

Estimation of glomerular filtration rate in dogs by plasma clearance of gadolinium diethylenetriamine pentaacetic acid as measured by use of an ELISA

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Objective—To evaluate use of gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) to estimate glomerular filtration rate (GFR) by plasma clearance and use of an ELISA as the method of Gd-DTPA quantification.

Animals—16 dogs of various sexes and breeds (12 dogs were clinically normal, and 4 dogs were polyuric and polydipsic with no other clinical or biochemical abnormalities).

Procedures—GFR was estimated by measuring the plasma clearance of Gd-DTPA and io-hexol by use of an ELISA and high-performance liquid chromatography (HPLC), respectively. The GFR was determined by use of a 1-compartment model for both methods. The GFRs obtained by Gd-DTPA plasma clearance were compared with those obtained by io-hexol plasma clearance by use of correlation analysis, paired *t* tests, and limits of agreement analysis. A paired *t* test was used to evaluate differences between the 2 plasma clearance methods.

Results—A strong linear correlation ($r^2 = 0.90$) was found between GFRs derived from the plasma clearance of Gd-DTPA and those derived from the plasma clearance of io-hexol. By use of limits of agreement analysis, almost all (13/14) dogs had Gd-DTPA GFRs that were within 12% of io-hexol GFRs. The remaining dog had a Gd-DTPA GFR that was 45% higher than the io-hexol GFR. There was no significant difference between Gd-DTPA GFRs and those obtained with io-hexol.

Conclusions and Clinical Relevance—This study revealed that plasma clearance of Gd-DTPA measured by use of an ELISA is an effective method to estimate GFR in dogs because it compared favorably with results for the io-hexol-HPLC method. (*Am J Vet Res* 2009;70:547–552)

Determinations of BUN and serum creatinine concentrations are the tests most commonly used to evaluate renal function in clinical and research settings. However, both are insensitive in that increases to concentrations higher than the respective reference ranges are not evident until there is loss of > 75% of renal function.¹ Concentrations of BUN are also nonspecific because they are affected by numerous factors, including dietary protein intake and renal blood flow. Therefore, diagnosing subclinical renal disease is a challenge for veterinarians. In addition, alterations in renal function are directly or indirectly related to many research applications, and changes in BUN and serum creatinine concentrations may not reflect these alterations.

Glomerular filtration rate is the most sensitive and accurate indicator of renal function. The GFR is the

ABBREVIATIONS	
AUC	Area under the plasma concentration-versus-time curve
CV	Coefficient of variation
Gd-DTPA	Gadolinium diethylenetriamine pentaacetic acid
GFR	Glomerular filtration rate
HPLC	High-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
NSF	Nephrogenic systemic fibrosis
OD ₄₅₀	Optical density at 450 nm

rate at which the kidneys remove a filtration marker from a given amount of plasma per unit of time. Several methods are used to estimate GFR in dogs, with urinary clearance of inulin as the criterion-referenced standard.² Urinary clearance is the most accurate assessment of GFR, and inulin satisfies the requirements for an ideal filtration marker. It is freely filtered across the glomerulus, eliminated only by the kidneys with no systemic metabolism, not secreted or absorbed in the renal tubules, and not bound by protein in blood. In addition,

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inulin is nontoxic. Urinary clearance of endogenous and exogenous creatinine also yields accurate assessments of GFR in dogs.^{3,4} However, there are practical concerns with the use of urinary clearance protocols. They are time-consuming and require meticulous urine collection with urinary catheters or metabolic cages. Protocols that involve the use of urinary catheterization have the risk of causing an iatrogenic urinary tract infection. For these reasons, other methods have been explored, including plasma clearance,^{5,6} nuclear scintigraphy,^{7,8} and functional computed tomography.^{9,10}

Measurement of GFR by plasma clearance of a filtration marker avoids the difficulties of urinary clearance techniques. The marker is injected IV, and its serum concentration is determined at various time points after injection. The GFR is determined by calculating the rate of decrease in plasma concentration of the marker over time. The most widely used marker is iohexol,^{5,11} which, similar to inulin, has the characteristics of an ideal filtration marker. However, use of iohexol is limited because there is only 1 reference laboratory^a in the United States that performs the HPLC assay to detect this marker. Other filtration markers^{6,12} have been evaluated for plasma clearance to determine GFR, but all have their limitations, and none have proven to be superior to iohexol.

