

## General Protocols for GFR Measurements

BioPAL's FIT-GFR kits provide researchers with a convenient, low-cost, in-house analytical method to accurately measure the renal clearance of a nonradioactive ideal filtration marker in order to measure the glomerular filtration rate (GFR) in experiment subjects. The FIT technology relies on immunoassay to measure the concentration of the GFR probe in collected blood and/or urine samples. Most analytical laboratories run ELISA-based tests on a regular basis. Therefore, these laboratories already have the equipment and trained personnel capable of running BioPAL's FIT-GFR kit. Instructions for running the kit are provided as a PDF download on BioPAL's web site ([www.BioPAL.com/FIT-GFR.htm](http://www.BioPAL.com/FIT-GFR.htm)). This document is designed to help researchers in designing and running the GFR procedure, i.e., injection and sample collection protocols. Below are a few important highlights that researchers should consider while developing an experimental protocol.

- 1. The GFR Probe:** BioPAL's (Gd-DTPA) FIT-GFR kit measures the renal clearance of Gd-DTPA. Gd-DTPA meets all the criteria for an ideal filtration marker and is clinically used to measure GFR using MRI imaging technology.<sup>1</sup> Gd-DTPA is clinically available for human use (Magnevist®, Bayer Pharmaceuticals), is not expensive and is available in more than 100 countries. Therefore, researchers have the ability to use the same diagnostic test throughout the drug development program (preclinical → clinical trials). For researchers interested in measuring GFR in animal models that do not have access to a clinical pharmacy, BioPAL provides Gd-DTPA for injection, which is sold separately from the kit.
- 2. Choice of a GFR Protocol:** There are many GFR protocols published in the literature that a researcher can choose from, but they all have one thing in common. If properly executed, all protocols will obtain the same GFR value. The choice of which GFR protocol to use is left to the end-user's scientific preference. This document provides two methods that have wide application in both preclinical and clinical research. However, the FIT-GFR kit can be used to analyze samples generated for any GFR protocol wherein Gd-DTPA is used as the renal probe.
- 3. Understanding the Relationship between the Administered Dose and the Sample Dilution:** BioPAL's FIT-GFR kit provides a sensitive assay for the measurement of Gd-DTPA in blood and urine samples. Researchers can measure GFR using a small dose of Gd-DTPA as the renal probe. The amount of the administered dose is an academic decision based on the requirements of the experimental model. Published references can provide guidance.<sup>2,3</sup> For example, a published human clinical trial used a dose of 10 µl/kg of Magnevist (Gd-DTPA) for all subjects.<sup>2</sup> This dose is 10x lower than the standard MRI imaging dose and proved successful in measuring GFR in all subjects. At this dose level, blood samples required a 1:300 dilution in order to fall within the active range of the standard curve of the ELISA, whereas the more concentrated urine samples require a 1:3,000 dilution.

There is an approximately linear relationship to the administered dose and the resulting sample dilution. For the human clinical study discussed above, if the researcher had chosen to administer 1 µl/kg of Gd-DTPA rather than 10 µl/kg, one would expect that the required dilution will in turn be reduced by a factor of 10, i.e., 1:30 for blood and 1:300 for urine. On the other hand, if the researcher was measuring GFR in combination with MRI imaging using the standard MRI dose of 0.1 ml/kg, one would expect that the needed sample dilution would increase by 10. Although this is a good rule-of-thumb, researchers should verify the required dilution at the start of a new protocol by evaluating a series of dilutions from one subject. Once the correct dilution factor has been determined, it should hold for most subjects enrolled in the protocol. Nevertheless, outliers can occur and therefore the stock sample should be reserved until the measurement is achieved. Researchers using animal models can use the above example as a guide, but should verify the dose and dilution factor for their model.

