge tube box.

Place boxed centrifuge tubes in the gallon sized Ziploc® plastic bag and seal. Place the Ziploc® bag inside the liner bag with 3 frozen ice packs and the absorbent sheet. Place the liner bag inside the insulated shipment container and close the shipping container lid.

Collection Record Instructions

Complete the Oral Fluids Collection Record Form (attached below). Skip the first row that is labeled "lab use only." **Place the original (white) collection record on top of the insulated cooler box lid.** Leave the yellow copy of the collection record with the producer. Secure the shipping box.

Shipping and Results

Keep samples cool in a refrigerator, attach shipping airbill (addressed to ISU) to the shipping box, and ship **within 24 hours of collection on Sunday-Friday**. The samples will be tested for the nucleic acid of Seneca Valley virus and results will be returned to the Producer in approximately 90 days after collection.

Swine 2021: Oral Fluids Collection Record

State Coordinator Name: _____

Submitter: NAHMS (lab use only)		Owner: Swine 2021 (lab use only)		Site: Use Farm ID (lab use only)		Reference: Use Kit Number (lab use only)		Animal ID: Use Barn ID (lab use only)	
Farm ID	# of People Involved ___ Fed VMO ___ Fed AHT ___ Producer ___ State VMO ___ St AHT ___ Priv Vet ___ Others-specify: _____		Key Collector Initials	Collection Date	Hours to Take and Prep Samples	Hours of Overall Travel Time	Kit Number		
6-digits: Op, Site		Enter number for each category		MM/DD/YY		In quarter hours		In quarter hours	
								Must match labels	

Rope Number	Facility/Barn ID ¹	Pen ID ¹	Facility Type ²	Where was rope hung? ³	Approximate # of pigs in pen	# of pigs share air space ⁴	Average age of pigs in pen (weeks)	Gender of pigs in pen ⁵	Vaccination codes ⁶			
									1st	2nd	3rd	4th
1			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O				<input type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M				
2			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O								
3			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O				<input type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M				
4			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O								
5			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O				<input type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M				
6			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O								
7			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O				<input type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M				
8			<input type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O								

¹Any designation will do whether text or numeric as long as it differentiates between row entries.

²**T=Total Confinement, O=Open building.** Total confinement means solid exterior walls and mechanical ventilation. An open building generally has open sides or the potential for sides to be opened weather permitting.

³**B=Bar (e.g., top bar of pen enclosure), G=Gate, O=Other**

⁴This would be the number of pigs in the barn or room within a barn usually.

⁵**G=Gilts, B=Barrows and M=Mixed.**

⁶Up to 4 different vaccinations may be entered. The codes and what they stand for are listed below. These are vaccinations that may have been given at any time in the pigs' lives.

Vaccination codes	
1=APP (Actinobacillus pleuropneumoniae)	11=Influenza
2=Actinobacillus suis (autogenous)	12=Leptospirosis
3=Atrophic rhinitis (Bordetella/Pasteurella)	13=Mycoplasma hyopneumoniae
4=Clostridium difficile (autogenous)	14=Porcine circovirus 2
5=Clostridium perfringens Type A	15=PRRS
6=Clostridium perfringens Types C and D	16=Porcine epidemic diarrhea
7=Erysipelas	17=Rotavirus
8=E. coli (K88, K99, 987P, F41)	18=Salmonella
9=Glasser's disease (Haemophilus parasuis)	19=Streptococcus suis
10=Ileitis (Lawsonia intracellularis)	20=TGE (transmissible gastroenteritis)

Oral Fluids Collection Record Example

Swine 2021: Oral Fluids Collection Record

State Coordinator Name: Dr. Bob Smith

Submitter: NAHMS <small>(lab use only)</small>		Owner: Swine 2021 <small>(lab use only)</small>		Site: Use Farm ID <small>(lab use only)</small>		Reference: Use Kit Number <small>(lab use only)</small>		Animal ID: Use Barn ID <small>(lab use only)</small>	
Farm ID 0999-12	# of People Involved <input type="checkbox"/> Fed VMO <input type="checkbox"/> Fed AHT <input type="checkbox"/> Producer <input type="checkbox"/> State VMO <input type="checkbox"/> St AHT <input type="checkbox"/> Priv Vet <input type="checkbox"/> Others-specify:		Key Collector Initials AW	Collection Date 10/16/21	Hours to Take and Prep Samples 1.5	Hours of Overall Travel Time 1.25	Kit Number 101		
6-digits: Op, Site		Enter number for each category		MM/DD/YY	In quarter hours	In quarter hours	Must match labels		