New filtration markers are needed that have the aforementioned characteristics and that can be assayed in a simple, timely, and inexpensive manner. This would allow GFR to be routinely determined, which could considerably impact the diagnosis and management of renal disease in dogs. It could also facilitate research that needs accurate assessment of GFR. The compound Gd-DTPA is an attractive candidate for a new filtration marker. It possesses the required attributes¹³⁻¹⁵ and can be readily assayed by use of an inexpensive ELISA.^b The purpose of the study reported here was to evaluate the use of Gd-DTPA to estimate GFR by plasma clearance and to evaluate a commercial ELISA kit as a method of Gd-DTPA quantification. The Gd-DTPA estimates of GFR were validated by comparing them with GFRs determined by plasma clearance of iohexol. The stability of Gd-DTPA in stored serum was also evaluated.

Materials and Methods

Animals—Sixteen dogs were included in the study; 3 were clinically normal purpose-bred adult Beagles, and 13 were client-owned dogs. Seven dogs were spayed females, and 9 were neutered males. Body weights of the dogs ranged from 11.0 to 39.9 kg. Twelve dogs were clinically normal, and 4 had polyuria and polydipsia. The latter 4 dogs were otherwise healthy as determined on the basis of results of physical examination, history, CBC, serum biochemical analysis (SUN and serum creatinine concentrations were within reference ranges), urinalysis, bacterial culture of urine samples, thoracic radiography, and abdominal ultrasonography. The cause of the polyuria and polydipsia remained unknown. All experimental procedures were approved by the Tufts Cummings School of Veterinary Medicine Institutional Animal Care and Use Committee. Owners provided written consent for participation of client-owned dogs in the study.

Plasma clearance procedures (Gd-DTPA and iohexol)—Food was withheld from each dog for at least 12 hours prior to the experiments. Dogs were allowed free access to water throughout the study. The morning of the procedure, dogs were weighed and a catheter was placed in a cephalic vein. Aliquots of Gd-DTPA^c (46.9 mg•kg⁻¹) and iohexol^d (300 mg of iodine•kg⁻¹) were injected sequentially, and the exact times of injection were recorded. The Gd-DTPA was always administered first as a bolus injection; iohexol was administered second during a 5-minute period to minimize discomfort. Blood samples (3 mL) were collected from a jugular vein at baseline (immediately before injection of Gd-DTPA) and at 2, 3, and 4 hours after injection. Blood was placed into serum collection tubes and allowed to clot for 10 minutes. The tubes were then centrifuged at 1,163 × g and the sera harvested for analysis.

Iohexol measurement—Samples for serum iohexol concentrations were shipped frozen to a reference laboratory.^a Samples were assayed by use of HPLC within 7 days after the clearance procedure.

Gd-DTPA measurement—Concentrations of Gd-DTPA were measured by use of a commercial ELISA kit^e within 4 hours after the clearance procedure. The diluent for the standards and samples was composed of 0.1% bovine serum albumin and 0.01% thimerosal in PBS solution (0.0098M dibasic sodium phosphate, 0.138M sodium chloride, and 0.00268M potassium chloride). Standards were prepared at 0, 0.003, 0.01, 0.03, 0.1, and 0.3 μg of Gd-DTPA•mL⁻¹, and serum samples were diluted at 1:400. Aliquots (50 μL) of standard or diluted sample were added to goat anti-rabbit IgG-coated microtiter plate wells, followed by sequential addition of 50 μL of horseradish peroxidase-Gd-DTPA conjugate containing a yellow dye and then 50 μL of rabbit anti-Gd-DTPA containing a blue dye. The plates were incubated for 90 minutes at 25°C and then washed with a Tween 20-PBS solution. Substrate (100 μL) was added to all wells and incubated at 21°C for 30 minutes. Stop reagent (100 μL) was then added, and the OD₄₅₀ for each well was recorded.

