Feline renal biomarkers: from reference intervals to clinical application

Rebekah Mack DVM, DACVIM-SAIM, IDEXX Medical Affairs Specialist

Objectives

- Understanding the development and utilization of a reference interval
- Understanding the difference between use of a reference interval for evaluating change in renal function and applying the IRIS CKD staging guidelines
- Exploring additional renal biomarkers to improve diagnosis of kidney disease
- Discussing feline kidney disease and concurrent conditions
- Exploring a case example

Understanding the development and utilization of a reference interval

Timeline of the development of reference intervals

1969 First introduced in human medicine in

Healthy Human Populations + Disease-State Human Populations

Healthy Human Populations

1977 Human guidelines established CLSI

1978 First comprehensive veterinary publication

1996 established American Society for Veterinary Clinical Pathology

2001 established ECVCP

Healthy Veterinary Populations

1) Gra¨sbeck R, Saris NE. Establishment and use of normal values. Scand J Clin Lab Invest. 1969;26:S62–S63.
2) Lumsden JH, Mullen K. On establishing reference values. Can J Comp Med. 1978;42:293–301.
3) Friedrich K, Harr K, Freeman K, et al. ASCVP reference interval guidelines: determination of de novo reference intervals in the veterinary species and other related topics. *Vet Clin Path* 2012; 41; 441-453

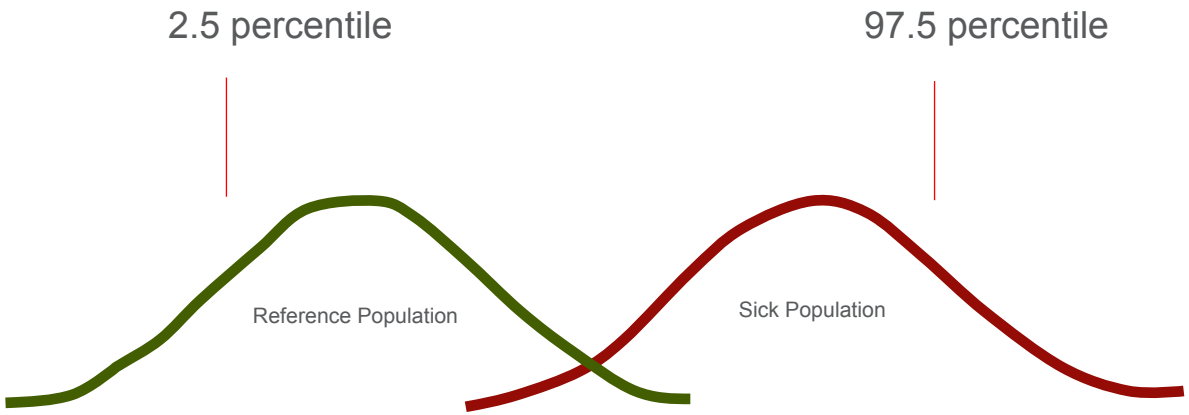
IDEXX

Definition of a reference interval

Definition: “is an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others.”

Mechanics of reference interval (RI):

- No RI is completely “right” or “wrong.”
- The majority of RIs in use today refer to the central 95% of the reference population of subjects.
- By definition, 5% of all results from “healthy” individuals will fall outside of the reported RI and, as such, will be flagged as being “abnormal.”



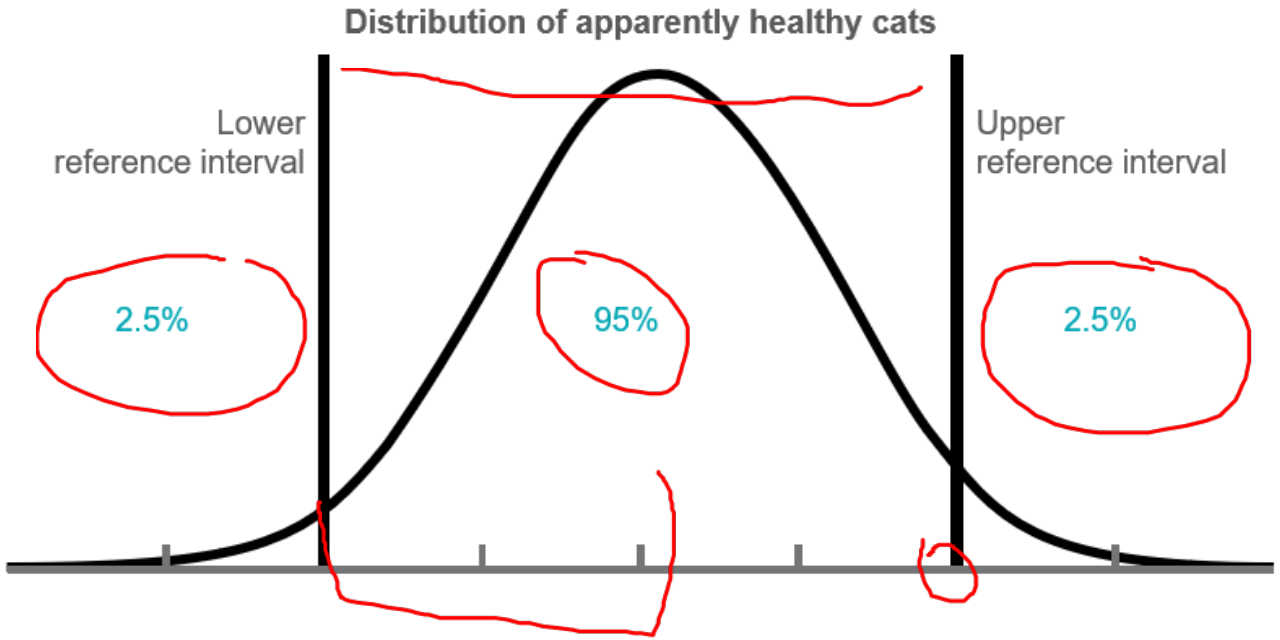
1) https://acutecaretesting.org/en/articles/reference-intervals-and-percentiles-implications-for-the-healthy-patien
2) Sunderman FW Jr. Current concepts of “normal values,” “reference values,” and “discrimination values” in clinical chemistry. Clin Chem. 1975;21:1873–1877.
3) Ekelund, Suzanne. Reference Intervals and percentiles – implications for the healthy patient. Acutecaretesting.org

How do you “develop” a reference interval?

