Dear JoVE editors,

Thank you for editorially reviewing our manuscript JoVE52425 'Physiology Lab Demonstration: Renal Function in a Rat'.

We have addressed your comments below. We have used the "track-changes" function in Microsoft Word to identify all of our manuscript edits. We have also uploaded this document labeled, ‘Response to Editor’s Comments”. Thank you for your comments. We are confident that your suggestions for revisions have significantly improved the quality of our submitted manuscript.

Editorial comments:

1.Please state the goal of your protocol in the Short abstract.

**Response:**  The Short Abstract has been revised to “Assessing renal function is critical in evaluating the renal effects of drugs and disease. The purpose of this protocol is to demonstrate the principles and techniques for measuring and calculating glomerular filtration rate, urine flow rate, and excretion of sodium and potassium a presented as a hands-on teaching laboratory.

2.Please specify the pore size of the filter in step 1.3 and please remove the word “Millipore”.

**Response**: The pore size has been included and the word “Millipore” has been removed from the revised manuscript.

3.Please specify the dose of isoflurane in step 2.1.

4.Is the animal secured before cannula implantation? If so please specify.

**Response:** In response to comments #3 and #4, Step 2.1 has been revised to, “Induce anesthesia in the rat with 5% isoflurane. Record body weight and place the rat on a heated surgical platform to maintain 37 °C body temperature. Gently secure the rat to the platform with laboratory tape over the paws. Maintain anesthesia with 1.8% isoflurane.”

5.Please write step 2.2 in imperative tense, “Record the blood pressure and heart rate…”.

**Response:** Corrected version: “Record blood pressure and heart rate using data acquisition software and display on a computer screen in real-time [5](#_ENREF_5).

6.Please write the “Post-Drug sample 1, 2, 3, in a sentence format.

**Response:** Corrected version:

Collect Post-Drug Sample 1 five minutes after furosemide.

Collect Post-Drug Sample 2 ten minutes after furosemide.

Collect Post-Drug 3 fifteen minutes after furosemide.

7.Please write step 4.1 in imperative tense. “Measure…”, “Centrifuge…” Additionally please specify the centrifugation speed in “ x G” and the time.

**Response:** Corrected version: “Measure urine volume gravimetrically with a digital scale. Centrifuge whole blood samples with a table-top centrifuge (18,000 x G) to separate plasma. Transfer plasma samples to small labeled vials.

8.Please mention step 4.2 in imperative tense. “Analyze Na and K concentrations..”

**Response:** Corrected version: “Analyze Na and K concentrations in urine and plasma samples with sodium/potassium analyzer.”

9.Please split step 4.3 in to smaller steps such that each step contains only two to three action items per step.

**Response**: This step has been divided into 4 smaller steps.

4.4) Measurement of FITC-inulin in plasma and urine

4.4.1) Dilute pre-drug urine (from 1:200 to 1:400), and post-drug urine (1:10) with HEPES buffer (500 mM, pH 7.4).

4.4.2) Add 40 ul of standard or sample and 60 ul of HEPES buffer in a 96 well plate (one sample per well) and allow to mix for 10 minutes while covered with aluminum foil.

4.4.3) Generate a standard curve for FITC-inulin for concentrations of 6.25, 12.5, 25, 50, 100, 200, 400 μg/ml (Figure 1). Determine FITC-inulin fluorescence in samples and standards using a microplate reader with excitation and emission wavelengths of 485 and 538 nm, respectively.

4.4.4) Fit the fluorescent values for the standards to a 4-paramter logistic function regression analysis and the regression function parameters are used to calculate FITC-inulin in plasma and urine samples (Table 1).

10.Please write section 5 in correct number format. And please define what each of the terms in the formula stand for.

**Response**: Section 5 has been revised to show the correct number format. All term is the formulas are defined.

5. Post-Lab Analysis of Results: Calculations

5.1) Urine Flow Rate (UV; ml/min) = volume of urine collected (ml)

time of collection (min)

5.2) Glomerular Filtration Rate (GFR; ml/min) = Urine inulin conc. (μg/ml) x UV (ml/min)

Plasma inulin conc. (μg/ml)

5.3) Filtered Sodium Load (uM/min) = Plasma sodium concentration (μM/ml) x GFR (ml/min)

5.4) Sodium Excretion Rate (UNaV; uM/min) = Urine sodium concentration (μM/ml) x UV (ml/min)

5.5) Fractional Excretion of Sodium (FE Na; %) = UNaV (μM/min) X 100

Filtered Sodum Load (μM/min)

5.6) Filtered Potassium Load (uM/min) = Plasma potassium concentration (μM/ml) x GFR (ml/min)

5.7) Potassium Excretion Rate (UKV; uM/min) = Urine potassium concentration (μM/ml) x UV (ml/min)

5.8) Fractional Excretion of Potassium (FE K; %) = UKV (μM/min) X 100

Filtered Potassium Load (μM/min)

11.In step 4.3 since you are measuring the FITC fluorescence in sample, it would be ideal to have a data showing the standard curve and the bar graph or table with the resulting concentration.

**Response:** A figure of the standard curve (Figure 1) and a table of resulting concentrations (Table 1) have been included in the revised manuscript. The original tables have been re-numbered to Table 2 and Table 3.

12.JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Please remove all commercial sounding language from your manuscript and replace it with a more generic term as much as possible throughout the entire manuscript. All commercial products should be sufficiently referenced in the table of materials/reagents. Examples of commercial sounding language in your manuscript are “Fluoroskan Microplate Reader”, “Millipore”, etc.

**Response:** The manuscript has been revised to remove all commercial sounding language. All commercial products are referenced in the table of materials.

13.After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is no page limit for the protocol text, but there is a 3 pages limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages (or less) of text to identify which portions of the protocol are most important to include in the video; i.e. which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVEs instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

**Response:** The Protocol Section is less than 3 pages.

14.Please provide a figure legend as per JoVE format for the figure, including title, description.

**Response:** All figures and tables have a title and legend in the revised manuscript.

15.Please make sure that the “Discussion” section covers the following pints running between 3 – 6 paragraphs.

a.Critical steps within the protocol.

b.Modifications and troubleshooting.

c.Limitations of the technique.

d.Significance of the technique with respect to existing/alternative methods.

e.Future applications or directions after mastering this technique.

**Response:** The revised Discussion section now covers the 5 points outlined above.

16.Issue number and DOI’s are missing. Please make sure that your references comply with JoVE instructions for authors. In-text formatting: corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text of the manuscript. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then et al.): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, doi:DOI, (YEAR).]

**Response**: The authors have included the issue numbers and the DOI’s for the references which had this information available. Some references did not have issue numbers and/or DOI numbers.