A standard curve⁴⁵⁰ was generated for each plate by fitting the data from the standards to a 4-parameter logistic function by use of commercial software.^f The Gd-DTPA concentration in each sample was determined by interpolation.

Sensitivity of the Gd-DTPA ELISA—Stock solutions of canine plasma^g at dilutions of 1:30 and 1:300 were prepared. Aliquots (n = 15) of each stock solution and the diluent were processed on a single plate. For each respective blank diluent and stock solution sample, the OD₄₅₀ was measured and the blank values determined by use of the standard curve. For each solution, the LOD was expressed as the mean blank value + 3 SD, and the LOQ was expressed as the mean blank value + 10 SD.¹⁶ Potential differences among the measured values for diluent and the stock solutions were evaluated by use of ANOVA.

Accuracy and precision of the Gd-DTPA ELISA—Intra-assay (n = 15) and interassay (5) replicate analyses at 2 concentrations of Gd-DTPA (low, 0.007 μg•mL⁻¹;

high, $0.055 \mu\text{g}\cdot\text{mL}^{-1}$) were performed. The aliquots were processed in the same manner as the serum samples, and the Gd-DTPA concentrations were calculated by interpolation from the standard curve. A sample at each Gd-DTPA concentration was assayed once weekly for 5 weeks for the interassay replicate analysis. Accuracy was defined as the range of percentage differences between the mean \pm 2 SD of measured concentrations and known standard values.¹⁶ Precision was expressed as the CV of the measured concentration (ie, $\text{CV} = [100\cdot\text{SD}]/\text{mean}$).¹⁶

GFR calculation—The plasma clearances of Gd-DTPA and iohexol for each dog were calculated by use of a 1-compartment plasma clearance model. This model, which uses the value of the marker concentration from 3 samples, provides a viable estimate for GFR determined by iohexol plasma clearance.⁵ The same model was used to estimate GFR determined by Gd-DTPA plasma clearance to allow comparison between the 2 clearance methods. The measured concentrations of Gd-DTPA and iohexol in each serum sample were plotted as a function of time. The data were fitted to a 1-exponential decay function, wherein $y = B \times e^{-bx}$ (where y = concentration of marker, B = intercept of the curve at time 0, e = the natural logarithm, b = the slope, and x = time). The function was integrated (limits of zero to infinity) to obtain the AUC (ie, $\text{AUC} = B/b$). The GFR was then obtained by dividing the administered dose by the AUC. The GFR was further adjusted on the basis of the dog's body weight, and these adjusted GFRs were used for statistical analyses.

Stability of Gd-DTPA in serum—Serum samples from 1 dog were stored at 4°C. After storage for 1, 7, 14, 41, 61, and 120 days, the samples were used to determine the GFR estimated by use of Gd-DTPA plasma clearance to evaluate Gd-DTPA stability in serum.

Data analysis—The calculated slopes for the iohexol and Gd-DTPA disappearance curves were compared by use of a paired t test. The GFRs obtained by Gd-DTPA plasma clearance were compared with GFRs obtained by iohexol plasma clearance by use of correlation analysis, paired t tests, and limits of agreement analysis.¹⁷

Results

Dogs—Two client-owned dogs vomited during IV administration of iohexol and were therefore excluded from the study. The remaining 14 dogs did not have any adverse effects following injection with Gd-DTPA or iohexol.

