IDEXX FGF-23 Test: a new tool for use in the management of chronic kidney disease in cats

Introduction and Background

Chronic kidney disease (CKD) affects an increasing percentage of cats as they age, reported as 0.1% of cats less than 9 years old but between 30%–40% above age 10, and the percentage is as high as 80% of cats over the age of 15.[1–3] CKD causes significant morbidity and mortality in the older cat population.[4] Kidneys are essential for phosphate homeostasis. As CKD develops and a decline in glomerular filtration rate (GFR) occurs, phosphorus concentration increases, which causes an imbalance in phosphate-calcium homeostasis.[5] This is labeled chronic kidney disease–metabolic bone disease (CKD-MBD; also referred to as mineral bone disorder) and in totality describes a complex syndrome that involves fibroblast growth factor 23 (FGF-23), parathyroid hormone (PTH), 1,25-dihydroxy D_3 (1,25 vitamin D_3, calcitriol), calcium, and phosphorus (figure 1).[6] CKD-MBD leads to chronically elevated FGF-23 concentrations in most patients. There is strong clinical evidence from both human and veterinary literature that FGF-23 identifies mineral disruption and phosphorus overload (CKD-MBD) earlier than total serum phosphorus and is a valuable tool in the management of cats with CKD.[7–10]

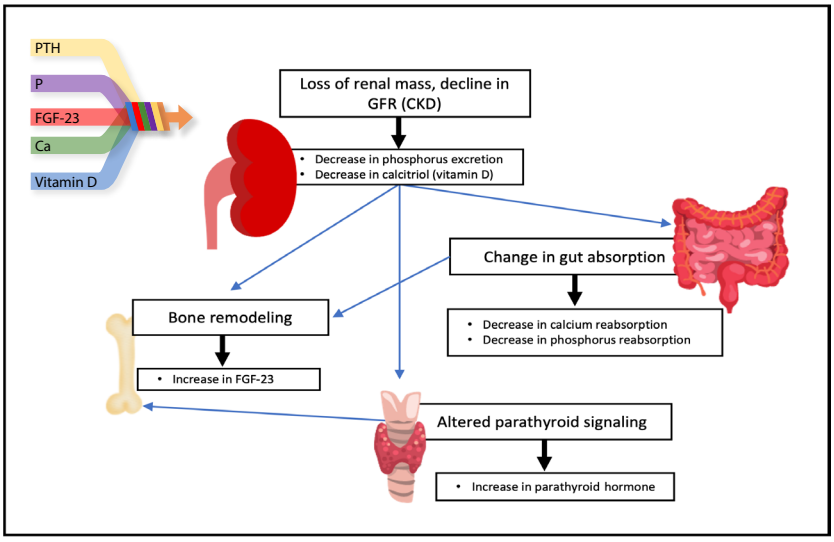

Figure 1: Simplified diagram of FGF-23 physiology in CKD. Loss of GFR leads to a decrease in phosphorus excretion and calcitriol production, leading to bone remodeling and increases in circulating FGF-23. Mineral imbalances in calcium and phosphorus alter gut metabolism and mineral reabsorption, which further promote metabolic bone disease. Due to decreased calcium absorption, a secondary increase in PTH is eventually seen, which leads to secondary renal hyperparathyroidism.

The IDEXX FGF-23 Test can be used in cats with IRIS* CKD Stages 1 and 2 to determine the need for targeted therapy, such as dietary management to reduce phosphate intake or phosphate binders. There is no current gold standard for measurement of FGF-23. Published assays for FGF-23 include sandwich enzyme-linked immunosorbent assays (ELISA) that measure either the C-terminal fragment of FGF-23 or intact FGF-23. The IDEXX FGF-23 Test measures intact FGF-23, which is more accurate in cats.[11] This assay has been validated in published research and by IDEXX for use in cats.[7,9,16]

Methods and results

With the IDEXX FGF-23 Test, FGF-23 is measured using a sandwich ELISA. The test was validated in feline serum by evaluating precision, accuracy, potential sample interferents, and sample stability.

Mean FGF-23 (pg/mL)	% CV	n	Mean FGF-23 (pg/mL)	% CV	n
Lot A			Lot B		
58	45	72	45	65	60
218	10	72	221	16	72
539	8	72	514	9	72
688	7	72	687	11	72
1,345	8	72	1,399	12	72
1,056	10	72	1,048	15	72

Table 1: % CV for two lots evaluating FGF-23 precision across clinical range.

