Assembling Materials for Environmental Sampling of Viral Pathogens

How to Prepare Cotton Gauze Samples

Supplies needed:

- 4 × 4 inch surgical cotton gauze
- Pre-moistening solution:
  - Preferred pre-moistening solution – phosphate buffered solution (PBS) of 1X concentration and pH of 7.4
  - Other option if unable to get PBS – 0.9% sterile saline
- 50 mL conical tubes
- Nitrile or latex gloves
- Disinfectant
  - Consult the manufacturers label for the dilution and contact time that is appropriate for the pathogen of interest
  - Some examples include:
    - 70% ethanol spray with contact time of 10 minute
    - 1:256 diluted quaternary ammonium/glutaraldehyde (Synergize) spray with contact time of 10 minutes
    - 1:16 diluted accelerated hydrogen peroxide (Intervention) spray with contact time of 5 minutes
    - 10% bleach spray with contact time of 10 minutes
- Paper towels
- Reusable plastic storage container

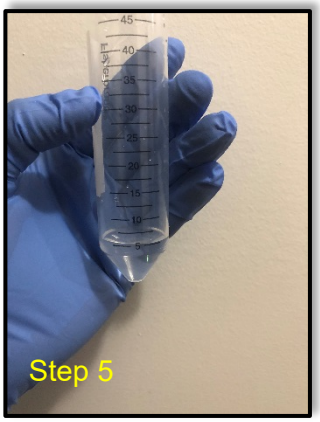
1. Clear off an area where supplies can be placed in one location for assembling sample materials. Once the work space has been cleared, apply disinfectant of choice following the recommendations on the manufacturer's label for dilution of disinfectant and time that is required for the disinfectant to sit on a surface for pathogen of interest. Wipe away with paper towels.

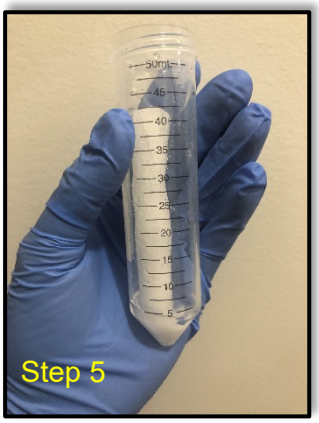
2. Place all needed supplies on the clean and disinfected surface. Put on a new pair of gloves.

3. Roll up a piece of cotton gauze so that it can fit into a 50 mL conical tube (as shown in picture below).

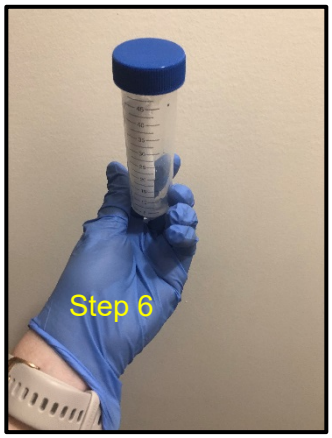
4. Open a 50 mL conical tube and put one rolled up cotton gauze inside the tube (as shown in pictures below).

5. Measure 5 mL of pre-moistening solution and put it into the 50 mL conical tube that has cotton gauze inside (as shown in the pictures below). Close the 50 mL conical tube.





6. Agitate the conical tube so that the cotton gauze absorbs all of the pre-moistening solution (as shown in the pictures below).



7. Repeat steps 3-6 until the appropriate number of samples are made.

8. Close the pre-moistening solution and cotton gauze packaging then place all supplies in the reusable plastic storage container. Take off and discard used gloves. Store reusable plastic storage container in a dry and temperature stable area.

