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Temporal Assessment of Immune Response

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes Vascular Access Button (VAB) catheter blood collection intended for later assessment of immune responses across time to different IP injected substances in awake mice. This protocol is preceded by "Mouse Catheter Maintenance" protocol.

Guidelines

- A maximum of 1% of the animal's body weight in blood can be collected within a two-week period. Check mouse body weight before the start of the experiment to calculate the maximum volume permitted to take.



Materials

-  OHAUS™ Compass CR Series Scale **Fisher Scientific Catalog #01-922-341** .
- Container for weighing mouse.
-  Dumont Forceps - Micro-Blunted Tips **Fine Science Tools Catalog #11253-25**
-  BD Slip Tip Sterile Syringes **Fisher Scientific Catalog #309656**
-  BD® Needle 3/4 in. single use, sterile, 23 G **Becton Dickinson (BD) Catalog #305143**
- Insulin syringes, used for IP injection protocol (BD, Cat#: BD328438).
-  Hamilton® syringe, luer tip **Merck MilliporeSigma (Sigma-Aldrich) Catalog #24531**
- Ethanol swabs (Uline, Cat#: 13-680-63).
-  Hanna Pharmaceutical Supply Co., Inc. 0.9% SODIUM CHLOR. INJ.10ML **Fisher Scientific Catalog #NC9054335**
- 500 units/mL heparinized 50% glycerol (Instech, Cat#: USP-HGS-500-10-VB-5).
-  Hamilton® syringe, luer tip **Merck MilliporeSigma (Sigma-Aldrich) Catalog #24531**
-  PinPort™ Injectors **Instech Laboratories, Inc. Catalog #PNP3M**
-  Sarstedt Inc SC Micro tube 1.3ml K3 EDTA, 100/pack, 10 packs/case **Fisher Scientific Catalog #NC9414041**

Safety warnings

- ❗ Wear required PPE consisting of lab coat and gloves.
- If performing terminal collection, ensure proper ventilation and airflow in a fume hood or air extraction system to reduce exposure.
- When handling syringes with needles, always ensure needles are pointed away from you to avoid any accidental sticks. Report any accidental sticks to EHS.
- Used needles and syringes should be disposed in a biohazard sharps container.

Ethics statement

This protocol has been approved by the Allen Institute Animal Care and Use Committee (IACUC).

PHS Assurance: D16-00781

AAALAC: Unit 1854

Before start

- Implant or acquire mice with VAB Insteck implanted catheters from animal vendor.
- For at least 3 days prior to the experiment, handle mice so they become familiar with your scent and ensure minimal stress on the experiment day. Handling days do not need to be consecutive and can be done on or between maintenance days.

Protocol



NAME

Mouse VAB Catheter Maintenance

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Avery Weaver

Preview

Prepare Flush and Lock Solution Syringes

1m

- 1 Prefill a flush syringe(s) (slip tip syringes Fisher Scientific catalog # 309656) with  50 μL of sterile saline (1 per mouse). 
- 1.1 Wipe septum of vial with 70% ethanol swab and let air dry for  00:00:30 prior to puncturing septum. 30s
- 1.2 Use a 1 mL syringe with 23 Gauge needle attachment to withdraw ~  100 μL saline solution to account for any dead volume when pinport injector is added. 
 - After withdrawal, point the syringe upward and away from you and gently flick the syringe to knock any bubbles up towards the needle.
- 1.3 Remove the needle attachment and attach pinport injector.
 - Ensure the needle is pointed away from you and grip the base of the needle while pulling the syringe out. Then dispose of needle in sharps waste container.
- 2
 - Prefill Hamilton syringe with  14 μL of 500 units/mL of heparinized 50% glycerol. 
- 2.1 Wipe septum of vial with 70% ethanol swab and let air dry for  00:00:30 prior to puncturing septum. 30s
- 2.2 Use 1 mL syringe with 23-gauge needle attachment to pull ~  100 μL of 500 units/mL of heparinized saline in 50% glycerol. 
- 2.3 Remove plunger from Hamilton and insert needle of filled syringe into the back of the Hamilton syringe and fill until the liquid can be seen in a drop at the tip of the Hamilton syringe.
- 2.4 Remove needle from the back of the Hamilton syringe and reinsert plunger, ensuring no air is in the Hamilton syringe.
- 2.5 With the remainder of the 500 units/mL of heparinized saline in 50% glycerol in the 1 mL syringe, fill the pinport injector such that no air is present.



- 2.6 Connect the Hamilton syringe to the pinport injector, then lower the plunger to  14 μL .
 - Use paper towel to dab away excess liquid from the pinport tip.
- 2.7 Between mice, fill a new pinport attachment with 500 units/mL of heparinized saline in 50% glycerol, then pull Hamilton syringe plunger up to 14 μL , then swap to the newly filled pinport attachment while ensuring no air in the syringe.

Prepare Mouse for Blood Collection

- 3 Place mouse cage in fume hood with prepared syringes and remove the lid.
- 4 Place mouse in container on scale and record body weight.
- 5 Do a health assessment per IACUC protocol and continue if mouse meets criteria to proceed.

Perform Catheter Blood Collection (Time Zero Collection)

- 6 Place mouse on grid of food hopper and remove cap from the Vascular Access Button (VAB) on the mouse's back.
- 7 Use empty withdrawal syringe to remove lock solution and a small amount of blood, discard in sharps waste container.
- 8 Use a new withdrawal syringe and take a maximum of 1% of the mouse's body weight divided by the number of non-terminal time points.

Note

For example, a 20g mouse receiving 4 blood collections means a maximum of 50 μL of blood collected per timepoint.

- 9 Remove withdrawal syringe and flush the catheter with 50 μL sterile saline using a flush syringe.



- 10 Remove flush syringe and attach Hamilton syringe, then fill the catheter with lock solution.
- 11 Remove Hamilton syringe and place cap on VAB.
- 12 Quickly expel drawn blood into a K3 EDTA coated tube and mix well then store  On ice .



Inject Mouse with the Substance to be Tested

- 13 Refer to:

Protocol

NAME

Intraperitoneal Injection in an Adult Mouse

CREATED BY

Allen Institute

Preview

- 14 Return mouse to its cage until the next time point of blood collection.
- 15 Record the time of injection for reference for any future blood collection time points.

Repeat Catheter Blood Collection

- 16 Repeat  [go to step #6](#) -  [go to step #12](#) for as many non-terminal blood collections timepoints as planned.

(Optional) Perform Terminal Blood Collection

- 17 At the final time point (terminal collection), mouse will be anesthetized with isoflurane and blood can be collected via one of the following methods:



17.1 Catheter blood collection:

- While the mouse is immobile with shallow respiration and unresponsive to toe pinch, perform cervical dislocation.
- Repeat  [go to step #7](#) -  [go to step #12](#) , collecting as much blood as possible.

17.2 Cardiac puncture:

- Attach 23-gauge needle to empty 1 mL syringe
- Switch Isoflurane flow to nose cone and position the mouse supine
- While the mouse is immobile with shallow respiration and unresponsive to toe pinch lay mouse on its back and gently insert needle below mouse's sternum and through the diaphragm targeting the heart.
- Gently pull the plunger and if blood is seen, continue pulling until syringe is filled. If blood is not seen, adjust the position of the needle and try again.

18 After completion of experiment, blood can be processed with the protocol "Processing blood intended for Olink assay."