Standard curve—A standard curve of OD_{450} versus serum Gd-DTPA concentration was used to calculate Gd-DTPA concentrations in all samples (Figure 1). The curve fit a 4-parameter logistic model having the equation $\log y = d + (a - d)/(1 + [x/c]^b)$, as provided by the plate reader software.^f For the equation, y is the OD_{450} and x is the concentration of Gd-DTPA; the variables a , b , c , and d are coefficients from the fitted polynomial curve generated by the plate reader software.^f

Sensitivity of the Gd-DTPA ELISA—The mean \pm SD values for blank samples of diluted canine plasma

and diluent were determined (1:30 dilution, $5.236 \times 10^{-5} \pm 1.168 \times 10^{-4} \mu\text{g}\cdot\text{mL}^{-1}$; 1:300 dilution, $5.716 \times 10^{-5} \pm 1.321 \times 10^{-4} \mu\text{g}\cdot\text{mL}^{-1}$; diluent, $2.997 \times 10^{-5} \pm 8.288 \times 10^{-5} \mu\text{g}\cdot\text{mL}^{-1}$). The LOD was approximately $0.00003 \mu\text{g}\cdot\text{mL}^{-1}$ for diluent and was approximately $0.0004 \mu\text{g}\cdot\text{mL}^{-1}$ for each of the diluted canine plasma samples. The LOQs for diluent and the 1:30 and 1:300 dilutions of canine plasma were $0.00086 \mu\text{g}\cdot\text{mL}^{-1}$, $0.0012 \mu\text{g}\cdot\text{mL}^{-1}$, and $0.0014 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. There were no significant ($P = 0.21$; ANOVA) differences between the OD_{450} s for diluent and canine plasma diluted 1:30 or 1:300. Therefore, the presence of canine plasma had no significant effect on the baseline measurement.

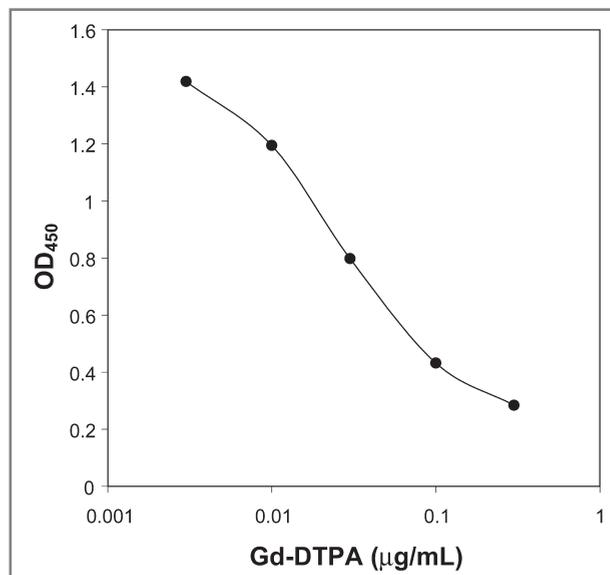


Figure 1—Standard curve (2 aliquots/standard) used to determine Gd-DTPA concentrations in canine plasma samples.

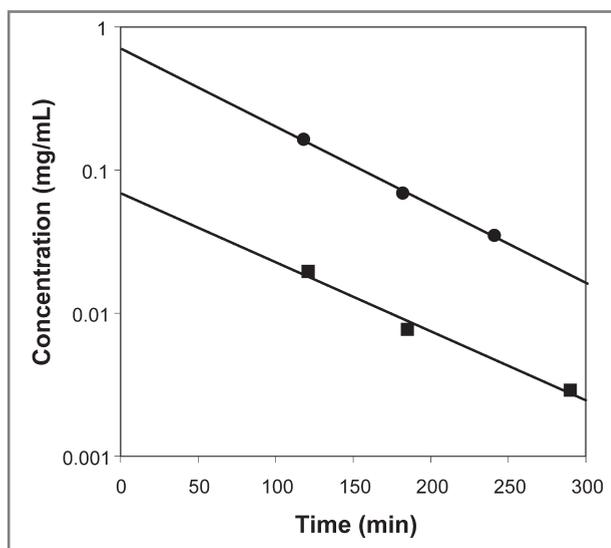


Figure 2—A comparison of the plasma disappearance curves for iohexol (circles) and Gd-DTPA (squares) for 1 dog. The slopes for iohexol and Gd-DTPA were $0.0126 \text{ minutes}^{-1}$ and $0.0155 \text{ minutes}^{-1}$, respectively. The Gd-DTPA was injected as a bolus, whereas iohexol was injected during a 5-minute period. Time 0 was designated as the end of each respective injection. Serum samples were diluted at 1:400 prior to analysis for Gd-DTPA concentration.

