

Measuring Mouse GFR by FITC-Inulin using the Thermo Scientific NanoDrop 3300 Fluorospectrometer

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Introduction

The measurement of glomerular filtration rate (GFR) is the gold standard in kidney function assessment, and as such is used as an indicator of kidney disease (1). Currently, GFR is determined by measuring the level of the endogenous biomarker creatinine (2) or radioactively (³H or ¹⁴C) labeled inulin in blood plasma and/or urine (1). Rodents, particularly mice, have high concentrations of non-creatinine chromagens in plasma and urine that interfere with commercially available creatinine assays. As a consequence, these assays, which are based on the alkaline picrate Jaffé reaction or enzymatic reactions, overestimate creatinine concentration in plasma compared with HPLC determination (2, 3). In addition to this, creatinine has also been proven to be secreted in the proximal tubule of the mouse, further adding to the inaccuracy of creatinine-based estimates of GFR (4). For these reasons, alternate tracer molecules have been sought to help increase the accuracy of GFR measurements.

GFR measurements using radioactive labeled inulin clearance, the traditional gold-standard, typically require approximately 3-5 μ l of sample per measurement. This inulin radioassay can be used in both anesthetized and conscious mice, but harbors the disadvantages of numerous safety regulations and strict handling issues inherent with the use of radioactive isotopes. An alternative protocol presented by Qi et al. (5) utilizes FITC-labeled inulin as the exogenous marker to measure GFR in conscious mice. This protocol retains the sensitivity of the traditional assay by using fluorescence detection and circumvents the disadvantages of radiolabel-based assays. The FITC-inulin assay has been used in a reduced volume format to take advantage of the microvolume capability of the Thermo Scientific NanoDrop 3300 Fluorospectrometer in order to further reduce the total volume of the assay (4, 6-12). The NanoDrop™ 3300 uses a patented “cuvette-less” sample retention system for fluorescence measurements using samples as small as 1-2 μ l.

Samples are pipetted onto the optical surface and held in place during the measurement by surface tension (fig. 1). After measurements, the samples are quickly and easily removed from the optical surfaces with a dry laboratory wipe.

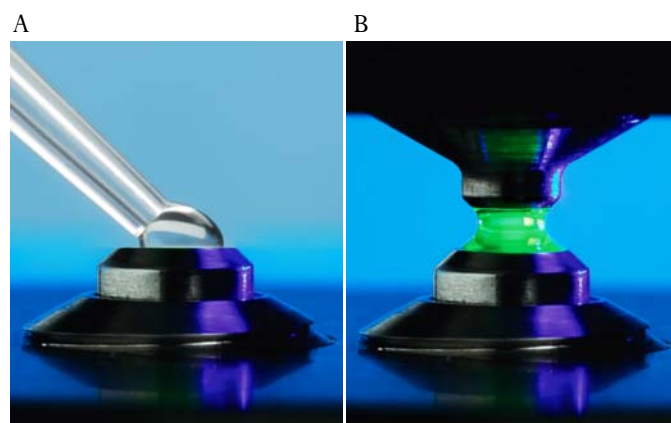


Figure 1: A) Loading of a 1-2 μ l FITC sample on the optical surface with a low volume pipette and B) measurement of a FITC sample using the 470 nm blue LED.

The use of the FITC labeled inulin method in conjunction with the microvolume technology of the NanoDrop 3300 Fluorospectrometer enabled researchers to reduce the volumes of blood required from 20 μ l to 2-10 μ l per collection (4, 6-12). This reduced blood volume per collection facilitated studies using serial measurements from the same individual mouse without compromising the accuracy of the GFR measurement data or the health of the mouse. This application note briefly summarizes the experimental protocol used and results obtained by Vallon et al. (6), who use a microvolume FITC-inulin procedure for use on the NanoDrop 3300 Fluorospectrometer.

