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Virus Inactivation Using PrimeStore® Molecular Transport Media (PSMTM)

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Nathaniel Higdon^{1,2}, Jennifer Welch¹, Rachel Palinski¹, Suelee Robbe-Austerman¹

¹Diagnostics and Biologics, National Veterinary Services Laboratories, Animal Plant Health Inspection Service, United States Department of Agriculture;

²Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University of Science and Technology

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Nathaniel B.A. Higdon

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We use this protocol and it's working.

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Disclaimer

This protocol is provided for informational, diagnostic, and research purposes only. The USDA does not endorse any specific method, company, product, or reagent. It is not intended to supersede or serve as official USDA or U.S. Government determination or policy, nor to replace institutional SOPs or regulatory requirements. The workflow described was developed using PrimeStore® Molecular Transport Media (PSMTM) and Amicon™ Ultra-15 50 kDa ultracentrifugal filters. However, equivalent media or validated alternatives may be used with appropriate verification. Users are strictly cautioned that PrimeStore® MTM reacts with sodium hypochlorite (bleach) to produce toxic gas. Appropriate alternative disinfectants, such as Virkon® S, must be used. Users are responsible for ensuring compliance with all applicable biosafety, quality assurance, and regulatory guidelines. The authors make no representations that this document is complete, accurate, or error-free and assume no responsibility for any errors, omissions, damage, or loss resulting from its use, even if the protocol is followed as written.

Abstract

This protocol describes a general methodology for inactivating viruses using PrimeStore® Molecular Transport Media (PSMTM) to allow for the safe transfer of diagnostic samples from high containment laboratories (BSL3/4) to lower biosafety levels (BSL1/2). PSMTM eliminates the need for standard cold chain transport, enabling ambient temperature shipment, and reduces stringent transport conditions while ensuring rapid viral nucleic acid stabilization. Proper inactivation is achieved by maintaining specific media to sample ratios and incubation time conditions. Upon successful execution complete viral inactivation is expected and may be confirmed through TCID50 titration assays and polymerase chain reaction (PCR) testing. For cytopathic effect (CPE) producing viruses' success is shown through the absence of any CPE and can also be seen by increases in cycle thresholds (Ct) values across a minimum of three serial passages. For non CPE producing viruses Ct values must be seen increasing across three serial passages.

Attachments



Inactivation Certifi...

15KB



titration worksheet....

14KB

Guidelines

****Definitions****

- **PrimeStore® Molecular Transport Media (PSMTM):** A proprietary reagent that has been approved by the Food and Drug Administration (FDA) to be used for the chemical inactivation of infectious pathogens and the stabilization of nucleic acids for downstream molecular testing.
- **Biosafety Level (BSL):** The level of biocontainment and safety precautions required for the handling, manipulation, use, and study of potentially dangerous infectious agents.
- **Cytopathic Effect (CPE):** Morphological changes to the structure of a host cell caused by viral infection, such as cell rounding or detachment. Can be used to assess infection progression and viral viability.
- **TCID₅₀ (50% Tissue Culture Infectious Dose):** A quantitative measure of viral infectivity based on the dilution at which 50% of cells are infected.
- **Buffer Exchange:** A process to remove cytotoxic components within a solution prior to cell culture inoculation. Within this protocol it is done with the addition of a neutral buffer and centrifugal filter separation.

****Personnel Qualifications****

- Personnel performing this protocol must be trained in appropriate BSL practices, aseptic technique, and the proper handling of infectious samples and hazardous agents.
- Users should be trained in the maintenance and use of laboratory equipment, such as Biosafety Cabinets and centrifuges. Users should also be familiar with calibration of equipment and calibration schedules.
- Familiarity with cell culture, titration assays, and PCR workflows are required.
- For protocols involving select agents, personnel must meet all institutional and federal training requirements for high containment laboratory work.

****Contamination Prevention****

- Perform all work inside a certified Class II Biosafety Cabinet (BSC).
- Use centrifuge buckets which can be locked and have O-ring seals in the event of tube breakage.
- Laboratorians should use appropriate PPE (gloves, lab coat, eye protection, respiratory protection as required) as required and should change PPE in the event of contamination or damage.



