# 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.