Population

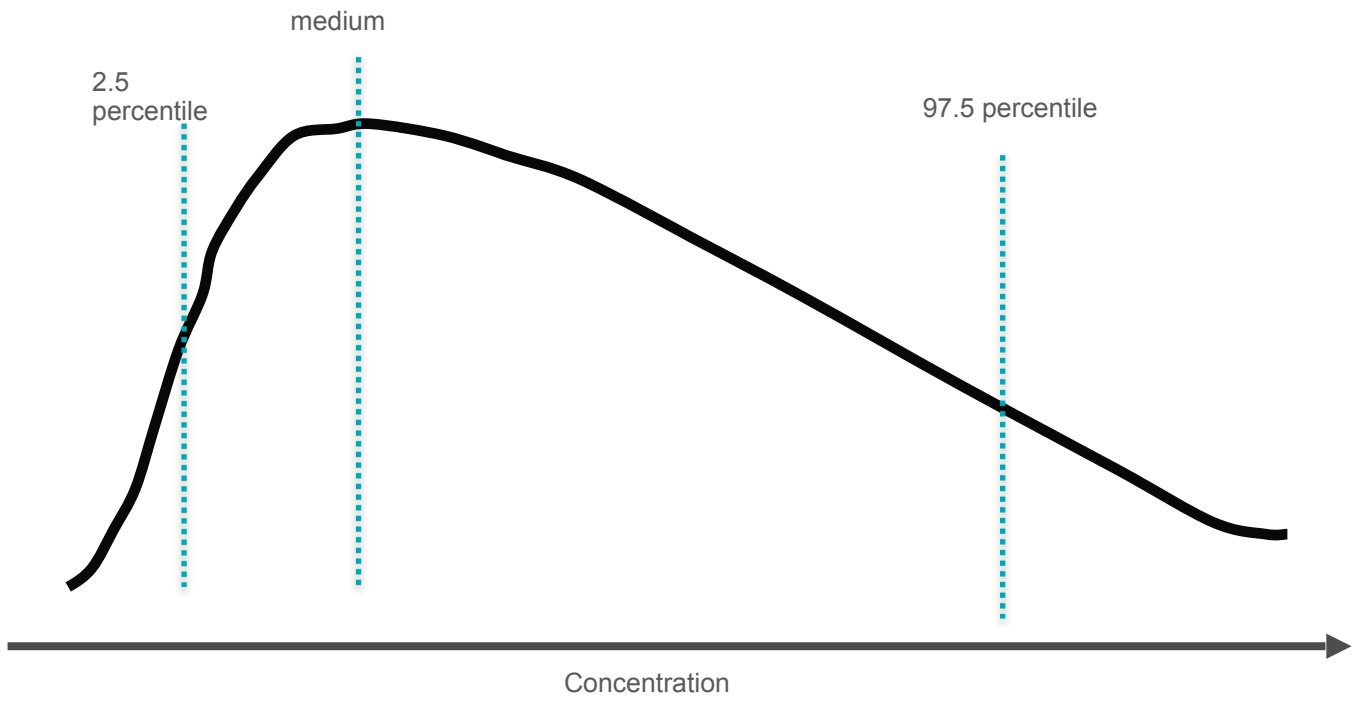
- 120 clinically healthy individuals
- Equal sex representation
- Geographic representation
- Appropriate age (>1 year)

Calculate using **nonparametric** methods



1) Ekelund, Suzanne. Reference Intervals and percentiles – implications for the healthy patient. Acutecaretesting.org
2) Ceriotti F, Hinzmann r, Panteghini m. Reference Intervals: the way forward. *Ann Clin Biochem* 2009;46:8-17
3) Friedrich K, Harr K, Freeman K, et al. ASCVP reference interval guidelines: determination of de novo reference intervals in the veterinary species and other related topics. *Vet Clin Path* 2012; 41; 441-453

In case you forgot…



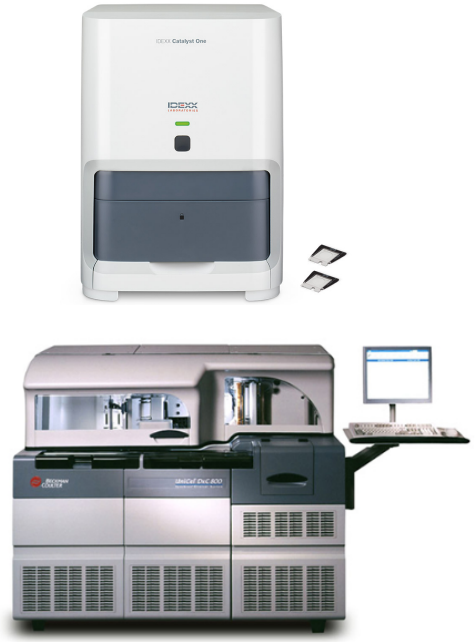
Nonparametric data:

- Does not have a normal distribution (not a perfect bell curve)
- Better for large data sets
- Considers outliers without having to remove them

1) Ekelund, Suzanne. Reference Intervals and percentiles – implications for the healthy patient. Acutecaretesting.org
2) Geffre A, Friedrichs K, Harr K, et al. Reference values: a review. *Vet Clin Ptath 2009: 38; 288-298*

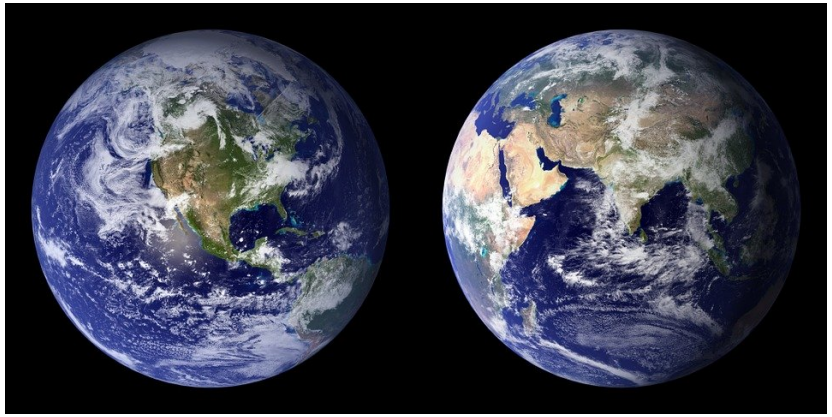
How do you “develop” a reference interval?