- Change gloves frequently and disinfect work surfaces with Virkon® S or equivalent disinfectant following recommended contact times.
- Utilize secondary containment vessels when removing samples, infection flasks, etc. when going from biosafety cabinets to storage.
- Avoid bleach (sodium hypochlorite) when working with PSMTM due to toxic gas formation. Use Virkon® S or other approved disinfectants for decontamination.

****Areas and Equipment****

- All work involving the manipulation of tubes, infection of flasks, and handling of samples should be performed in a certified class II biosafety cabinet.
- Separate areas for sample handling, molecular testing, and cell culture are recommended.
- Dedicated calibrated pipettes and equipment should be used for each area.
- Ensure all equipment (pipettes, centrifuges, incubators) is calibrated and certified per institutional SOPs/protocols.

****Validation****

- Inactivation is considered valid if nucleic acid is detected in the original sample material for both PSMTM and Control Condition samples.
- Ct values of original material of PSMTM and Control Condition must be within 1 log (3.3 Ct).
- For PSMTM conditions the Ct values must increase (reduction of virus titer) with each serial passage and by the third passage they should reach the threshold cutoff.
- If the viral titer is high, then four to five passages may be needed to reach cycle threshold.
- For Control Conditions the cycle threshold should decrease (increase in viral titer) by the third passage or must not have increased from starting material by greater than 1 log (3.3 Ct).

Materials

****Reagents****

- PrimeStore® Molecular Transport Media (Longhorn Vaccines & Diagnostics, catalog No. varies by tube type, or equivalent)
- Phosphate Buffered Saline (PBS), pH 7.4 (Thermo Fisher Scientific or equivalent)
- Virkon® S Disinfectant (LANXESS)
- Cell Culture Media and Additives (Appropriate for cell line used, e.g., DMEM with FBS and antibiotics)
- Purified and Characterized Virus (For validation testing; minimum 4-log₁₀ titer required)

Note: PSMTM reacts with bleach (sodium hypochlorite) to produce toxic gas; do not use bleach for decontamination.

Use appropriate PCR protocol and associated reagents for PCR assay and analysis.

****Supplies****

- Amicon™ Ultra-15 50 kDa Ultracentrifugal Filters (MilliporeSigma, catalog No. UFC9050 or equivalent)
- Pipette tips, aerosol-resistant, sterile
- Microcentrifuge tubes (1.5–2.0 mL, nuclease-free)
- Centrifuge tubes (15 mL, 50 mL)
- 96-well plates, tissue culture treated
- 6-well plates, tissue culture treated
- Serological pipettes (5 mL, 10 mL)
- Laboratory gloves, powder-free nitrile

****Equipment****

- Class II Biological Safety Cabinet (BSC)
- Calibrated single and multi-channel pipettes (covering 1 µL to 1 mL ranges)

- Centrifuge (capable of 4500 ×g)
- Inverted light microscope, preferable with camera attachment
- Incubator (temperature and CO₂ settings appropriate for cell line)
- Ultra-low freezer (−80°C ± 10°C)
- Vortex mixer or equivalent
- Tube racks
- Timers
- Real-Time PCR System: (e.g., Applied Biosystems™ QuantStudio™ 5 for validation of inactivation via Ct values)

Note on Equipment Calibration: All equipment, including pipettes, centrifuges, and PCR platforms, must be calibrated and certified according to manufacturer instructions or institutional SOPs.

Safety warnings

- ! ▪ PSMTM reacts with bleach (sodium hypochlorite) to produce toxic gas. Never use bleach for decontamination of PSMTM tubes or surfaces where PSMTM and or PSMTM/Sample may have spilled. Use Virkon® S or other validated disinfectants for specific pathogen being worked with instead. Ensure appropriate contact time is achieved (Virkon® S requires a 10 min contact time).
- Ensure proper PPE and BSL containment based on the pathogen being handled.
- Inactivation validation requires strict adherence to ratios and incubation times. Deviating may result in incomplete inactivation.
- PSMTM is cytotoxic to cell cultures at low dilutions and so a buffer exchange is required before inoculation with cells for validation assays.
- Always check tubes for leakage and disinfect any tubes that have leaked and transfer sample to a new tube if damaged.
- While this protocol facilitates safe transfer of samples, users are responsible for compliance with all institutional, federal, and select agent regulations.