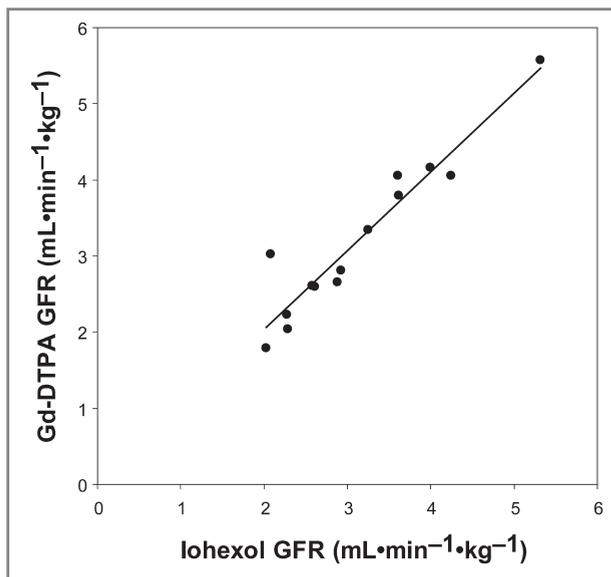


Figure 3—Plot of GFRs in 14 dogs determined by plasma clearances of Gd-DTPA versus iohexol. The linear regression equation is $y = 1.03x - 0.025$ and revealed a good correlation ($r^2 = 0.90$).

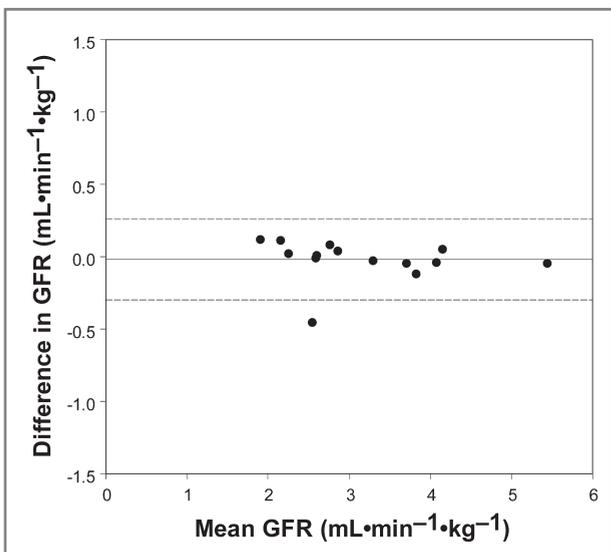


Figure 4—Plot of difference in GFRs in 14 dogs determined by plasma clearances of Gd-DTPA and iohexol versus the mean GFR $[(\text{Gd-DTPA GFR} + \text{iohexol GFR})/2]$ for each dog. The solid line represents the mean difference between Gd-DTPA and iohexol GFRs ($-0.02 \pm 0.28 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and dashed lines represent the 95% limits of agreement as described by the mean difference $\pm 2 \text{ SD}$.

Accuracy and precision of the Gd-DTPA ELISA—

The calculated mean \pm SD and CV for the low and high concentrations for the intra-assay (low, $0.0069 \pm 0.0003 \mu\text{g}\cdot\text{mL}^{-1}$ and 3.3%; high, $0.0548 \pm 0.0025 \mu\text{g}\cdot\text{mL}^{-1}$ and 4.3%) and interassay (low, $0.0070 \pm 0.0001 \mu\text{g}\cdot\text{mL}^{-1}$ and 1.3%; high, $0.0562 \pm 0.0026 \mu\text{g}\cdot\text{mL}^{-1}$ and 4.6%) replicate analyses were determined. Intra-assay accuracy and precision were $101.2 \pm 6.8\%$ and 3.3% for the low concentration and $98.8 \pm 8.6\%$ and 4.3% for the high concentration. The interassay accuracy and precision were $101.4 \pm 6.4\%$ and 3.2% for the low concentration and $101.1 \pm 6.6\%$ and 3.2% for the high concentration.