Experimental procedures

Briefly, FITC-inulin (5% in 0.85% NaCl) is dialyzed for 24 h against 0.85% NaCl which results in a ~2% solution and serves to establish the standard curve. The dialyzed FITC-inulin solution is sterile filtered and injected into the retro-orbital plexus (2 $\mu\text{l/g/body weight}$) during brief isoflurane anesthesia. At 3, 7, 10, 15, 20, 40 and 60 min after injection, blood is collected from a small tail nick the end of the tail into a Na⁺-heparinized 10 μl microcap (Hirschmann Laborgeräte, Eberstadt, Germany). After centrifugation, 1 μl of plasma is diluted 1:10 in 0.5 mol/l HEPES (pH 7.4) and fluorescence is determined in 2 μl samples using the NanoDrop 3300 Fluorospectrometer. GFR is calculated using a two-compartment model of two-phase exponential decay (GraphPad Prism, San Diego, CA). The mathematical modeling to obtain GFR by single-bolus injection method was described in detail earlier (13). The standard curve is created by diluting the FITC-Inulin (1:100, 1:1000, 1:2000, 1:10000, 1:20000 and 1:100000). Because of the autofluorescence of mouse plasma, it is necessary to use mouse plasma (diluted 1:10 with HEPES) from the same strain as diluent for the standard curve. Of note, FITC-inulin can also be used for determination of GFR in anesthetized mice using steady-state infusion clearance (4).

Results

To measure GFR in conscious mice, plasma kinetics of FITC-inulin following a single-dose intravenous injection were used. For calculation of GFR, a two-compartment model is employed. In the two-compartment model, the initial, rapid-decay phase represents redistribution of FITC-inulin from the intravascular compartment to the extracellular fluid. The later phase with slower decay in FITC-inulin concentration predominantly reflects systemic clearance from the plasma (fig. 2). An example from Vallon et al. (6) of individual mouse GFR measurements and the population average GFR result derived from the FITC-inulin clearance decay curve is shown in fig. 3.

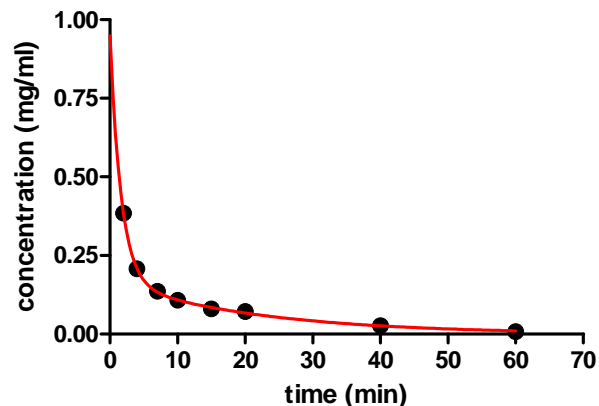


Figure 2: Representative plasma clearance kinetics of FITC-inulin using the microvolume procedure.

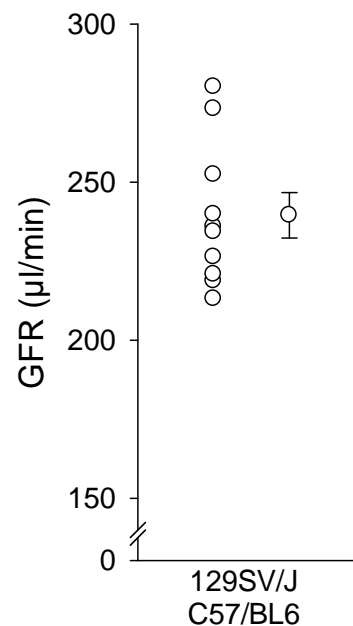


Figure 3: Single measurements (left) and average (right) GFR in mice with a mixed genetic background of 129Sv/J and C57/BL6. Data are from Reference 6.

Conclusion

The method described in this application note demonstrates the feasibility of non-radioactive and repeated determination of GFR in conscious mice without surgical procedures (implantation of osmotic mini pumps) or metabolic cage experiments. Using this method, diabetic hyperfiltration as well as reduced kidney function after nephrectomy have been shown (5, 6). The data shown in this study clearly demonstrate the advantage of measuring GFR using FITC-inulin with a NanoDrop 3300 fluorospectrometer. By combining the sensitivity of the FITC-inulin technique with the microvolume format of the NanoDrop 3300, required blood volumes were significantly reduced. This reduction in blood draw volume facilitates further studies that until now were not possible because of the physiological effects of the removal of larger fluid volumes. Further, this method negates the need for radioisotopes, the use of which can be inconvenient considering the high levels of radioactivity used (especially in micropuncture experiments) and the associated high costs.

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