How to Prepare Paint Roller Cover Samples

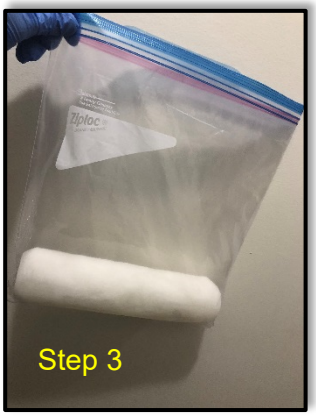
Supplies needed:

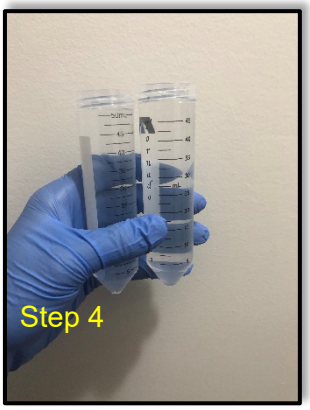
- Synthetic paint roller cover
- Plastic storage bag
- Pre-moistening solution:
    - Preferred pre-moistening solution – phosphate buffered solution (PBS) of 1X concentration and pH of 7.4
    - Other option if unable to get PBS – 0.9% sterile saline
- 50 mL conical tube for measuring
- Nitrile or latex gloves
- Disinfectant
    - Consult the manufacturers label for the dilution and contact time that is appropriate for the pathogen of interest
    - Some examples include:
        - 70% ethanol spray with contact time of 10 minute
        - 1:256 diluted quaternary ammonium/glutaraldehyde (Synergize) spray with contact time of 10 minutes
        - 1:16 diluted accelerated hydrogen peroxide (Intervention) spray with contact time of 5 minutes
        - 10% bleach spray with contact time of 10 minutes
- Paper towels
- Reusable plastic storage container

1. Clear off an area where supplies can be placed in one location for assembling sample materials. Once a workspace has been cleared, apply disinfectant of choice following the recommendations on the manufacturer's label for dilution of disinfectant and time that is required for the disinfectant to sit on a surface for pathogen of interest. Wipe away with paper towels.

2. Place all needed supplies on the clean and disinfected surface. Put on a new pair of gloves.

3. Open a plastic storage bag and put one paint roller cover in the plastic storage bag (as shown in the picture on the right).





4. Measure 100 mL of pre-moistening solution (can use conical tubes if needed) and add to the plastic storage bag containing the paint roller cover (as shown in the two pictures on the left).

5. Close the plastic storage bag and agitate the plastic storage bag so that the paint roller cover absorbs all of the pre-moistening solution (as shown in the pictures on the right).

6. Repeat steps 3-5 until the appropriate number of samples are made.

7. Close the pre-moistening solution and paint roller cover packages then place all supplies in the reusable plastic storage container. Take off and discard used gloves. Store reusable plastic storage container in a dry and temperature stable area.