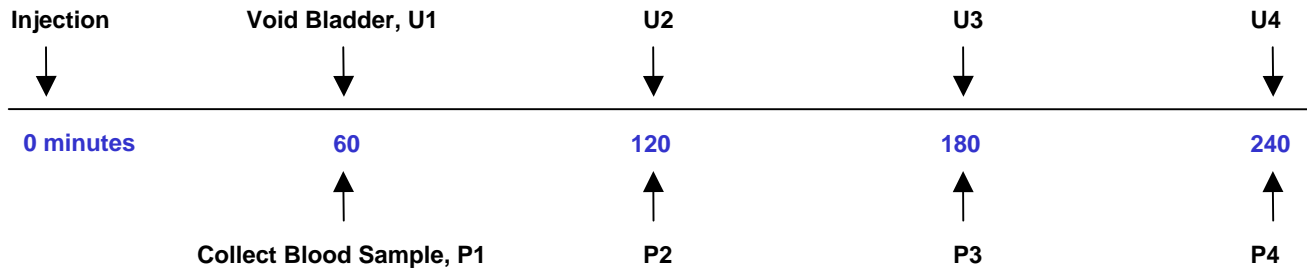
- 4. Analyte Specific Reagents:** For researchers conducting preclinical or clinical research, the FIT-GFR test is available in kit format. For clinical reference laboratories assisting in clinical trials, analyte specific reagents are available.

### References

1. *J Magn Reson Imaging* **22**:406-414 (2005)
2. *J Am Soc Nephrol* **18**:547A (2007)
3. *J Am Soc Nephrol* **18**:898A (2007)

# Protocol I: Classical UV/P Method

This protocol is an adaptation of the classic UV/P method to measure GFR using a single bolus injection of the GFR probe. This protocol will generate three UV/P measurements that are averaged to obtain the GFR value.



1. For normalizing to the body surface area, record the height and weight of the subject. Encourage the subject to drink fluids throughout the study. The subject should be instructed to “completely” void the contents of his/her bladder into the collection vessel to the best of their ability. The subject should also be instructed to inform the technician of any significant loss of urine during the voiding process.
2. *Via* an intravenous bolus injection, administer the required dose of Gd-DTPA and record the time of the injection to the nearest minute.
3. At 60-minutes post injection, the subject should void his/her urine into a collection vessel (U1). The technician should record the moment of voiding to within the nearest minute and measure the volume of the collection to the nearest ml (V1). After voiding and using the opposite arm, a venous blood collection is drawn in a serum separation tube without anticoagulant and the time of collection is recorded to the nearest minute (P1). Centrifuge blood sample to obtain a serum sample.
4. Step 3 is repeated at approximately 120, 180, and 240 minutes post injection. Remember, it is important to record the “correct” time of each collection.

Subject's Name: \_\_\_\_\_  
Date: \_\_\_\_\_

Height (cm): \_\_\_\_\_  
Weight (kg): \_\_\_\_\_

	Record the Time	Record the Measured Volume
Injection		
Urine Collection – U1		
Blood Collection – P1		
Urine Collection – U2		
Blood Collection – P2		
Urine Collection – U3		
Blood Collection – P3		
Urine Collection – U4		
Blood Collection – P4		

Note any problems with urine collection: \_\_\_\_\_

5. Follow the instructions provided with the FIT kit for running the immunoassay to determine the Gd-DTPA concentration in each sample and then use BioPAL's GFR calculator or similar spreadsheet program to calculate the GFR value (ml/min/1.73m<sup>2</sup>).

# Frequently Asked Questions

## Protocol I: Classical UV/P Method

**Question:** If the subject recently underwent a Gd-DTPA enhanced MRI procedure, can I still run a GFR study?

**Answer:** For the vast majority of applications, FIT-GFR test can be conducted post one week of an enhanced MRI procedure. Nevertheless, it is recommended that the researcher obtain a pre-injection blood and urine sample to confirm the baseline.

**Question:** Why is the first urine collection not used to calculate GFR and, if it's not needed, do I need to analyze this sample?

**Answer:** This first urine collection MUST be obtained in order to void the bladder in preparation for the first GFR UV/P measurement. The second urine collection (U2) is the total accumulative urine volume the body generated between P1 and P2. Although the U1 sample must be obtained, it does not need to be analyzed.