Most universities, and commercial labs develop independent reference intervals unique to them.



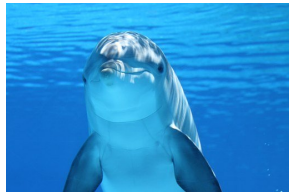
Analyzers

Reagent & Methodology



Population & Geography

Veterinary reference interval development can differ



Limitations:

- Unique species
- Sample quantities
- Individual samples

These limitations can lead to the use of different statistical methods and less “robust” reference intervals

Friedrich K, Harr K, Freeman K, et al. ASCVP reference interval guidelines: determination of de novo reference intervals in the veterinary species and other related topics. *Vet Clin Path* 2012; 41; 441-453

Benefits of Reference Intervals

Medical Decision Point – provide guidance for when to institute further testing or care

Can allow for a single moment in time (testing) to help determine the health of an individual

12/2/15	9:35 AM	
TEST	RESULT	REFERENCE VALUE
Glucose	119	74 - 143 mg/dL
Creatinine	1.2	0.5 - 1.8 mg/dL
BUN	16	7 - 27 mg/dL
BUN: Creatinine Ratio	13	
Sodium	151	144 - 160 mmol/L
Potassium	5.1	3.5 - 5.8 mmol/L
Na: K Ratio	30	
Chloride	120	109 - 122 mmol/L
Total Protein	5.4	5.2 - 8.2 g/dL
Albumin	3.2	2.3 - 4.0 g/dL
Globulin	**2.2**	**2.5 - 4.5 g/dL**
Albumin: Globulin Ratio	1.5	
ALT	**520**	**10 - 125 U/L**
ALP	86	23 - 212 U/L
Osmolality	303	mmol/kg

Leads to differentials and medical action

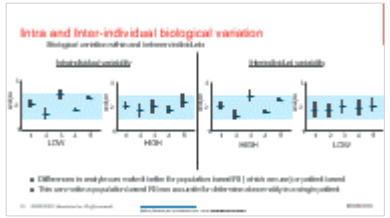
Downside of reference intervals

95% of the population represented – 5% will fall outside the confidence intervals

Population based vs Individual based - describe fluctuations in healthy populations or individuals which make establishing health status critical for interpretation

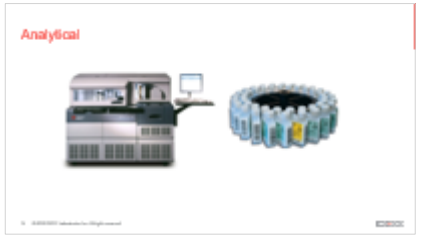
Difficult, time-consuming, and expensive to establish

Intra- and Interindividual biological variation



Preanalytical Aspects

Analytical Aspects

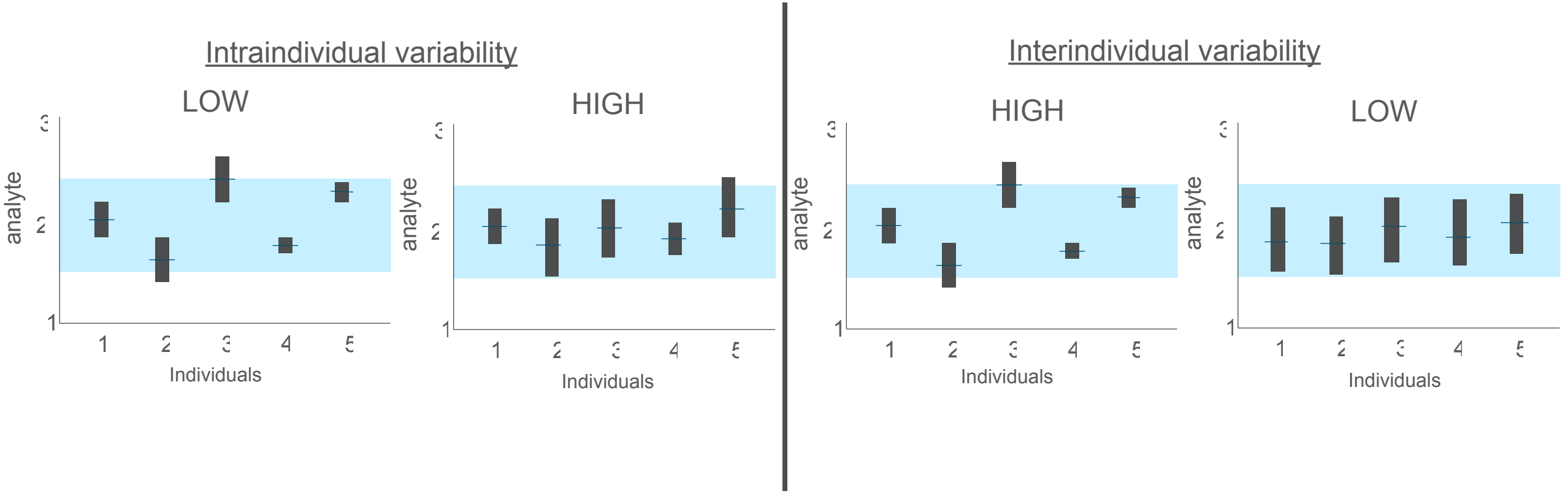


Calculations

Ceriotti F, Hinzmann r, Panteghini m. Reference Intervals: the way forward. *Ann Clin Biochem* 2009;46:8-17