Rope Number	Facility/Barn ID ¹	Pen ID ¹	Facility Type ²	Where was rope hung? ³	Approximate # of pigs in pen	# of pigs share air space ⁴	Average age of pigs in pen (weeks)	Gender of pigs in pen ⁵	Vaccination codes ⁶			
									1st	2nd	3rd	4th
1	1A	8	<input checked="" type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input checked="" type="checkbox"/> G <input type="checkbox"/> O	6	200	21 W	<input checked="" type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
2	1A	10	<input checked="" type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input checked="" type="checkbox"/> G <input type="checkbox"/> O	6	200	21 W	<input checked="" type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
3	1A	6	<input checked="" type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input checked="" type="checkbox"/> G <input type="checkbox"/> O	8	200	22 W	<input checked="" type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
4	1A	4	<input checked="" type="checkbox"/> T <input type="checkbox"/> O	<input type="checkbox"/> B <input checked="" type="checkbox"/> G <input type="checkbox"/> O	8	200	22 W	<input checked="" type="checkbox"/> G <input type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
5	2B	1	<input type="checkbox"/> T <input checked="" type="checkbox"/> O	<input type="checkbox"/> B <input checked="" type="checkbox"/> G <input type="checkbox"/> O	10	100	21 W	<input type="checkbox"/> G <input checked="" type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
6	2B	3	<input type="checkbox"/> T <input checked="" type="checkbox"/> O	<input checked="" type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O	7	100	24 W	<input type="checkbox"/> G <input checked="" type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
7	2B	5	<input type="checkbox"/> T <input checked="" type="checkbox"/> O	<input checked="" type="checkbox"/> B <input type="checkbox"/> G <input type="checkbox"/> O	6	100	24 W	<input type="checkbox"/> G <input checked="" type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1
8	2B	7	<input type="checkbox"/> T <input checked="" type="checkbox"/> O	<input type="checkbox"/> B <input type="checkbox"/> G <input checked="" type="checkbox"/> O	6	100	21 W	<input type="checkbox"/> G <input checked="" type="checkbox"/> B <input type="checkbox"/> M	9	11	14	1

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 13=Mycoplasma hyopneumoniae
 14=Porcine circovirus 2
 15=PRRS
 16=Porcine epidemic diarrhea
 17=Rotavirus
 18=Salmonella
 19=Streptococcus suis
 20=TGE (transmissible gastroenteritis)

Oral Fluids Producer Report Example

National Animal Health Monitoring System (NAHMS) Oral Fluids Report

Dear participant,

Thank you for participating in the oral fluids testing portion of the NAHMS Swine 2021 Study. This report contains testing results for Seneca Valley virus performed on samples from swine pens at your site.

If you have questions about your results, please contact Dr. Alyson Wiedenheft, the NAHMS biologics coordinator at (970) 494-7290 or alyson.m.wiedenheft@usda.gov.

Background on Seneca Valley virus:

Seneca Valley virus was unknown until 2002. Lesions and clinical signs mimic other vesicular diseases and include ulcerations on the distal limbs, lameness, and vesicles around the oral mucosa. Since this disease is clinically indistinguishable from foreign animal diseases, diagnostic testing must be used to identify this virus.

Overview of Oral Fluids Testing Performed and Results Reported:

Oral fluid samples collected from finishing pens on your site were tested for exposure to Seneca Valley virus.

The presence ("Positive") or absence ("Negative") of the viruses in the samples are reported for each rope sample.

Date of report: **11/1/2021**

Oral Fluids test results for Farm ID: **9999-99**

SENECA VALLEY VIRUS RESULTS:

Individual Rope Results:

Rope #	Seneca Valley virus
1	Negative
2	Negative
3	Negative
4	Positive
5	Negative
6	Negative
7	Negative
8	Negative