Before start

- Visually inspect all PSMTM tubes to ensure there is enough reagent for proper inactivation.
- Verify that all required reagents, controls, and equipment are available and calibrated.
- Annotate all pertinent information, including but not limited to pathogen strains, sample identifiers, reagent lot numbers, and expiration dates for traceability, quality assurance, and select agent requirements if applicable.
- Record and keep inactivation records if required by specific host institution and or government regulations.

MTM Sample Tube Differences

- 1
 - There are several tubes offered by LongHorn for the PrimeStore® Molecular Transport Media (PSMTM).
 1. A 2 mL cryo tube with 1.0 mL of PSMTM in it.
 2. A 3 mL cryo tube with 1.5 mL of PSMTM in it.
 3. A 5 mL cryo tube with 1.5 mL of PSMTM in it. This tube enables the use of swabs.
 4. A 12 mL high durability tight seal cap tube with 3.0 mL of PSMTM in it. This tube is ideal for pooling of samples and high throughput automation. It has a bottom etching line that indicates minimum volume of PSMTM required prior to sample addition and a second higher etching that indicates maximum volume of sample that can be added to PSMTM to achieve proper inactivation ratio.

Samples Collection Submitting Laboratory

- 2
 - Visually inspect PSMTM tube to verify PSMTM within the tube reaches the appropriate volume level of PSMTM required for inactivation for the sample being tested.



Note: A test tube can be used to measure the amount to PSMTM within the tube. Then using a sharpie a mark can be made at the meniscus to compare with other tubes. It is important that you use enough PSMTM that has been validated for complete inactivation of the pathogen(s) being investigated.

- Add virus-containing sample to PSMTM tube.

Note: Add appropriate validated sample volume that achieves a proper inactivation ratio for the pathogen(s) of interest.

- In the event of sample spillage to outside of PSMTM tube during sample addition procedure, tubes should be decontaminated with Virkon® or other appropriate disinfectant.

Note: PSMTM reacts with bleach to produce toxic gas. Do not expose PSMTM to bleach (sodium hypochlorite).

- Ensure screw-top cap of PSMTM tube is properly fitted and tightened. Tighten ½ turn past once the user feels resistance. Overtightening of tubes should be avoided.
- Invert PSMTM tube containing virus sample three times to mix.

- Visually inspect PSMTM tube for signs of leakage following inversion. Tubes with visible leakage should be disinfected with Virkon® or other appropriate disinfectant.
- Allow inactivation to proceed for 1 hour $\pm$ 2 minutes at room temperature with tubes in upright position. Time may vary depending on validation of pathogen(s) of interest.
- Follow any institutional requirements for movement of virus to lower BSL level at your institution and complete any inactivation certificates or tracking logs required.
- Complete movement or shipment of tubes at ambient temperature in appropriate shipping container.

Receiving Laboratory

- 3
- Inside a certified BSC, visually inspect PSMTM tubes for signs of leakage.
 - Decontaminate tubes with visible leakage with Virkon® or other appropriate disinfectant. Further DNAway can be used to remove any possible nucleic acids, and 70% ethanol may be used to remove any residues. Be mindful of writing on tubes prior to cleaning.



Note: PSMTM reacts with bleach to produce toxic gas. Do not expose PSMTM to bleach (sodium hypochlorite).

- Complete molecular testing according to appropriate SOP and protocols.
- Complete work with PSMTM tubes inside a BSC and utilize a secondary containment vessel(s) for removal from BSC and storage of PSMTM tubes until disposal.
- Disposal recommendations are up to the end user and should follow proper institutional and local regulations for the disposal of toxic/harmful substances.

Routine Inactivation Validation

- 4
- Complete sample collection submitting laboratory steps.



Note(s):

1. Virus-containing sample(s) should contain a minimum of 4-log₁₀ virus.
2. Sample collection submitting laboratory steps should also be completed with no PSMTM appropriate media (such as PBS) as a control.

3. Validation procedure should be completed in appropriate BSL level laboratory with PPE appropriate for virus being utilized.
 4. PSMTM and control procedures should be completed in triplicate at a minimum.
- Complete virus titration assay (TCID₅₀) by standard 1:10 dilution of PSMTM and control-treated viruses.