**Question:** What should I do if the subject needs to urinate before the programmed time point?

**Answer:** This is not a problem and the technician has two choices. First, if this event occurs close to the programmed timed collection (within ~15 minutes), simply collect and measure the volume of the urine and record the time of collection, followed by the blood collection. Second, if the event occurs well before the programmed timed collection, collect the urine in the collection vessel and reserve. At the programmed time point, have the subject void again and record the time of the second voiding. Combine the two collections and then record the total volume. The two collections MUST be combined and mixed prior to analysis in order to obtain the correct Gd-DTPA concentration.

**Question:** Should the three UV/P values be equivalent? If so, what does it mean if there is a significant difference between the three values?

**Answer:** For a normal subject with no evidence of kidney dysfunction, the three values should be very similar. If there is one value that appears to be an outlier, it most likely is. The problem may be due to an error during urine collection or an error made during the immunoassay procedure for either blood or urine. The researcher should review the lab notes for recordings of suspected problems. If the researcher believes the outlier is an artifact, then that UV/P value can be eliminated from the average. However, the kidney is not a machine. Its function can vary in time and may vary to a greater degree in a subject with underlying kidney dysfunction. The power of this method is that the final GFR value is an average of three measurements. Therefore, it is advised that a researcher should not quickly eliminate a value without justification.

**Question:** Can I shorten the protocol by collecting only one UV/P value?

**Answer:** Yes, although this will eliminate the benefit of the three-point averaging. However, to ensure that sampling occurs within the second compartment of the bi-exponential clearance of the GFR probe, it is recommended that the U1 and P1 sampling be programmed for 90 minutes post injection and the U2 and P2 be programmed for 150 minutes post injection.

**Question:** Can I run the assay using plasma, rather than serum?

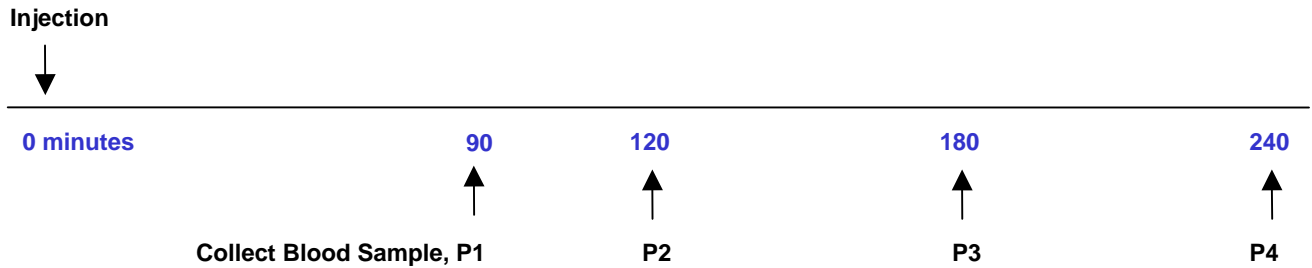
**Answer:** Yes.

**Question:** What is the difference between Bayer's Magnevist® (Gd-DTPA) and BioPAL's Renal**RATE**™ (Gd-DTPA)?

**Answer:** Chemically there is no difference. However, BioPAL's Renal**RATE** is for animal research ONLY and is provided for those researchers who do not have access to a clinical pharmacy. For clinical research use in human subjects, researchers MUST use Magnevist.

# Protocol II: Blood Clearance Method

This protocol is an adaptation of the one compartment, blood clearance method to measure GFR using a single bolus injection of the GFR probe. No urine collection is required.



1. For normalizing to the body surface area, record the height and weight of the subject. Encourage the subject to drink fluids throughout the study.
2. Draw the required dose of Gd-DTPA into the injection syringe. Accurately measure and record the mass of the filled syringe.
3. *Via* an intravenous bolus injection, administer the required dose of Gd-DTPA and record the time of the injection to the nearest minute. If an injection port is used, follow the bolus with a saline wash.
4. Accurately re-measure and record the mass of the emptied syringe. The difference in the mass - pre and post injection - is the amount of Gd-DTPA administered to the subject.
5. At 90-minutes post injection and using the opposite arm, a venous blood collection is drawn in a serum separation tube without anticoagulant. The time of collection is recorded to the nearest minute (P1). Centrifuge the blood sample to obtain a serum sample.
6. Step 5 is repeated at approximately 120, 180, and 240 minutes post injection. Remember, it is important to record the “correct” time of each collection.