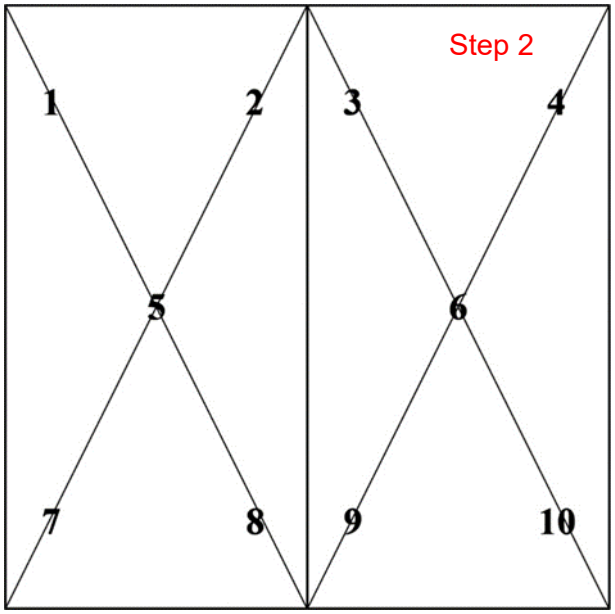
Collecting Feed Samples

How to Collect Feed Samples with Sleeved Feed Probes

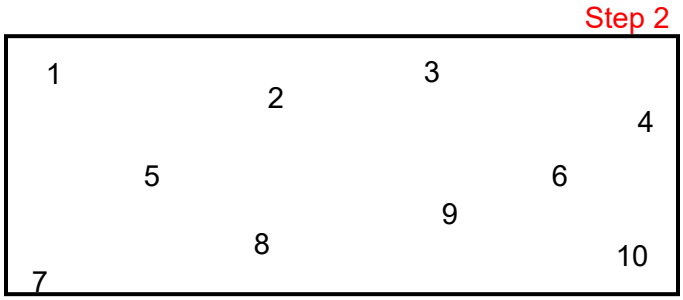
Supplies needed:

- Sleeved feed probe (an example shown below)
- Whirlpack or plastic storage bag
- Nitrile or latex gloves
- Disinfectant wipes
- Permanent marker

1. Get to a location where the sampling person can have an overview of the container with the desired feed ingredient or complete feed to be sampled. Wipe down the exterior and interior of the double sleeved feed probe with a disinfectant wipe. Let dry before using.



2. Put on a new pair of gloves. When looking down at the container, position the probe in the locations shown by the numbers in the picture on the left. When inserting the probe into the feed ingredient or complete feed, the depth of the feed probe should vary with each sample (as shown in the picture below which is a side view of the container; methodology based on the American Association Feed Control Officials Feed Inspectors Guide and bulk sampling methodology by Jones et al., 2019).



3. After each probe sample is taken from the container, deposit the feed collected from the probe into a Whirlpack or plastic storage bag. All ten probe samples should be placed in the same Whirlpack or plastic storage bag.

4. Seal plastic bag. With the permanent marker, write the following information on the bag: sample number, sampled material, and sample date. Wipe down the exterior and the interior of the feed probe with a disinfectant wipe as best as possible. Take off and discard used pair of gloves.

How to Collect Feed Samples via Cut Stream

Supplies needed:

- 8 ounce cup or container
- Whirlpack or plastic storage bag
- Nitrile or latex gloves
- Permanent marker
- Disinfectant wipes

1. Determine how long it will take to unload the feed ingredient in minutes. Convert that length of time into seconds and divide by ten. This is will be referred to as the sampling interval. Wipe out the exterior and interior of the 8 ounce cup with a disinfectant wipe and let dry before using.

2. Put on a new pair of gloves. When the feed ingredient begins unloading, place the 8 ounce cup into the stream of the feed ingredient or complete feed, approximately 4 inches from the edge of stream (as shown in the picture below). Let the cup fill up half way then remove the cup from under the stream. Open plastic storage bag or Whirlpack, dump feed ingredient or complete feed from the cup into the bag.

3. Wait for the designated sampling interval and take another sample from the stream using the same 8 ounce cup. Each time the cup is filled half way, dump the sample from the cup into the same plastic storage bag or Whirlpack.

4. Repeat this process until a total of 10 samples have been taken with the cup.

5. When done, seal the Whirlpack or plastic storage bag. With the permanent marker, write on the bag the following information: sample number, sample material, and sample date. Disinfect the exterior and interior of the 8 ounce cup with a disinfectant wipe. Take off and discard used pair of gloves.

How to Collect Feed Samples with Single Tube Trier

Supplies needed:

- Single tube trier, at least 25 inches long (an example shown below)
- Whirlpack or plastic storage bag
- Nitrile or latex gloves
- Disinfectant wipes
- Permanent marker
- Duct tape

1. Select 10 bags of the desired feed ingredient or complete feed to be sampled that are in the same lot of manufacturing. Wipe off the exterior and interior of the single tube trier with a disinfectant wipe, let dry before using.

2. Lay one of the bags on the floor. Put on a new pair of gloves. Rotate the single tube trier so that the opening is facing down. Then, with the slot still facing down, insert the single tube trier into the top left corner of the bag.