Table 1—The GFRs determined by use of Gd-DTPA plasma clearance for serial analysis of serum samples from the same dog that were stored at 4°C for various intervals.

Day	GFR ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Difference (%)
0	4.05	NA
1	3.94	-3.0
7	4.04	-0.2
14	3.77	-7.0
41	3.98	-2.0
61	3.96	-2.0
120	3.79	-6.0

NA = Not applicable.

Disappearance curves—Iohexol and Gd-DTPA disappearance curves for 1 representative dog were plotted (Figure 2). The equation for iohexol disappearance was $y = 0.1305 \times e^{-0.0155x}$ and the equation for Gd-DTPA disappearance was $y = 0.7105 \times e^{-0.0126x}$. Paired *t* tests comparing slopes of disappearance curves for iohexol and Gd-DTPA revealed a significant ($P = 0.008$) difference between the 2 methods.

GFR values—The GFRs determined by plasma iohexol clearance ranged from 2.02 to $5.32 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. The GFRs determined by plasma Gd-DTPA clearance ranged from 1.79 to $5.57 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. A strong linear correlation ($r^2 = 0.90$) existed between GFRs derived from the plasma clearance of Gd-DTPA and those derived from the plasma clearance of iohexol (Figure 3). There was no significant ($P = 0.42$) difference between Gd-DTPA GFRs and those obtained with iohexol.

The data were also compared by use of limits of agreement analysis. A limits of agreement plot comparing the differences in GFR versus the mean GFR between the clearance methods was created (Figure 4). The distribution around the mean difference revealed no significant linear correlation ($r^2 = 0.06$) in these data. The difference between GFRs determined by the plasma clearances of iohexol and Gd-DTPA was $-0.02 \pm 0.28 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. All of the difference values, except for 1, were within 2 SDs of the mean difference (95% limits of agreement). Almost all (13/14) dogs had Gd-DTPA GFRs that were within 12% of the iohexol GFR. The remaining dog had a Gd-DTPA GFR that was 45% higher than the iohexol GFR.

Stability of Gd-DTPA in serum—The stability of Gd-DTPA in serum was tested by using the ELISA to determine GFR on the same samples from 1 dog after storage at 4°C for 1, 7, 14, 41, 61, and 120 days. All GFRs were within 7% of the original value (Table 1), which indicated good stability of Gd-DTPA.

Discussion

The study reported here revealed that the plasma clearance of Gd-DTPA, as measured by a commercial ELISA kit, is an effective method to estimate GFR in dogs. Good correlation ($r^2 = 0.90$) was evident when comparing GFRs determined by plasma clearance of Gd-DTPA with those determined by plasma clearance of iohexol (Figure 3). However, the strength of correlation describes the relation between 2 variables and not necessarily their agreement.¹⁷ To assess agreement,

it is better to compare the differences between associated values. This can be accomplished by calculating the bias of the test being evaluated against an accepted standard test, in this case the GFR determined by plasma clearance of Gd-DTPA versus the GFR determined by plasma clearance of iohexol. Bias is defined as the mean difference \pm 2 SD. This is expressed graphically as a limits of agreement plot.

In evaluating a limits of agreement plot, it is important to determine whether the mean and SD are acceptable and constitute good agreement between methods. In this case, the spread of data as described by the bias represented good agreement between the Gd-DTPA and iohexol plasma clearance methods because almost all of the differences were within \pm 2 SD. Another way to examine the data was in terms of the percentage differences among the GFR values obtained by use of the Gd-DTPA and iohexol plasma clearance methods. The GFRs determined by Gd-DTPA plasma clearance were within 12% of those determined by iohexol plasma clearance for all but 1 dog, which indicated good agreement. The remaining dog had a Gd-DTPA GFR that was 45% higher than the iohexol GFR, which indicated poor agreement. This is the same dog whose data point was outside the 95% limits of agreement (Figure 4). Possible explanations for this aberrant value include normal variation, laboratory error, or different processing of the filtration marker by that particular dog.