Subject's Name: \_\_\_\_\_ Height (cm): \_\_\_\_\_  
 Date: \_\_\_\_\_ Weight (kg): \_\_\_\_\_

	Record the Time
Injection	
Blood Collection – P1	
Blood Collection – P2	
Blood Collection – P3	
Blood Collection – P4	

Pre-injection Syringe Mass [**A** = Gd-DTPA + syringe (g)]: \_\_\_\_\_  
 Post-injection Syringe Mass [**B** = syringe after injection (g)]: \_\_\_\_\_  
 Mass of delivered Gd-DTPA [**A-B** (g)]: \_\_\_\_\_

7. Follow the instructions provided with the FIT kit for running the immunoassay to determine the Gd-DTPA concentration in each sample and then use BioPAL's GFR calculator or similar spreadsheet program to calculate the GFR value (ml/min/1.73m<sup>2</sup>).

# Frequently Asked Questions

## Protocol II: Blood Clearance Method

**Question:** Why do I have to accurately know the delivered dose of Gd-DTPA for the Blood Clearance Method, but it is not required for the UV/P Method?

**Answer:** The UV/P method measures GFR by evaluating the rate of clearance from the blood over its simultaneous appearance in the urine. As long as the administered dose is sufficient for measurement by FIT, the exact dose delivered is not relevant for the UV/P calculation. Based on the injected dose, the Blood Clearance method measures GFR by calculating total amount of Gd-DTPA removed from the blood as a function of time.

**Question:** Why is the first blood sample collected at 90 minutes post injection?

**Answer:** Gd-DTPA clears from the blood bi-exponentially and this is a one-compartment blood clearance method. Therefore, it is important that the measurement starts after the first exponential clearance. By having the first programmed blood withdrawal at 90 minutes post injection ensures that the subject –normal or renal insufficient – is within the second exponential period.

**Question:** Can I run the assay using plasma, rather than serum?

**Answer:** Yes.

**Question:** Can I change the duration of the protocol?

**Answer:** Yes. The protocol can be lengthen if GFR is being measured in subjects with known renal insufficiency. For example, a 24-hour sample can be collected. For subjects with normal or near normal kidney function, the protocol can be shorten by an hour, i.e., the last programmed blood collection can be at 180 minutes post injection.

**Question:** Can I add or reduce the number of programmed collections?

**Answer:** Yes. For the one-compartment blood clearance method, researchers can add as many additional programmed collections post 90-minutes as desired. However, at minimum three programmed collections are needed for this method.

**Question:** If I use the UV/P protocol, can I also calculate GFR *via* the blood clearance method? If so, will they provide the same value?

**Answer:** Yes. Using the UV/P protocol there is sufficient information to also calculate the GFR using the one-compartment blood clearance method. Because Gd-DTPA is an ideal filtration marker that does not bind to serum proteins or disassociate from the chelate, the two GFR values should be comparable thereby providing confirmation of the measurement. A significant deference between the two values would likely be due to either an error in urine collection, resulting in an error in the UV/P GFR value, or an error in measuring the injected dose, resulting in an error in the Blood Clearance GFR value. By reviewing the lab note, the researcher should be able to discern the problem and select the correct GFR value. However, if combining the two methods, it is suggested that the researcher include an addition programmed blood sample at the 90 minutes post injection and do not include the 60 minute collection as one of the four time points in the blood clearance GFR calculation.

**Question:** Can I measure GFR in small animal models?

**Answer:** Yes. Two important advantages of the FIT-GFR method is that (1) the test uses a small injectate volume and (2) only a small amount of sample is required for the analysis. As a result, GFR of small animal models, such as mice and rats, can be measured. Published protocols that use radioactive tracers to measure GFR can provide guidance concerning experimental design for small animal models.