Precision

Precision was measured using feline serum samples containing native analyte or calibration buffer spiked with recombinant human FGF-23. Serum samples were diluted 5 times in calibration buffer, and FGF-23 was measured over two kit lots (A and B) by two operators over 3 days to determine precision (table 1). The assay had good precision (< 15% CV) at concentrations of 300 pg/mL (limit of quantification) and higher (table 1).

Accuracy

Accuracy was measured across the full range of expected feline serum FGF-23 concentrations. There is no gold standard FGF-23 method with which to compare the measured FGF-23 concentration, so accuracy was determined by comparing actual FGF-23 to the expected FGF-23 concentration. Feline serum samples with different expected concentrations of FGF-23 were generated by mixing feline serum samples with high or low FGF-23 concentrations in different ratios. FGF-23 was measured 8 times at each expected concentration. A linear regression was used to compare the mean-corrected FGF-23 concentration for all replicates against the expected concentration. There was good dilutional linearity across the expected biological range (slope = 1.07, r^2 = 0.99) (figure 2).

To calculate recovery, the mean values of each dilution were compared to the expected values. Recovery was acceptable (± 20%) for all samples with expected FGF-23 concentrations > 235 pg/mL (lower limit of quantification 300 pg/mL) (table 2).

Interference

The potential for interference due to hemolysis, lipemia, and icterus was evaluated in native human serum samples. Five interferent concentrations of each analyte were tested, ranging from 0–500 mg/dL for hemolysis, 0–3,000 FTU for lipemia, and 0–50 mg/dL for icterus. No clinically relevant interference was seen from hemolysis, lipemia, or icterus in the concentrations tested.

Sample stability

The stability of FGF-23 in feline serum was measured in samples held at 4°C for 14 days. Six feline serum samples were aliquoted and frozen at -80°C (Day 0) or stored at 4°C for 14 days (Day 14). FGF-23 was measured in parallel for both aliquots in undiluted serum samples, samples 2 and 5 were below the limit of quantification. Storage for 14 days at 4°C did not decrease FGF-23 recovery (table 3).

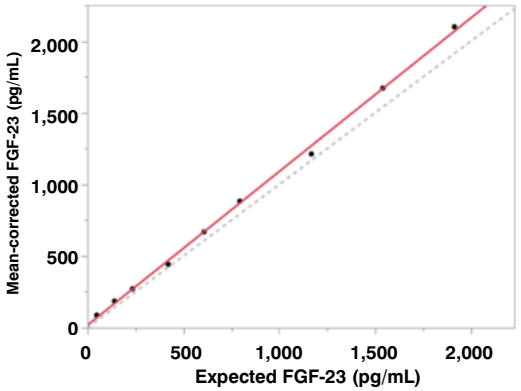

Figure 2: Regression plot showing the relationship of mean-corrected FGF-23 concentration measured in 8 replicates compared to the expected FGF-23 concentration.

Sample number	Percent sample	N rows	Mean	Expected FGF-23 (pg/mL)	Percent recovery to expected
1	0	8	87	48	182%
2	0.05	8	186	141	131%
3	0.1	8	271	235	116%
4	0.2	8	443	421	105%
5	0.3	8	668	608	110%
6	0.4	8	883	794	111%
7	0.6	8	1212	1167	104%
8	0.8	8	1671	1540	109%
9	1	8	2100	1913	110%

Table 2: Percent recovery of FGF-23 at different expected concentrations.

Sample number	FGF-23 (pg/mL)		Percent recovery
	Day 0	Day 14	Day 14
1	128	131	100%
2	30	48	157%
3	86	90	104%
4	196	188	96%
5	45	76	169%
6	157	160	102%

Table 3: Percent recovery of FGF-23 in six undiluted feline serum samples stored at 4°C for 14 days.