Note(s):

1. Virus titration assays are only applicable to CPE producing viruses.
 2. Virus titration steps may be omitted for viruses that do not produce CPE.
 3. The appropriate cell line should be used for the virus being utilized.
 4. Proper titration assay for specific virus should be utilized. The use of a no PSMTM control condition may be used to aid in the determination of assay endpoint.
 5. PSMTM is highly toxic to cell viability (cytotoxic). It is expected that cell death will occur at lower dilutions (1:10 – 1:100).
 6. Inactivation is considered valid if no CPE is observed at non-cytotoxic concentrations for PSMTM condition. CPE must be observed at corresponding non-cytotoxic concentration for control condition.
 7. Virus titer of control condition must reach 4-log₁₀.
- Complete at a minimum three serial passages of PSMTM and control-treated viruses.

Note(s):

1. To minimize PSMTM cytotoxicity it is recommended to complete buffer exchange prior to adding to susceptible cells for the first passage.
- Dilute PSMTM and control-treated viruses at a ratio of 1:10 in appropriate cell culture viral propagation media (example 200uL sample + 1.8mL cell media)
 - Add total diluted volume to Amicon™ Ultra-15 50 kDa ultracentrifugal filters.
 - Depending on the starting volume of diluted sample centrifuge times may vary. It is recommended for total dilution volume to be 15 mL.
 - For 15 mL centrifugation to ~200 µL of original sample volume will require 15 minutes at 4500 xg.

Note(s):

1. Lower volumes may require less time or centrifugal speeds.
2. Depending on viscosity of the sample, cellular debris, or large particles and debris within the solution longer times or multiple spin cycles may be required to fully concentrate to starting pre diluted volume.

- Repeat dilution and buffer exchange steps.
- After final concentration to original volume (~200 μ L), dilute sample at a ratio of 1:10 in appropriate cell culture media (200 μ L sample + 1.8mL cell culture media) to generate final inoculum.
- Add total 1:10 diluted volume to appropriate cells (2mL/well for 6-well plate format).

Note: Buffer exchange is not required for second and third passage.

- Duration of passage assay should be appropriate to virus utilized and no PSMTM control may be used to aid determination of assay endpoint.
- Collect passage material by freeze/thawing cells and the supernatant together three times.
- Centrifuge collected material at 1500 xg for 10 minutes $\pm$ 2 minutes to remove cellular debris after final freeze/thaw.
- Add 1/10th volume of clarified passage material to naïve cells for each subsequent passage (200 μ L passage material + 1.8mL cell media for 2mL/well of 6-well plate format).

Note: Inactivation is considered valid if no CPE is observed in any passage for PSMTM condition for CPE-producing viruses. CPE should be observed at minimum for third passage in control condition for CPE-producing viruses.

- Complete virus appropriate PCR assay on PSMTM and control-treated viruses from original material and on each serial passage product according to appropriate SOP/protocol.

Note(s):

1. Inactivation is considered valid if nucleic acid is detected in original material for both PSMTM and control condition.
2. Ct values of original material of PSMTM and control condition must be within 1-log (3.3Ct).
3. For PSMTM condition, Ct values must increase (reduction of viral titer) with each serial passage and by the third passage CT must reach cycle threshold cutoff (depending on virus and assay this may be a Ct value of 40 to 45).
4. For high titer virus, four or five serial passages may be required to reach cycle threshold cutoff.



5. For control-treated viruses, third passage material Ct value must show reduced (increased virus titer) Ct of original material Ct or must be no greater than 1-log (3.3 Ct) of original material Ct.
 - All inactivation validity criteria for each assay (titration and PCR) must be confirmed for inactivation validation to be satisfied for CPE-producing viruses.
 - For viruses that do not produce CPE, inactivation validity criteria must be satisfied for PCR assay.
 - All inactivation certificates and tracking logs should be completed as required for end user institution requirements.

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PrimeStore® MTM Instructions for Use (Longhorn Vaccines & Diagnostics):

<https://www.lhnvd.com/primestore-mtm>

Amicon™ Ultra-15 50 kDa Ultracentrifugal Filter User Guide (MilliporeSigma):

<https://images-na.ssl-images-amazon.com/images/I/71A8g+u8BrL.pdf>

Virkon® S Safety and Usage Guidelines (LANXESS):



<https://lanxess.com/en-us/products-and-brands/brands/virkon-s>

WHO Laboratory Biosafety Manual, 4th Edition:

<https://www.who.int/publications/i/item/9789240011311>

WOAH (formerly OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals:

<https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>