3. Continue to pass the single tuber trier within the bag until the tip of the single tube trier makes contact with, but does not puncture, the bottom right corner of the bag.

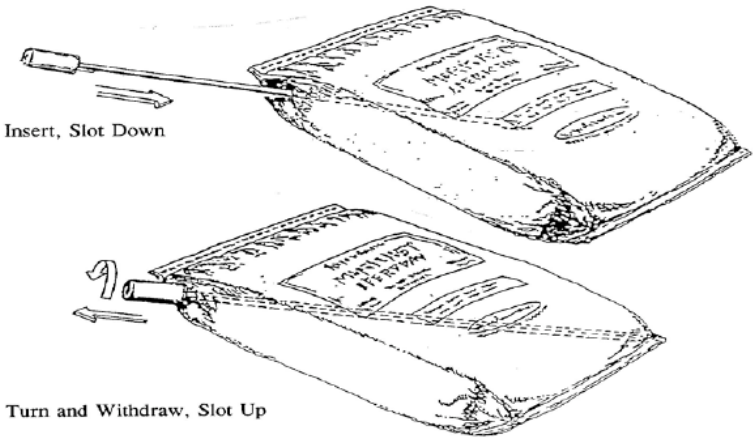
4. With the trier still positioned in the bag diagonally, rotate the single tube trier in a counter clock wise rotation so that the empty compartment is filled with the material present in the bag. Remove the single tube trier from the bag, making sure that the compartment is facing up (picture for steps 2 through 4 is shown in figure 1).

5. Dump the contents of the single tube trier into the plastic storage bag or Whirlpack. Rip off a piece of duct tape and seal the hole made by the single tube trier in the bag.

6. Repeat steps 2-5 with each individual bag for a total of 10 single tube trier samples for each individual bag. All 10 subsamples should be placed in the same plastic storage bag or WhirlPack. With the permanent marker, write on the bag the following information: sample number, sampled material, and sample date. Take off and discard used pair of gloves.

Figure 1. How to take a single tube trier sample in bagged feed or feed ingredients based on the American Association Feed Control Officials Feed Inspectors Guide

Steps 2 - 4



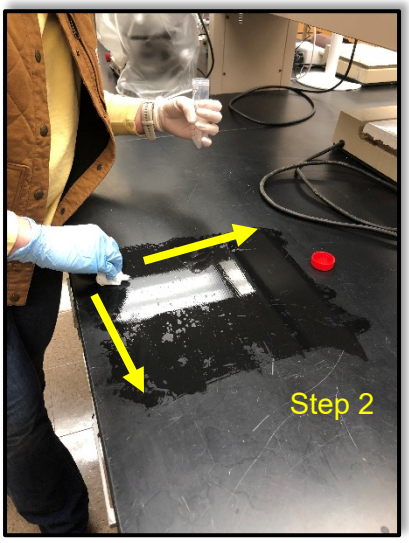
Collecting Environmental Samples

How to Collect Environmental Samples with Cotton Gauze

Supplies needed:

- Conical tube containing pre-moistened cotton gauze
- Nitrile or latex gloves
- Permanent marker

1. Put on a pair of gloves. Open the conical tube containing the cotton gauze with the non-dominant hand (in the picture on the left, it is the white gloved hand) and put the cotton gauze into the dominant hand (in the picture on the left, it is the blue gloved hand). For the remainder of the sampling, the cotton gauze should only touch the dominant hand and the sampling area (as shown in the picture below).



2. Identify a sampling surface that is approximately 8 × 8 inches (for reference, the average length of a human foot is 10 inches). Unroll the cotton gauze and pass over the desired sampling surface with 10 horizontal pushes and pulls and 10 vertical pushes and pulls (as shown in the picture on the left). Apply firm and even pressure with each pass.