	Assessment		
Criteria evaluated	**Clinically healthy (non-CKD)**	**IRIS CKD Stage 1**	**IRIS CKD Stage 2**
SDMA (µg/dL)	≤ 14	15–18	> 19
Creatinine (mg/dL)	< 2.3	< 1.6	1.6–2.8
Urine specific gravity	> 1.035	≤ 1.035	≤ 1.035
Other criteria	Normal physical exam, > 1 year of age, no history of UTI, or major illness within 6 months of exam; normal lab work (CBC, chemistry, urinalysis and UPC); commercial diet	No physical exam or history available; > 1 year of age, no major illness; no inflammation identified in serial lab work (CBC, chemistry, urinalysis)	No physical exam or history available; > 1 year of age, no major illness; no inflammation identified in serial lab work (CBC, chemistry, urinalysis)

Table 4: Description of IRIS CKD Staging Guidelines used to determine inclusion and exclusion criteria for clinically healthy and CKD cats' samples used to evaluate FGF-23 concentrations.

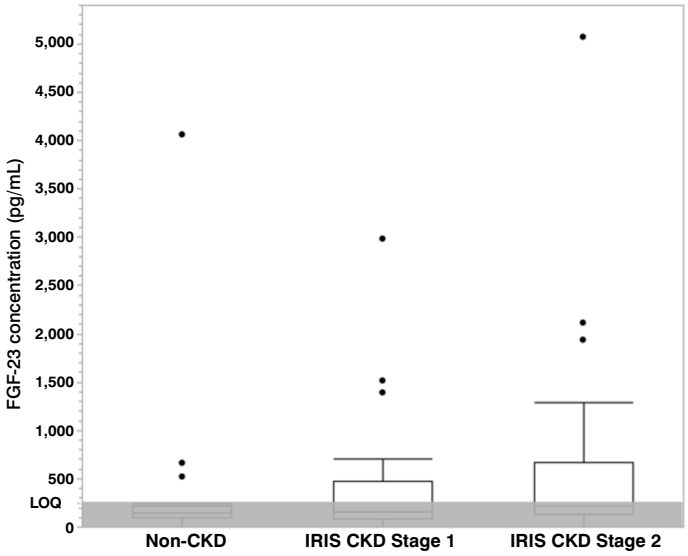

Figure 3: Box plots show the distribution of FGF-23 values in non-CKD, IRIS CKD Stage 1, and IRIS CKD Stage 2 cats. The central line represents the mean, edges of the box represent 25th and 75th percentiles, whiskers represent 95% confidence intervals, and dots represent outliers. FGF-23 concentrations increased with higher IRIS CKD stage but there was overlap between groups.

FGF-23 in cats with normal kidney function and cats with IRIS CKD Stages 1 and 2

Feline serum samples for healthy cats were collected prospectively through enrolled clinical practices. Feline serum samples for cats with CKD were collected from routine customer submissions to IDEXX Reference Laboratories; no clinical information was available. Clinical biochemistry and hematology results were independently reviewed by two veterinarians to classify results (table 4). Cats whose laboratory findings were consistent with CKD were then staged based on IRIS CKD staging guidelines. Staging was determined by either including two consecutive findings of SDMA and creatinine at least 14 days apart and/or trended increases of > 0.3 mg/dL of creatinine and/or > 5 µg/dL in SDMA concentration at least 14 days apart. Cats with results indicating hyperthyroidism, anemia, and/or an inflammatory leukogram were excluded from the study; previous studies suggest these comorbidities may impact FGF-23 concentrations.[12,13]

FGF-23 trended higher in CKD cats versus clinically healthy cats as well as in cats with IRIS CKD Stage 2 versus IRIS CKD Stage 1 (figure 3).[14] FGF-23 was below the limit of quantification for most healthy cats. An increasing proportion of cats with laboratory findings consistent with IRIS CKD Stages 1 and 2 had FGF-23 concentrations > 300 pg/mL. There was substantial overlap between the expected FGF-23 in healthy and CKD cats, which is consistent with the current understanding of metabolic bone disease, which is not correlated to the severity of kidney biomarkers in cats with CKD and which has a variable onset.

