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🌐 Determination of Glomerular Filtration Rate in Conscious Mice using FITC-inulin

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We use this protocol and it's working

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Abstract

Summary:

This protocol describes the procedures for estimating glomerular filtration rate in conscious mice based on Fluorescein Isothiocyanate-inulin (FITC-inulin) clearance following a single bolus intravenous injection.

Diabetic Complication:

Nephropathy

Reference:

1. Hem, A., Smith, A.J., and Solberg, P. 1998. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink. *Lab Anim 32:364-368.*

2. Sturgeon, C., Sam, A.D., 2nd, and Law, W.R. 1998. Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. *J Appl Physiol 84:2154-2162.*

3. Qi, Z., Whitt, I., Mehta, A., Jin, J., Zhao, M., Harris, R.C., Fogo, A.B., and Breyer, M.D. 2003. Serial Determination of Glomerular Filtration Rate in Conscious Mice Using FITC-Inulin Clearance. *Am J Physiol Renal Physiol.*

Materials

MATERIALS

⊗ FITC-inulin **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #F-3272

⊗ HEPES **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ Dialysis Membrane **Repligen (Spectrum Labs) Catalog** #132636

⊗ Closures **Repligen (Spectrum Labs) Catalog** #132736

⊗ Syringes (1ml 5ml)

⊗ Needles (30 G1/2 23 G1/4)

⊗ Filter (0.22μm) **Costar Catalog** #8110

⊗ Isoflurane drop jar

⊗ Isoflurane

⊗ Centrifuge (desktop)

Reagent Preparation:

1. 5% FITC-inulin: dissolved 100mg of FITC-inulin in 2 ml of 0.9% NaCl by heating the solution in boiling water.

2. 500 mM HEPES buffer: dissolved 59.6g of HEPES in 500 ml of deionized water and adjust pH to 7.4 using 10N NaOH.

Troubleshooting

1 **Preparation of 5% FITC-inulin solution:**

1. 5% FITC-inulin is dissolved in two ml of 0.9% NaCl -- facilitated by heating the solution in boiling water.

2. To remove residual FITC not bound to inulin, the solution is filled into a 1000 Daltons cut-off dialysis membrane (Spectra/Pro 6, Spectrum Laboratories Inc., Rancho Dominguez, CA).

3. Put the dialysis membrane filled with FITC-inulin into 1000 ml 0.9% NaCl for 24 hours at room temperature.

4. Prior to use, this dialyzed solution is sterilized by filtration through a 0.22 µm filter (e,g, Costar).

2 **Intravenous injection and blood collection:**

1. Mice are anesthetized using Isoflurane, which for approximately 20 seconds.

2. 5% FITC-inulin (3.74µl /g body weight) is injected retroorbitally under anesthesia within 10 seconds.

3. After fully regaining consiousness the mouse is restrained inside a 50- mL centrifuge tube with large air-holes drilled in the tip.

4. The inner thigh was closely shaven and wiped with 75% ethanol, revealing the saphenous vein. Approximately 20 µl blood is collected in a heparinized capillary tube (Fisher Scientific) by venepuncture using a sterile 23 gauges syringe needle (see Hem et. al.1998(1)). On average, this yields 10 µl of plasma following centrifugation (4,000 RPM, 10 min).

5. Blood was sampled via the saphenous vein at 3, 7, 10, 15, 35, 55, 75 minutes post injection of FITC-inulin.

3 **Determination of fluorescence of the sampled plasma:**

1. Since pH significantly affects FITC fluorescence value, each plasma sample is buffered to pH 7.4, by mixing 10 µl of plasma with 40 µl of 500 mM HEPES (pH 7.4).

2. The titrated samples were then loaded onto a 96-well plate, 50µl sample/well. Fluorescence was determined using a Fluoroscan Ascent FL (Labsystems, FIN-00811 Helsinki, Finland), with 485 nm excitation, and read at 538 nm emission.

4   **Calculation of GFR:**

A two – compartment clearance model may be employed for the calculation of GFR(2, 3). In the two-compartment model used, depicted in Fig 1, the initial, rapid decay phase represents redistribution of the tracer from the intravascular compartment to the extracellular fluid. Systemic elimination also occurs, but the distribution process is relatively dominant during this initial phase. During the later, slower decay in concentration of the tracer systemic clearance of the tracer from the plasma predominates. At any given time (t_x), the plasma concentration of the tracer (Y) equals to $Ae^{-\alpha tx} + Be^{-\beta tx}$ +Plateau (http://curvefit.com/id205.htm).

The parameters of above equation could be calculated using a non-linear regression curve-fitting program (GraphPad Prism, GraphPad Software, Inc., San Diego, CA.) (see below). GFR was calculated using the equation:
GFR= I/(A/α + B/β), where I is the amount of FITC-inulin delivered by the bolus injection; A (Span1) and B (Span2) are the y -intercept values of the two decay rates, and α and β are the decay constants for the distribution and elimination phases, respectively.
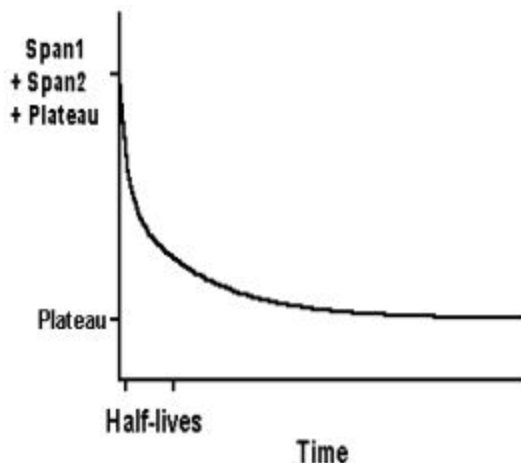
5   **Use of Prism:**

1. Open Prism program and click "New table".

2. Input time point into "X Values" column, and the corresponding fluorescence data into " Y" column.

3. Click " Analysis" and choose " Nonlinear regression (curve fit)"

4. Click " OK", and choose " Two phase exponential decay"

5. Set "Plateau" zero.

6. The parameters of the fluorescence decay curve are shown in a spreadsheet. SPAN1 and SPAN2 are Y intercepts , and K1 and K2 are constants of the distribution and clearance phases, respectively.