3. Once sampling is complete, place cotton gauze back in the conical tube and seal it (as shown in the picture on the right). With the permanent marker, write the following information on the conical tube: sample number, sample location, and sample date. Take off and discard used pair of gloves.

How to Collect Environmental Samples with Paint Roller Covers

Supplies needed:

- Plastic storage bag with pre-moistened paint roller cover
- Paint roller
- Paint roller extension set
- Latex or nitrile gloves
- Conical tube
- Permanent marker

1. Attach the paint roller to the extension set. Put on a new pair of gloves.

2. Open the plastic storage bag and maneuver the paint roller cover so it can be placed on the paint roller without being touched by hands or outside environment (as shown in the picture on the left). Slide the paint roller cover onto the paint roller.

3. Roll the paint roller cover over the desired sample location with 10 horizontal pushes and pulls and 10 vertical pushes and pulls (shown in the picture below on the left). Apply firm and even pressure with each pass.

4. When finished, put the paint roller cover back into the plastic storage bag, and detach it from the paint roller (as shown in the picture on the far right). Squeeze out excess air of the bag and seal the plastic storage bag.

5. Squeeze the paint roller cover in the plastic storage bag so that the liquid from the paint roller cover accumulates in the plastic storage bag (as shown in the picture below).

6. Get the conical tube, open it, open the plastic storage bag, and pour the liquid from the plastic storage bag into the conical tube (as shown in the picture on the right). There should be approximately 20 mL of liquid in the conical tube. If not, add 20 mL of pre-moistening solution, and squeeze the liquid out of the paint roller cover. Repeat this process until 20 mL has been collected from the paint roller cover. Once 20 mL are collected, seal the conical tube, take the permanent marker and write the following on the tube: sample number, sample location, and sample date. Take off and discard used pair of gloves.

How to Collect Environmental Samples with Stick Sponges

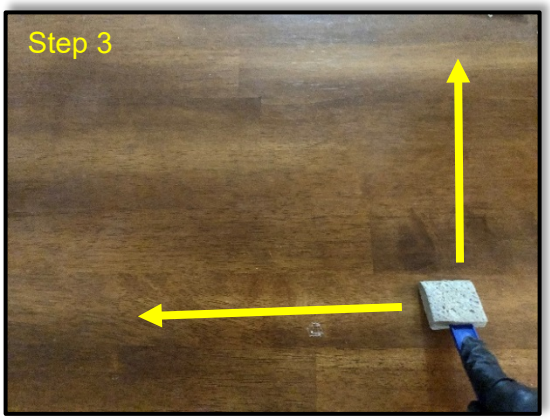
Supplies needed:

- 3M sponge sticks (3M reference number: SSL10BPW)
- Latex or nitrile gloves
- Permanent marker

1. Put on a new pair of gloves. Pull the red tabs on the bag away from each other to open the plastic storage bag (see picture on the left).

2. With one hand, maneuver the blue stick from outside the bag so the other hand can grab the blue stick without reaching into the bag (see picture on the right.

3. Identify a surface that is approximately 8 × 8 inches (for reference, the average length of a human foot is 10 inches). Swab the desired located with 10 horizontal pushes and pulls then 10 vertical pushes and pulls of the stick sponge across the sampling surface (as shown in the picture on the right). Apply firm and even pressure with each pass.



4. Place sponge back in the bag, ensuring the blue stick does not touch the inside of the bag. Disconnect the sponge from the blue stick (shown in the picture on the left).

5. Squeeze out excess air of the bag, fold down the top, and seal with the blue metal tabs on the side (shown in the picture on the right). With the permanent marker write the following on the bag: sample number, sample location, and sample date. Take off and discard used pair of gloves.

Recording Samples

How to Record Samples from Surveillance Sampling

Supplies needed:

- Information on the sampling location like sampling date, sample number, sample identification or location, sample zone, and sample type
- Excel workbook or piece of paper
    - An already formatted Excel workbook can be found in the SOP section, titled “Surveillance Sampling Excel Workbook”

1. In an Excel workbook or on a piece of paper, create a table with individual columns for the sampling date, sample number, sample location, sampling zone that corresponds with the sample location, sample type, and pathogen of interest.