Interpretive ranges

There are three clinical ranges for the IDEXX FGF-23 Test:

≤ 299 pg/mL	Normal	FGF-23 is within expected range for normal cats. For cats with IRIS CKD Stage 1 or 2, recommend rechecking with the IDEXX FGF-23 Test in 6–12 months alongside kidney biomarkers to identify progressive disease or onset of phosphorus overload.
300–399 pg/mL	Borderline	This result is higher than expected for normal cats and most cats with IRIS CKD Stage 1 or 2. In cats with diagnosed CKD, recommend rechecking with the IDEXX FGF-23 Test in 3–6 months alongside kidney biomarkers to identify onset of phosphorus overload. If indicated by clinical context and/or other kidney diagnostics, targeted therapies (such as diet changes) should be initiated.
≥ 400 pg/mL	Increased	Elevated result indicating phosphorus overload. Targeted therapy to reduce phosphorus concentrations should be added to existing CKD therapies.

Results that are less than 299 pg/mL will not be reported as discrete numerical values, but as < 299 pg/mL. Values 300 pg/mL–4,000 pg/mL will be reported as numerical values.

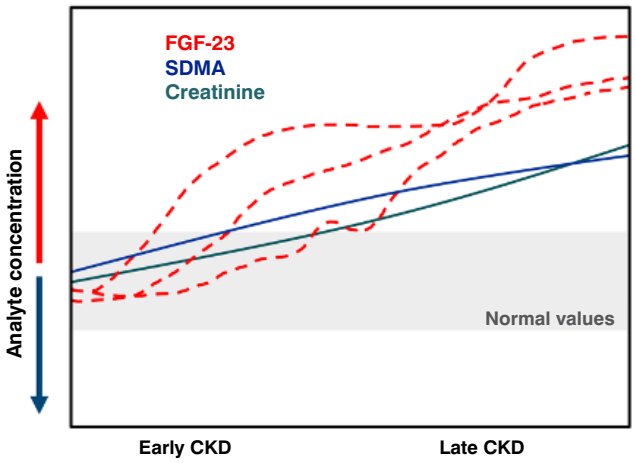

Figure 4: Potential behavior of FGF-23 in relation to traditional kidney biomarkers. FGF-23 does not have a linear relationship with kidney biomarkers in early-stage CKD due to variable onset of metabolic bone disease.

Clinical use and application

The IDEXX FGF-23 Test provides a new tool for use in the management of chronic kidney disease. It is recommended for use in cats diagnosed with IRIS CKD Stage 1 or 2. While extremely valuable for identification of MBD in early-stage kidney disease, it should not be used to diagnose CKD. The nonlinear relationship with CKD biomarkers, including SDMA and creatinine (figure 4), is expected in cats, given variation in CKD etiology and external influences (diet or other comorbidities). Some cats will have notable MBD-CKD early in CKD and others will have delayed onset of MBD-CKD. Below 4.6 mg/dL, total phosphorus does not accurately portray phosphorus balance due to myriad compensatory mechanisms. In contrast, FGF-23 can be used in early-stage kidney disease when total phosphorus is below 4.6 mg/dL to identify cats who are expected to benefit from phosphate reduction through diet or medication.

Therefore, the IDEXX FGF-23 Test can be used for kidney management after CKD diagnosis to monitor the response to phosphate reduction therapies. Based on previously published literature, it is expected that FGF-23 concentrations will remain stable or decline with phosphate reduction.[15–17] FGF-23 monitoring is recommended in cats with IRIS CKD Stage 1 and 2 disease; for cats with initial FGF-23 concentrations within normal limits or borderline, retest every 6 months. For cats being treated with phosphate restriction, test at 3 months postintervention and then every 6 months with other kidney biomarkers for long-term monitoring.

Conclusion

The IDEXX FGF-23 Test offers evidence-based results to guide targeted therapy for cats with early-stage CKD. Currently, IRIS guidelines recommend a therapeutic renal diet for cats with IRIS CKD Stage 2, but IRIS CKD Stage 2 is a wide category (creatinine of 1.6–2.8 mg/dL and SDMA of 18–25 µg/dL) and does not account for cats in IRIS CKD Stage 1 who might benefit from early diet change. CKD-MDB impacts the systemic and kidney health of a cat before clinical signs are apparent. The IDEXX FGF-23 Test allows the clinician to exercise their clinical acumen, combining the IDEXX FGF-23 Test with physical exam and history, traditional kidney biomarkers (SDMA and creatinine) and urinalysis, providing the pet owner an additional piece of reinforcement and motivation to begin phosphate reduction intervention, such as diet and/or phosphate binding medications. Early diet modification in chronic kidney disease has been shown to reduce total phosphorus and improve survival time of cats.[18]