Intra- and Interindividual biological variation

Biological variation within and between individuals



- Differences in analyte can make it better for population based RI (which we use) or patient based
- This can make a population based RI less accurate for determining abnormality in a single patient

1) http://eclinpath.com/test-basics/reference-intervals/
2) Geffre A, Friedrichs K, Harr K, et al. Reference values: a review. *Vet Clin Ptath 2009: 38; 288-298*

Preanalytical

- Samples must be from clinical practice

Subject Preparation	Methodological Factors: Specimen Collection	Methodological Factors: Specimen Collection
• Fasting vs non-fasting • Drug regimen • Physical activity	• Time of day • With or without tourniquet • Body posture • Anticoagulant, type • Sampling equipment • Interferents	• Transportation • Time before centrifugation • Storage before measurement

Analytical

Calculations

Proper statistical methods used for population and population distribution

Downside of individual reference intervals

- Often imperfect for evaluation of all patients
- Variability on how well an entire population can be represented within a RI
- Complex to properly establish
- Must be maintained as methodologies, analyzers, or patient populations change

Individual patient vs population reference interval

6 year old Male Domestic Shorthair

	3/9/17 2:52 PM	1/19/17 12:46 AM	12/9/16 12:25 AM	10/28/16 3:09 PM	9/23/16 3:20 PM	8/23/16 2:45 PM	5/20/16 12:45 AM
Creatinine (mg/dL)	1.8	1.7	1.6	1.7	2.0	2.1	2.3
(µmol/L)	159	150	141	150	176	185	203

Trending Up

Trending Down

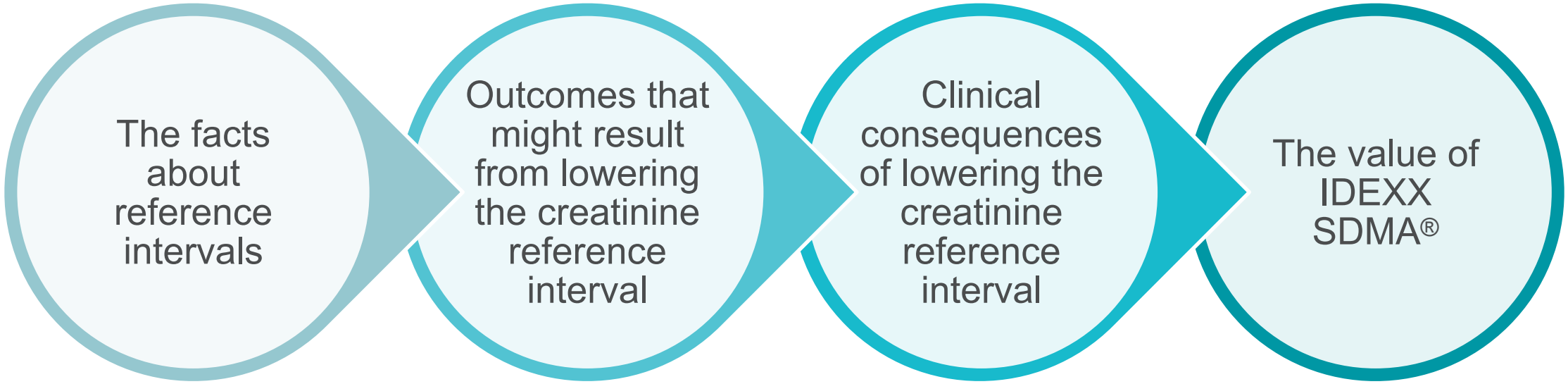
High Normal

Individual patient details:
Slow muscle and weight loss
Intermittent hyporexia

IDEXX SDMA (µg/dL)	aw 22	az 20	bc 19	bf 20	bi 22	bl 15

Application of Reference Interval vs Treatment Guideline: Creatinine as a clinical example

Diagnosis of kidney disease does not improve with lowering the creatinine reference interval—let us show you why



Reference intervals are determined for each laboratory test by universal standards protocols

IDEXX follows regulatory standards to establish reference intervals, specifically the **Clinical and Laboratory Standards Institute**.[1]

Determinations study **clinically healthy populations**.

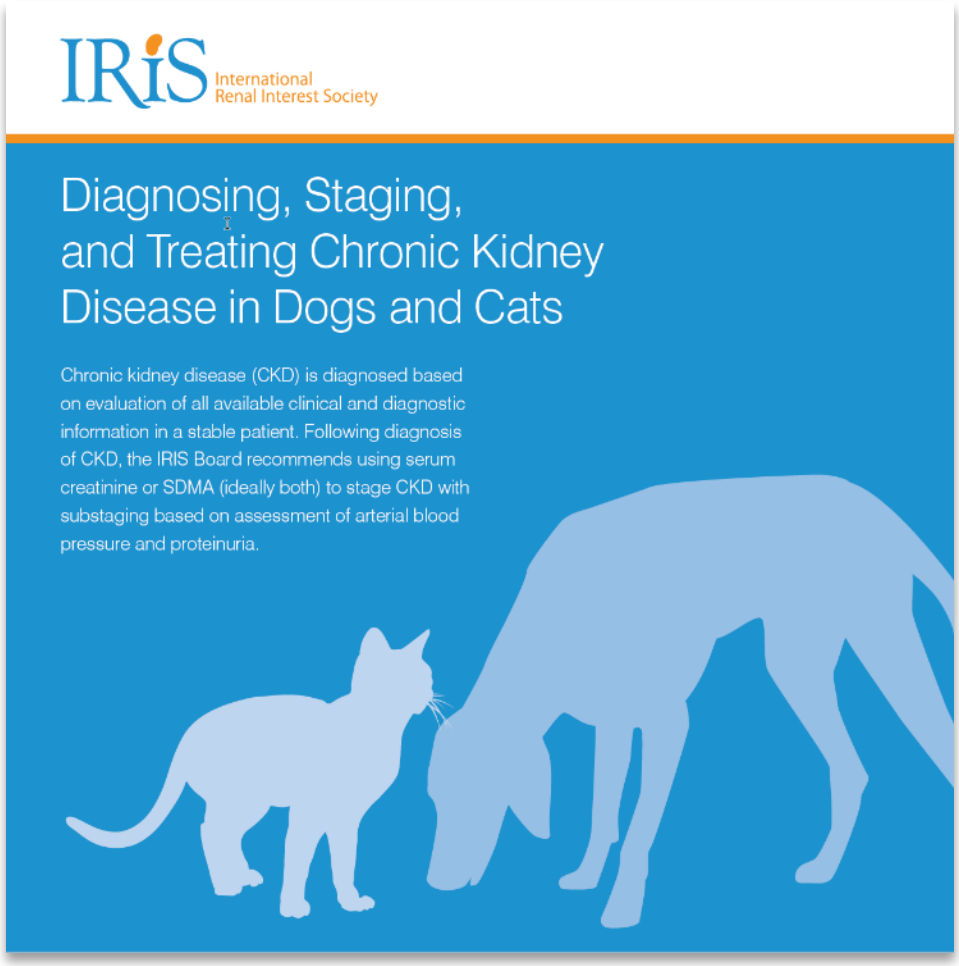
Reference intervals are **not universal and may differ** based on laboratory, methodology, and population.