2. Fill out the table with the appropriate information for each collected sample (an example of how to do this is listed below).

    - For environmental samples, sampling zone refers to the type of surface that was sampled. This can either be a feed contact surface, non-feed contact surface, or a transient surface.

Sample Log for Feed Mill X

Sampling Date (YYYY-MM-DD)	Sample Number	Sample Location	Sampling Zone	Sample Type	Pathogen of Interest
2017-01-06	100	Floor of Warehouse	Non-feed contact	Cotton Environmental	Virus
2017-01-06	101	Receiving Pit Grate Dust	Feed contact	Roller Environmental	Virus
2017-01-07	102	Complete Feed – Batch 2, Production Site Y	-	Feed	Virus
2017-01-07	103	Floor Dust in Feed Mill Office	Non-feed contact	Sponge Environmental	Bacteria

How to Record Samples from Suspected Contamination Sampling

Supplies needed:

- Information on the sampling location like sample identification, sample date, and test that samples were submitted for.
- Excel workbook or piece of paper
    - An already formatted Excel workbook can be found in the SOP section titled “Suspected Contamination Sampling Excel Workbook”

1. In an Excel workbook or on a piece of paper, create a table that has the total number of environmental and feed samples at the top then individual columns for sample identification or location, date collected, and what test the sample was analyzed for. Also add in two columns for each sample type for sample results after submission.

2. At the bottom of the table, create a row where the positive and negative samples can be totaled and a location to interpret sample results in relation to thresholds.

3. Fill out the table with the appropriate information for each collected sample (an example of how to make and fill out this table is listed below).

4. As results are returned from the laboratory, continue to update the table. Add up the number of the positive and negative samples and if totals are below threshold limits, then return to surveillance sampling. If totals are at or above the threshold, continue to conduct suspected contamination sample.

Sample Log for Suspected Contamination Sampling Event for the week of 3/13/2022

Environmental samples (total = 75)					Feed Samples (total = 25)				
Sample ID	Date collected (YYYY-MM-DD)	Test submitted for	Positive sample	Negative sample	Sample ID	Date collected (YYYY-MM-DD)	Test submitted for	Positive sample	Negative sample
1301 – Forklift tires	2022/3/14	PEDV		X	130 – Complete feed for Site A	2022/3/14	PEDV		X
1302 – Office floor	2022/3/14	PEDV	X		131 – Ingredient A	2022/3/14	PEDV		X
…					…				
1375 – Exterior of feed delivery truck A	2022/3/14	PEDV		X	155 – Feed sample from delivery truck A	2022/3/14	PEDV	X	
Totals:			1	74	Totals:			1	24

Return to surveillance: Y/N

Submitting Samples

How to Package Samples for Submission to a Laboratory

Supplies needed:

- Plastic storage bags
- Samples intended for submission
- Copies of sample record
- Completed laboratory submission form
- Ice packs
- Styrofoam cooler
- Cardboard box large enough for the Styrofoam cooler
- Permanent marker
- Packing or duct tape

