IBC Guidelines for Proper Hamilton Syringe Use with Viral Vectors

Overview

Hamilton syringes are used in research involving injections in animals of substances, drugs or microorganisms in small volumes with precision. This document addresses the specific application of Hamilton syringes to deliver viruses or viral vectors for recombinant and synthetic nucleic acids to animals.

Background

Hamilton syringes are manufactured to be accurate within $\pm 1\%$ of nominal volume and with precision within 1%, measured at 80% of total scale volume. The design of the barrel and plunger dimensions assure high levels of accuracy and precision. Since Hamilton syringes are very precise, researchers often use them to deliver extremely small volumes in microliters. [8, 9]

When using a Hamilton syringe, the volume in the needle (called dead volume) is considered constant and the volume being dispensed is absolute. The dead volume is maintained throughout aspirate and dispense stages. The amount of dead volume that remains in the syringe depends on the needle inner diameter and termination style. The Hamilton Company indicates that the dead volume is generally less than 1.0 μ L for small volume syringes and as much as 6.8 μ L for large volume syringes.

Choosing the Correct Hamilton Syringe for Viral Vector Delivery

Hamilton syringe is used for delivery of viral vectors for recombinant and synthetic nucleic acids in animals with or without use of stereotaxic equipment.

For viral vector applications, Hamilton syringes with port for removable needles (RN) must be used because the syringe can be disassembled for virus decontamination (for examples refer to the Hamilton 600 Series and some 700 Series) [1, 2, 8]. Do not use syringes with cemented needles because they cannot be autoclaved due to different rates of glass and metal expansion that compromise the glued connection.

Consider purchasing one syringe for each type of viral vector construct. If you perform multiple injections with different viral vector constructs at the same time, this will allow you to avoid cross contamination from residual volumes that do not eject from the syringe (Hamilton refers to this as the "dead volume").

Safety Precautions during Viral Vector Delivery When Using Hamilton Syringe

The appropriate protective personal equipment (PPE) should be worn.

Assemble the Hamilton syringe, plunger, and removable needle. Attach the Hamilton assembly to stereotaxic equipment (as needed).

Clear any blockages by drawing and expelling 100% acetone five times. Subsequently draw and expel sterile PBS five times to remove any residual acetone. Draw the maximum volume of

sterile PBS into the Hamilton syringe, taking care to not include any bubbles, and dispense into waste container [8].

Draw the desired volume of viral vector solution for single injection. Slowly insert the needle into the animal (or specific brain region of animal), dispense the viral vector solution, keep the needle in place at least 5 minutes, and then slowly withdraw it from the animal to avoid backflow and to prevent accidental exposure to the viral vector.

If performing multiple injections of the same viral vector construct with the same syringe, decontaminate the exterior and interior of syringe between use. Gently clean the exterior of the needle with a 70% ethanol-soaked cotton swab to remove debris. Use the swab such that fingers do not come in proximity with the needle. Dispose of the contaminated cotton swab in a biohazard waste container.

** Use extreme caution when handling needles to avoid accidental personal injury or damage to the needle.

Decontaminate the interior of the syringe by drawing 70 % ethanol in and out of the syringe barrel followed by sterile water or PBS rinse. Dispense washes into a beaker to be diluted to 10% bleach and washed down the drain in running water at end of use.

** Be aware that ethanol will not inactivate AAV or Adenovirus vectors, which may contaminate your subsequent experiments if not thoroughly cleaned. It is highly recommended to use multiple syringes to avoid cross contamination.

<u>Cleaning, Decontamination and Sterilization Procedures for Hamilton Syringes and Needles after Use in Viral Vector Delivery</u>

Cleaning with Solvents

Solvents suitable for routine cleaning of Hamilton syringes include methanol, acetonitrile and acetone. Use solvents of high purity grade. Halogenated hydrocarbons may damage the syringe and should not be used.

Sterilization

- 1) Hamilton syringes may be sterilized with appropriate sterilizing agents such as Ethylene Oxide.
- * Sterilization equipment containing Ethylene Oxide is located in the CCM facility. Ethylene oxide sterilization can be performed by CCM on fee-for-service basis. The CCM website can be accessed at https://ccm.northwestern.edu/. Scroll down to Quick Links and click on "AOPS System Login". This will take you to the Service Request (Veterinary Services) section. If you have further questions, please contact the CCM main office.

2) Autoclaving - Recommended

Acceptable for all viral vectors.

For syringes that are autoclave compatible, carefully disassemble syringe, plunger and needle and placed them in different bags prior to autoclaving.

Chemical Disinfection

Hamilton recommends the use of Microcide SQ® (p/n 3995-01); however, this is not recommended for some viral vectors including AAV and Adenovirus.

For AAV and Adenovirus, a 10% bleach solution can be used followed by an alcohol wipe to lessen the corrosive nature of the bleach, as long as the syringe is bleach compatible. Again, autoclaving a removable needle (RN) Hamilton syringe is recommended instead of chemical disinfection.

References:

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- 9. Enok Tjott et all, "Survival of HIV -1 Activity After Disinfection temperature and pH Changes, or Dying", J of Medical Virology 35: 223-227,1991 https://onlinelibrary.wiley.com/doi/pdf/10.1002/jmv.1890350402
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