References

1. Conroy M, Brodbelt DC, O'Neill D, Chang YM, Elliott J. Chronic kidney disease in cats attending primary care practice in the UK: a VetCompass study. *Vet Rec*. 2019;184(17):526. doi:10.1136/vr.105100
2. Marino CL, Lascelles BD, Vaden SL, Gruen ME, Marks SL. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. *J Feline Med Surg*. 2014;16(6):465–472. doi:10.1177/1098612X13511446
3. Sparkes AH, Caney S, Chalhoub S, et al. ISFM consensus guidelines on the diagnosis and management of feline chronic kidney disease. *J Feline Med Surg.* 2016;18(3):219–239. doi:10.1177/1098612X16631234
4. White JD, Malik R, Norris JM. Feline chronic kidney disease: can we move from treatment to prevention? *Vet J*. 2011;190(3):317–322. doi:10.1016/j.tvjl.2010.12.011
5. Slatopolsky E. The intact nephron hypothesis: the concept and its implications for phosphate management in CKD-related mineral and bone disorder. *Kidney Int Suppl*. 2011;79(121):S3–S8. doi:10.1038/ki.2011.23
6. Moe S, Drüeke T, Cunningham J, et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2006;69(11):1945–1953. doi:10.1038/sj.ki.5000414
7. Finch NC, Geddes RF, Syme HM, Elliott J. Fibroblast growth factor 23 (FGF-23) concentrations in cats with early nonazotemic chronic kidney disease (CKD) and in healthy geriatric cats. *J Vet Intern Med*. 2013;27(2):227–233. doi:10.1111/jvim.12036
8. Geddes RF, Elliott J, Syme HM. Relationship between plasma fibroblast growth factor-23 concentration and survival time in cats with chronic kidney disease. *J Vet Intern Med*. 2015;29(6):1494–1501. doi:10.1111/jvim.13625
9. Geddes RF, Finch NC, Elliott J, Syme HM. Fibroblast growth factor 23 in feline chronic kidney disease. *J Vet Intern Med*. 2013;27(2):234–241. doi:10.1111/jvim.12044
10. Seiler S, Heine GH, Fliser D. Clinical relevance of FGF-23 in chronic kidney disease. *Kidney Int Suppl*. 2009;76(114):S34–S42. doi:10.1038/ki.2009.405
11. Heijboer AC, Levitus M, Vervloet MG, et al. Determination of fibroblast growth factor 23. *Ann Clin Biochem*. 2009;46(Pt 4):338–340. doi:10.1258/acb.2009.009066
12. Williams TL, Elliott J, Berry J, Syme HM. Investigation of the pathophysiological mechanism for altered calcium homeostasis in hyperthyroid cats. *J Small Anim Pract*. 2013;54(7):367–373. doi:10.1111/jsap.12102
13. Edmonston D, Wolf M. FGF23 at the crossroads of phosphate, iron economy and erythropoiesis. *Nat Rev Nephrol*. 2020;16(1):7–19. doi:10.1038/s41581-019-0189-5
14. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.
15. Lin J, Lin L, Chen S, Yu L, Chen S, Xia Z. Serum fibroblast growth factor 23 (FGF-23): associations with hyperphosphatemia and clinical staging of feline chronic kidney disease. *J Vet Diagn Invest*. 2021;33(2):288–293. doi:10.1177/1040638720985563
16. Geddes RF, Elliott J, Syme HM. The effect of feeding a renal diet on plasma fibroblast growth factor 23 concentrations in cats with stable azotemic chronic kidney disease. *J Vet Intern Med*. 2013;27(6):1354–1361. doi:10.1111/jvim.12187
17. Schauf S, Coltherd JC, Atwal J, et al. Clinical progression of cats with early-stage chronic kidney disease fed diets with varying protein and phosphorus contents and calcium to phosphorus ratios. *J Vet Intern Med*. 2021;35(6):2797–2811. doi:10.1111/jvim.16263
18. Elliott J, Rawlings JM, Markwell PJ, Barber PJ. Survival of cats with naturally occurring chronic renal failure: effect of dietary management. *J Small Anim Pract*. 2000;41(6):235–242. doi:10.1111/j.1748-5827.2000.tb03932.x

*IRIS is the International Renal Interest Society.