Sources:
1. Horowitz GL, Altaie S, Boyd JC, et al; Clinical and Laboratory Standards Institute. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—third edition. https://clsi.org/media/1421/ep28a3c_sample.pdf. Published October 2010. Accessed December 10, 2019.
2. International Renal Interest Society. IRIS Guidelines. http://www.iris-kidney.com/guidelines/index.html. Updated 2019. Accessed December 10, 2019.

The IRIS guidelines do not propose reference intervals

International Renal Interest Society (IRIS) guidelines[2] are not reference intervals.

The IRIS guidelines provide disease-staging criteria to inform treatment after diagnosis of chronic kidney disease (CKD).



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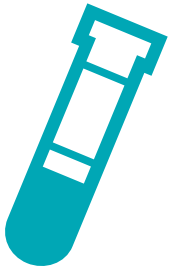
IDEXX creatinine reference interval study for cats: A reference interval is determined, not created

175 feline patients

Adults of all breeds

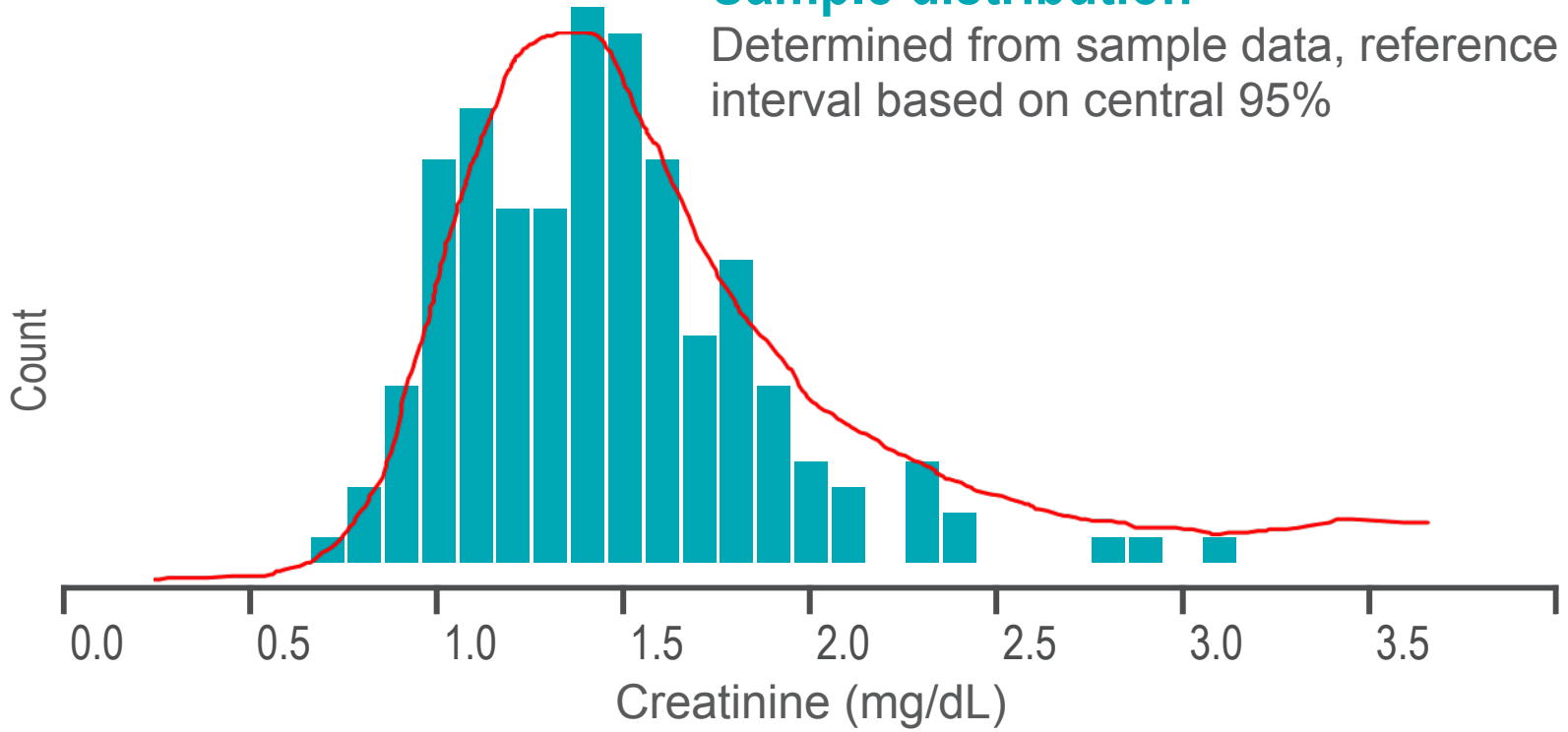
7 clinics + 3 reference laboratories



Small-animal private practices from diverse geographic locations

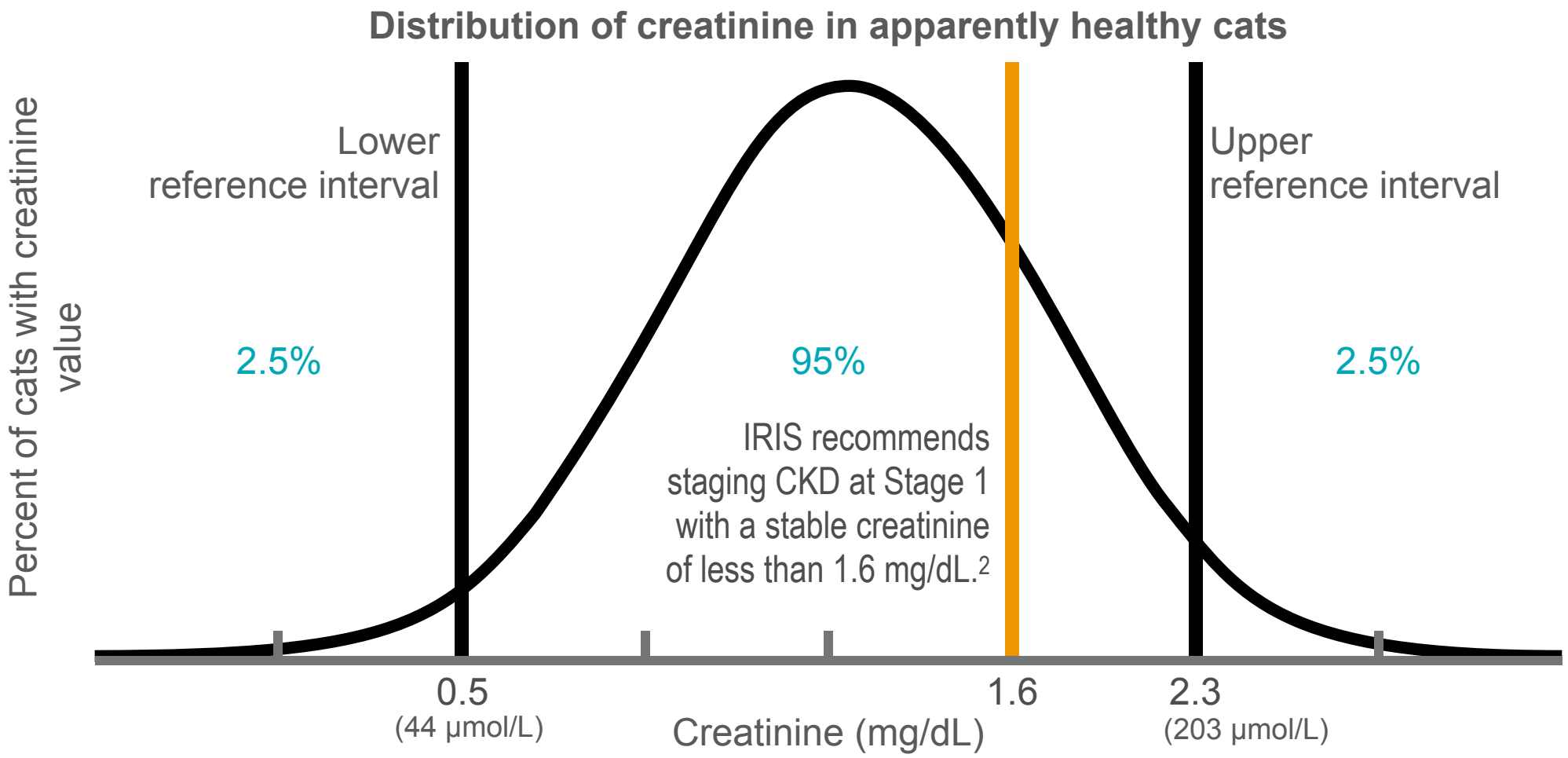
Sample distribution

Determined from sample data, reference interval based on central 95%



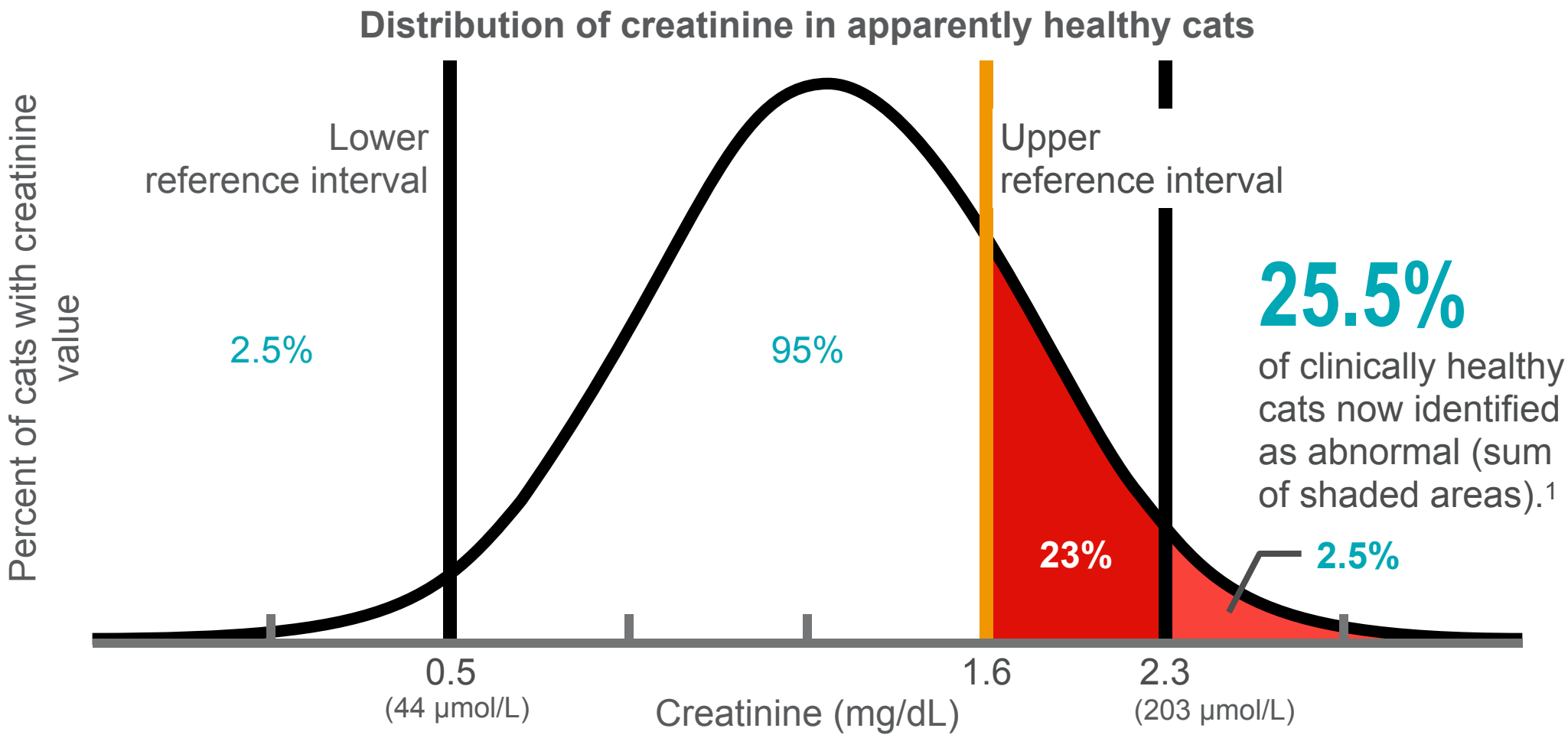
Source:
1. Data on file at IDEXX Reference Laboratories, Inc. Westbrook, Maine USA.

