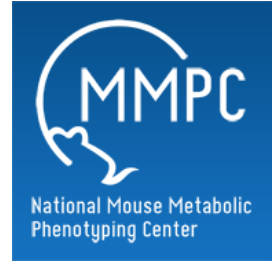


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## Vandy - Hyperinsulinemic-Hypoglycemic clamp

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**Protocol status:** Working

We use this protocol and it's working

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## Abstract

### Summary:

Mice with catheters implanted in the jugular vein (infusions) and carotid artery (sampling) are used for this procedure (V3002). The hyperinsulinemic hypoglycemic clamp involves a constant rate insulin infusion with a fall in blood glucose that is controlled by feed back from regular glucose measurements. Blood glucose is then clamped at a hypoglycemic level. The hypoglycemic clamp is used to test hypoglycemic counterregulation and the functionality of the hypothalamic-pituitary-adrenal axis.

## Materials

### MATERIALS

☒ Infusion Pumps **Harvard Apparatus Catalog #PY8 70-2208**

☒ Stand **Fisher Scientific Catalog #14-670A**

☒ Dual channel swivel **Instech Laboratories, Inc. Catalog #375/D/22QM**

☒ 3- and 4-way stainless steel connectors **Ziggy's Tubes and Wires Catalog #HSCY-25 or HSC4-25**

☒ Microrenathane tubing (0.033" OD) **Braintree Scientific Catalog #MRE-033**

☒ Glucose meter and strips **ACCU-CHEK aviva**

☒ Blunt needle with luer hub **Ziggy's Tubes and Wires Catalog #LHN-E011041 25ga x 0.5"**

☒ Wire stainless steel **Ziggy's Tubes and Wires Catalog #W020304V-1**

☒ Clamp extension **Fisher Scientific Catalog #05-769-7Q**

☒ Connector hook **Fisher Scientific Catalog #14-666-18Q**

### Reagent Preparation:

#### Reagent 1: Donor Blood

1. Collect ~ 1 ml of blood from donor mouse in 0.5 ml EDTA tubes.
2. Centrifuge blood (1 min at 16,000 g) and save plasma for preparation of insulin (see below).
3. Resuspend red blood cells (RBC) with heparinized saline (10U/mL).
4. Centrifuge (1 min at 16,000 g), discard supernatant, and resuspend RBC with an equal volume of heparinized saline. Transfer resuspended RBC (donor blood) to a 1.5 ml tube

### Note:

**Fisher Scientific, RRID:SCR\_008452**

- 1 Surgical catheterization of the carotid artery and jugular vein in mice at least 5 days prior to the day of the study (refer to protocol for Surgical Catheterization of the Carotid Artery and Jugular Vein).
- 2 Weigh mouse and start fast (suggested starting time between 7:00 and 8:00 AM) by placing mouse in a plastic container with fresh bedding.
- 3 Mouse is hooked up to the swivel 3 hours into fasting (refer to protocol for Hyperinsulinemic-Euglycemic Clamp for detailed set-up and connections).
- 4 After a total of 5 hr fast, a constant infusion of insulin starts and glucose is monitored every 10 min and is allowed to fall and is fixed at a hypoglycemic level (~60mg/dL) by adjusting the infusion of 20% glucose.
- 5 Donor blood is infused to jugular vein catheter throughout the study to prevent a fall of hematocrit.
- 6 Catecholamines and glucagon levels are measured at 0, 30, and 120 min. Insulin is measured at 0 and 120 min.
- 7 At the end of the study, mouse is anesthetized and tissues of interest are harvested and frozen in liquid nitrogen.



Time (min)	Sample (ul)	Glucose (mg/dl)	Time of Infusion Change	Glucose Infusion Rate		HCT	Comments
				(uL/min)	(mg/kg/min)		
0	200 (G,I,N,C,S)					*	Donor RBC
10	10 (G)						
15	10 (G)						
20	10 (G)						
30	160 (G,N,C,S)						
40	10 (G)						
50	10 (G)						
60	10 (G)						
70	10 (G)						
80	10 (G)						
90	10 (G)					*	
100	10 (G)						
110	10 (G)						
120	200 (G,I,N,C,S)						

**G:** Sample for plasma glucose concentration ~ taken every 10 minutes

**I:** Sample for plasma insulin concentration (25 µl plasma) ~ taken 0, 120 minutes

**N:** Sample for plasma glucagon concentration (25 µl plasma) ~ taken 0, 30, 120 minutes

**C:** Sample of blood for catecholamine (100 µl whole blood) ~ taken 0, 30, 120 minutes