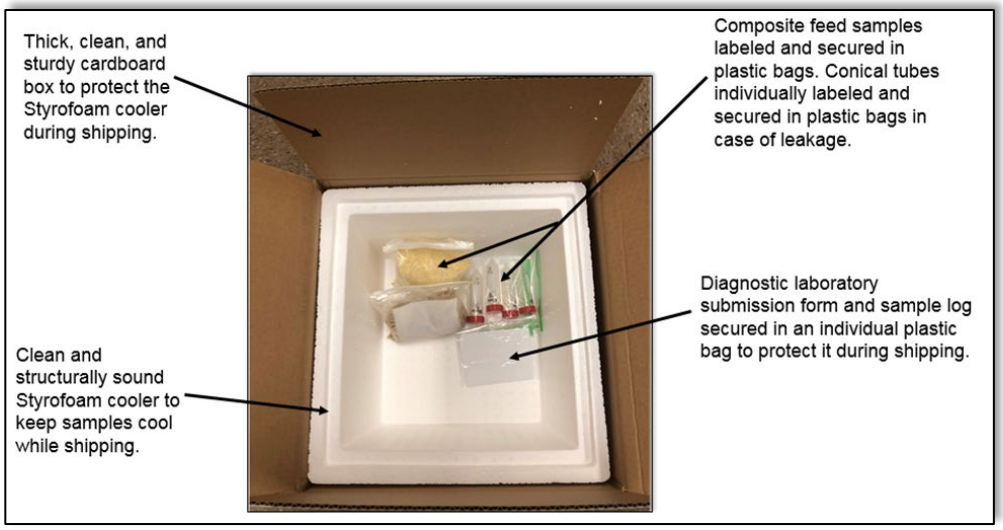
1. Inspect cardboard box and Styrofoam cooler for any holes or damaged sides. If both are structurally sound, put Styrofoam cooler into the cardboard box, and proceed with the following steps.

2. Gather all samples intended for laboratory submission. Put all conical tubes for environmental samples into a plastic storage bag.

3. Put all samples in plastic storage bags into the Styrofoam cooler, including environmental samples for viruses or bacteria, complete feed samples, or feed ingredient samples.

4. Put completed laboratory submission form and copies of sample record into a plastic storage bag. If laboratory of choice has an online submission form process, write the accession number from the online submission process in permanent marker on the plastic storage bag containing the submission forms.

5. Put plastic storage bag containing submission paperwork and copies of sample record into the Styrofoam cooler. An example of how all samples should be packaged is shown on the right.



6. Put ice packs into the Styrofoam cooler. Place lid on Styrofoam cooler. Fold over flaps of cardboard box onto the top of the cooler and tape the cardboard box closed.

7. Take cardboard box containing the Styrofoam cooler with samples to a shipping service of choice. Samples should arrive to the laboratory within 24-36 hours of when samples were placed into the shipping container.

Centrifugation of Environmental Samples for Viral Pathogens

Research suggests that centrifugation of environmental samples for viral pathogen detection prior to PCR analysis can increase the assay sensitivity (Elijah et al., 2021). When submitting the environmental samples to the laboratory of choice, communicate with the laboratory that all environmental samples for viral pathogens need to be centrifuged at 4000 × g for 10 minutes before PCR assay (Khanal et al., 2022). If centrifuging the environmental samples for viral pathogens before laboratory submission, follow this SOP on how to do so. This SOP can also be sent to the laboratory if they require an SOP to centrifuge the environmental samples for viral pathogens. For more information on diagnostic tests for samples, refer to the additional resource titled “Diagnostic Tests for Samples.”

Supplies Needed

- Centrifuge
- Graduated cylinder
- Pre-moistening solution
    - Same solution used when preparing samples for sampling viral pathogens
    - Phosphate buffered solution (PBS) or 0.9% NaCl sterile saline
- Disposable transfer pipettes
- Cryovials
    - Need to be DNA/RNase free
    - Need to store at least 1 mL of sample
- Vortex machine
- Storage container for cryovials
    - Plastic storage bags
    - Cardboard boxes with dividers for cryovial size
- Nitrile or latex gloves
- Disinfectant
    - Consult the manufacturers label for the dilution and contact time that is appropriate for the pathogen of interest
    - Some examples include:
        - 70% ethanol spray with contact time of 10 minute
        - 1:256 diluted quaternary ammonium/glutaraldehyde (Synergize) spray with contact time of 10 minutes
        - 1:16 diluted accelerated hydrogen peroxide (Intervention) spray with contact time of 5 minutes
        - 10% bleach spray with contact time of 10 minutes
- Paper towels
- Permanent marker

How to Centrifuge Cotton Gauze Environmental Samples

1. Locate an area that is the appropriate size for centrifugation and clear off all other items. Apply disinfectant of choice to work area following the manufacturer's recommendation of dilution and appropriate contact time for pathogen of interest. Wipe away with a paper towel when appropriate.