What happens if we change our upper reference limit[1] to 1.6 mg/dL (140µmol/L)?



Sources:
1. Horowitz GL, Altaie S, Boyd JC, et al; Clinical and Laboratory Standards Institute. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—third edition. https://clsi.org/media/1421/ep28a3c_sample.pdf. Published October 2010. Accessed December 10, 2019.
2. International Renal Interest Society. IRIS Guidelines. http://www.iris-kidney.com/guidelines/index.html. Updated 2019. Accessed December 10, 2019.

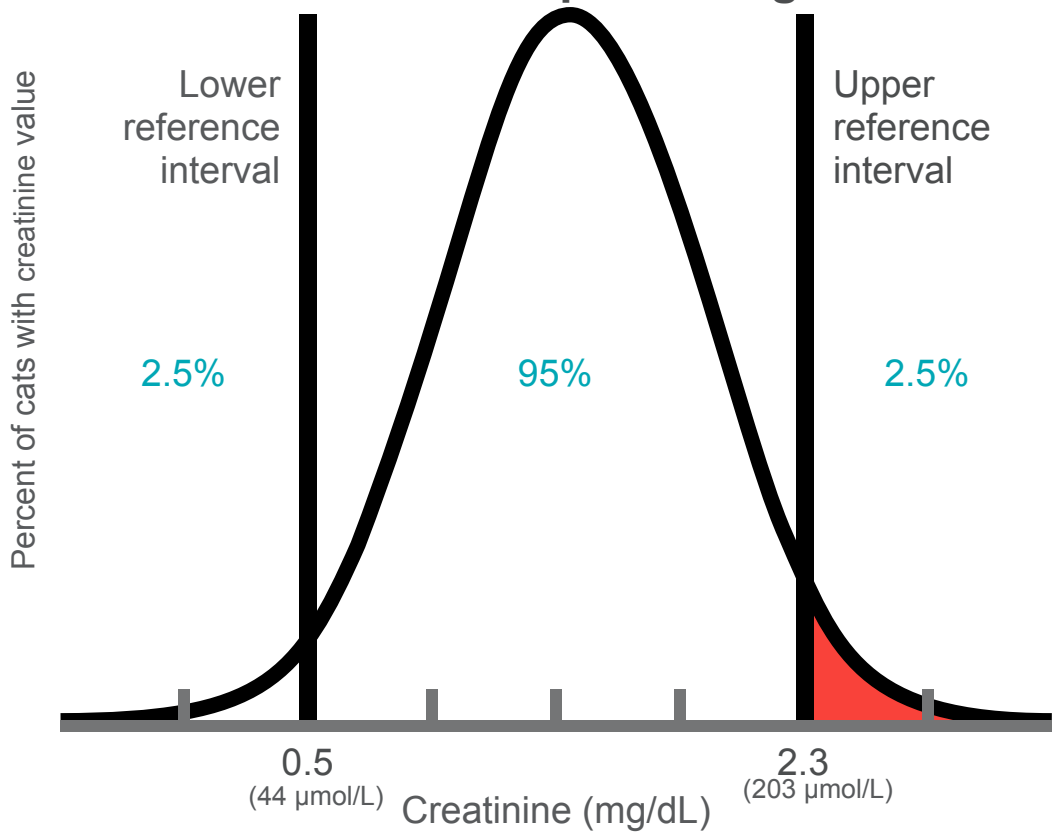
The math: The effect on our cat population