Iohexol was administered during a 5-minute period to minimize patient discomfort associated with the large volume ($1 \text{ mL} \cdot \text{kg}^{-1}$) and viscosity of the administered dose. The administered dose of Gd-DTPA was much smaller ($0.1 \text{ mL} \cdot \text{kg}^{-1}$) and thus given as a bolus. The extent to which this difference in administration biased the results is unknown, but it appeared negligible given the excellent correlation in GFRs obtained between iohexol and Gd-DTPA plasma clearance methods.

Assessment of GFR by Gd-DTPA plasma clearance has good agreement with the clinical standard and other advantages as well. The lack of an accessible test is a major impediment for measuring GFR in dogs in a clinical setting. The test most commonly used, plasma clearance of iohexol, requires the use of HPLC for measurement of plasma iohexol concentration. However, the HPLC assay is currently performed at only 1 laboratory in the United States, requires shipping of blood, and is expensive. The commercial ELISA kit for assay of plasma Gd-DTPA concentrations is more accessible and less expensive than HPLC or other methods currently used for quantification of GFR markers. The overall performance of the ELISA, in terms of accuracy and precision, is comparable with that of competing technologies. Additionally, the LOQ and LOD values indicate that this method has improved sensitivity for the measurement of Gd-DTPA, as compared with sensitivity of assays for other markers. This may allow a smaller dose of Gd-DTPA, thereby providing an additional margin of safety. Virtually all diagnostic laboratories use a variety of ELISAs on a daily basis and are relatively proficient with use of this technique. It should be simple for diagnostic laboratories to institute use of the ELISA kit described in the study reported here for the analysis of plasma Gd-DTPA concentrations, thereby greatly

facilitating measurement of GFR in dogs. Clearance of Gd-DTPA may also provide an alternative method to accurately measure GFR in other animal species.

Stability of Gd-DTPA in serum is another advantage of this method. Only a small variation was detected among GFRs obtained from initial analysis of samples from 1 dog and from repeat analyses on the same samples stored at 4°C for up to 120 days. These subsequent GFRs were within 7% of the initial value. This indicated that serum may be stored prior to analysis, which is often necessitated by convenience or shipping requirements. This stability also allows batch analysis of multiple samples, which is more efficient than processing individual samples.

Historically, contrast agents containing gadolinium have been considered safe and cause minimal adverse reactions, compared with effects after administration of iodinated contrast agents.¹⁸ Recently, high-dose administration of gadolinium-containing compounds to people with end-stage renal failure has been associated with an increased risk of developing NSF, a rare and sometimes fatal skin disorder.¹⁹⁻²² Although the exact mechanism of gadolinium in the pathogenesis of NSF is not fully understood, it is thought that free gadolinium (Gd^{3+}) plays a role.^{23,24} Most cases of NSF involve the use of gadodiamide,^{19,20} but there are a few NSF cases in which Gd-DTPA was the contrast agent.^{21,22} In addition, some cases of NSF have been reported in people who had no exposure to gadolinium.²⁵ Although a causative role has not been definitively established, the potential link prompted the US FDA to issue a public health advisory regarding the use of all gadolinium-containing contrast agents in humans.²⁶

To our knowledge, NSF related to gadolinium administration has not been reported in dogs. In this study, no adverse effects were detected that could be definitively attributed to Gd-DTPA. Two dogs vomited during iohexol injection. However, because iohexol was administered immediately after Gd-DTPA, it could not be determined whether the vomiting was attributable to iohexol, Gd-DTPA, a combined effect of these substances, or an unrelated cause.

Analysis of results of this study indicated that Gd-DTPA can be used as a filtration marker to estimate GFR in dogs. This is supported in that the GFRs obtained by plasma clearance of Gd-DTPA compared favorably with those obtained by plasma clearance of iohexol. In addition, the commercial ELISA kit used in measuring plasma Gd-DTPA concentrations was simple and easy to use and can be performed in most laboratories. Additional studies are necessary to evaluate the use of Gd-DTPA to estimate GFR in dogs with known renal disease and in other animal species for both research and clinical purposes.

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