2. For every environmental sample, get out a second conical tube and cryovial, then transfer the sample number from the environmental sample to the second conical tube and cryovial with a permanent marker. For every environmental sample, there should be three tubes - a conical tube with cotton gauze, an empty second conical tube, and an empty cryovial.

3. Place sample tubes, pre-moistening solution of choice, disposable transfer pipettes, cryovials, and graduated cylinder on the work station. Put on a new pair of gloves.

3. Measure 20 mL of pre-moistening solution, open the conical tube containing the cotton gauze, and pour the pre-moistening solution into the conical tube.

4. Close the conical tube still containing the cotton gauze and vortex for 10-15 seconds.

5. Let the conical tube containing the cotton gauze sit at room temperature for 1 hour.

6. After 1 hour, open the conical tube, transfer the liquid from the conical tube containing the cotton gauze with a serology transfer pipette to the second conical tube. Once all liquid has been pipetted off, discard the conical tube with the cotton gauze. Close the second conical tube.

7. Open the centrifuge and put the second conical tube into the centrifuge. Centrifuge at 4000 × g for 10 minutes.

8. Once centrifugation is completed and comes to a stop, carefully lift out the second conical tube from the centrifuge. Open the second conical tube and transfer the liquid to the corresponding cryovial. Once liquid from the second conical tube has been transferred, discard it.

10. Place cryovials into the storage container.

11. Close all used materials and store them in the appropriate place. Take off and discard gloves. Submit samples for laboratory analysis.

How to Centrifuge Paint Roller Cover Environmental Samples

1. Locate an area that is the appropriate size for centrifugation and clear off all other items. Apply disinfectant of choice to work area following the manufacturer's recommendation of dilution and appropriate contact time for pathogen of interest. Wipe away with a paper towel when appropriate.

2. For every environmental sample, get out a cryovial, then transfer the sample number from the environmental sample to the cryovial with a permanent marker. For every environmental sample, there should be two tubes - a conical tube containing the liquid from the paint roller cover and an empty cryovial.

3. Place sample tubes, pre-moistening solution of choice, disposable transfer pipettes, cryovials, and graduated cylinder on the work station. Put on a new pair of gloves.

4. Vortex the conical tube with the liquid from the paint roller cover for 10-15 seconds.

5. Let the conical tube sit at room temperature for 1 hour.

6. After 1 hour, place the conical tube in the centrifuge and centrifuge at 4000 × g for 10 minutes.

7. Once centrifugation is completed and comes to a stop, carefully lift out the conical tube from the centrifuge. Open the conical tube and transfer the liquid to the corresponding cryovial. Once liquid from the conical tube has been transferred, discard it.

8. Place cryovial into the storage container.

9. Close all used materials and store them in the appropriate place. Take off and discard gloves. Submit samples for laboratory analysis.

References

Elijah, C.G., Harrison, O.L., Blomme, A.K., Woodworth, J.C., Jones, C.K., Paulk, C.B., and Gebhardt, J.T. (2021). Evaluating the impacts of presence of organic matter on environmental samples and sample processing technique on RNA detection of PEDV. Kansas Agricultural Experiment Station Research Reports 7(11). doi:10.4148/2378-5977.8211.

Khanal, P., Olcha, M., and Niederwerder, M.C. (2022). Detection of African swine fever virus in feed dust collected from experimentally inoculated complete feed using quantitative PCR and virus titration assays. Transbound and Emerg Dis. 69:97-102. doi:10.1111/tbed.14176.