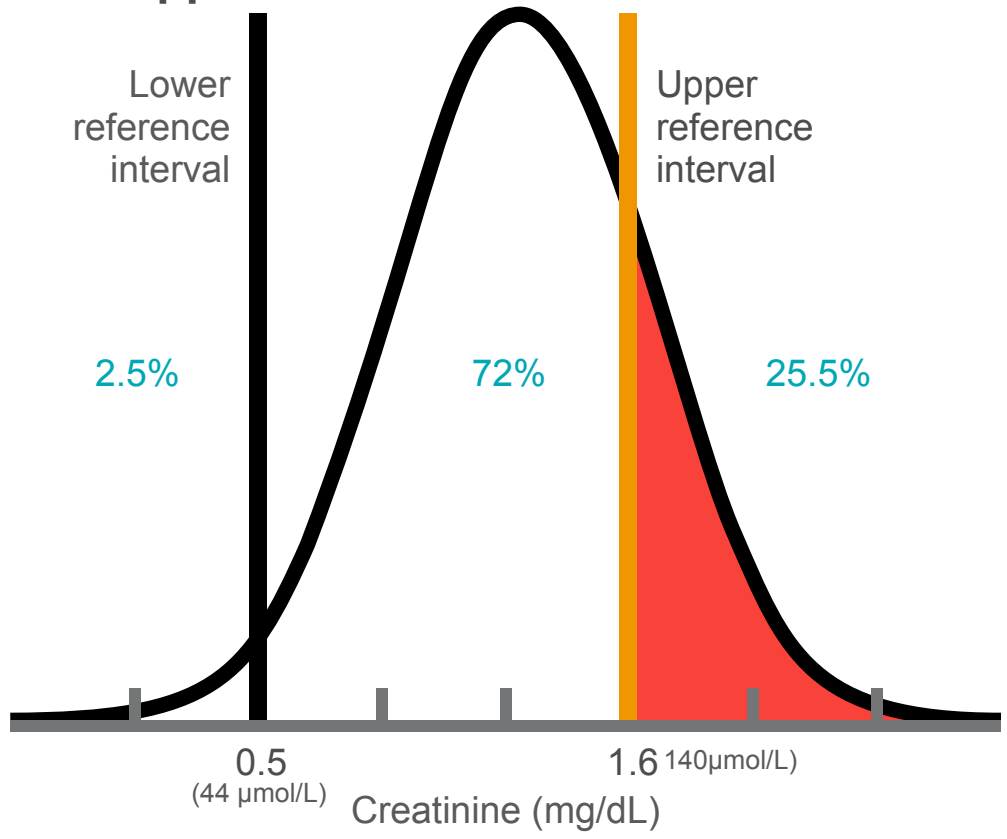
Source:
1. Data on file at IDEXX Reference Laboratories, Inc. Westbrook, Maine USA.

Using the IRIS Stage 1 threshold for the upper limit of the reference interval would misdiagnose one in four clinically healthy cats

IDEXX reference interval per CLSI guidelines



Using IRIS CKD Stage 1 threshold (140 µmol/L) as upper limit of reference interval



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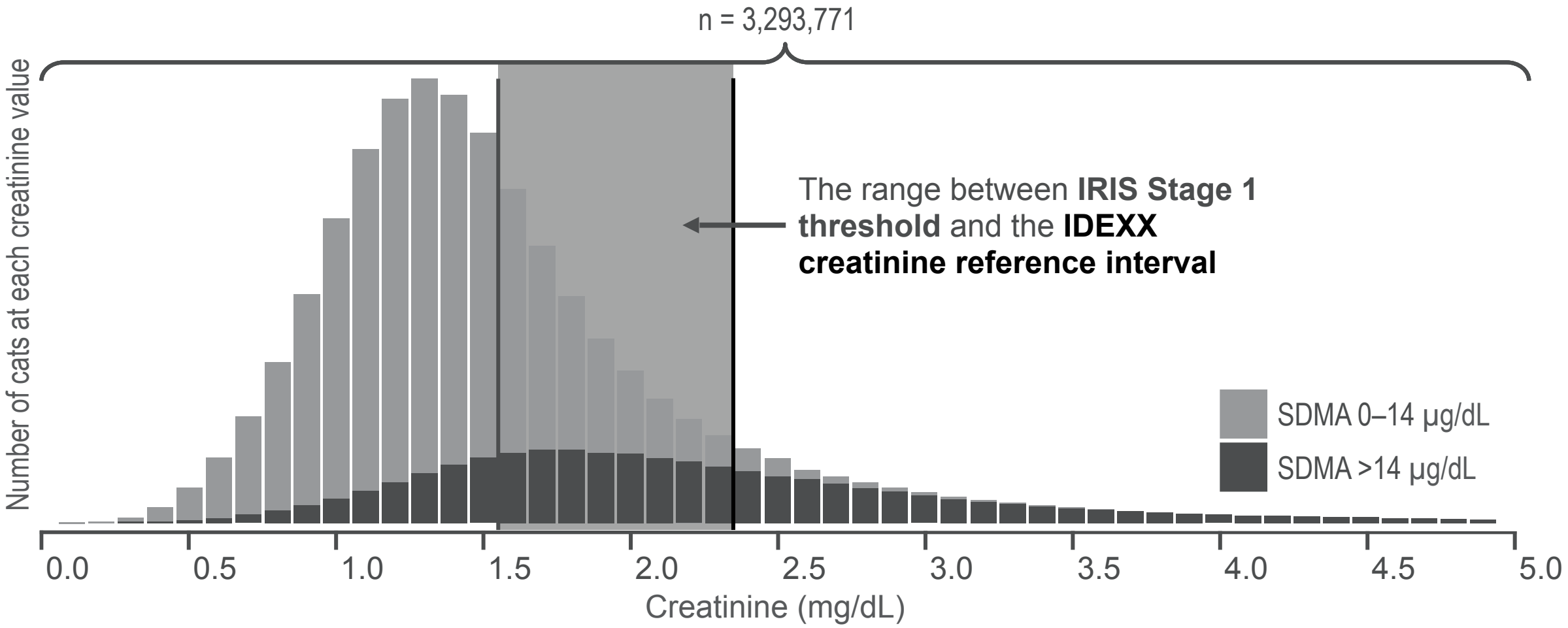
2. International Renal Interest Society. IRIS Guidelines. http://www.iris-kidney.com/guidelines/index.html. Updated 2019. Accessed December 10, 2019.

The consequence of misdiagnosis of disease

- Unnecessary diagnostics for healthy cat
- Unnecessary costs for the owner
- Unwarranted treatment for a healthy cat
- Inappropriate diagnosis of an irreversible and progressive condition for healthy cats

Exploring additional renal biomarkers to improve diagnosis of kidney disease

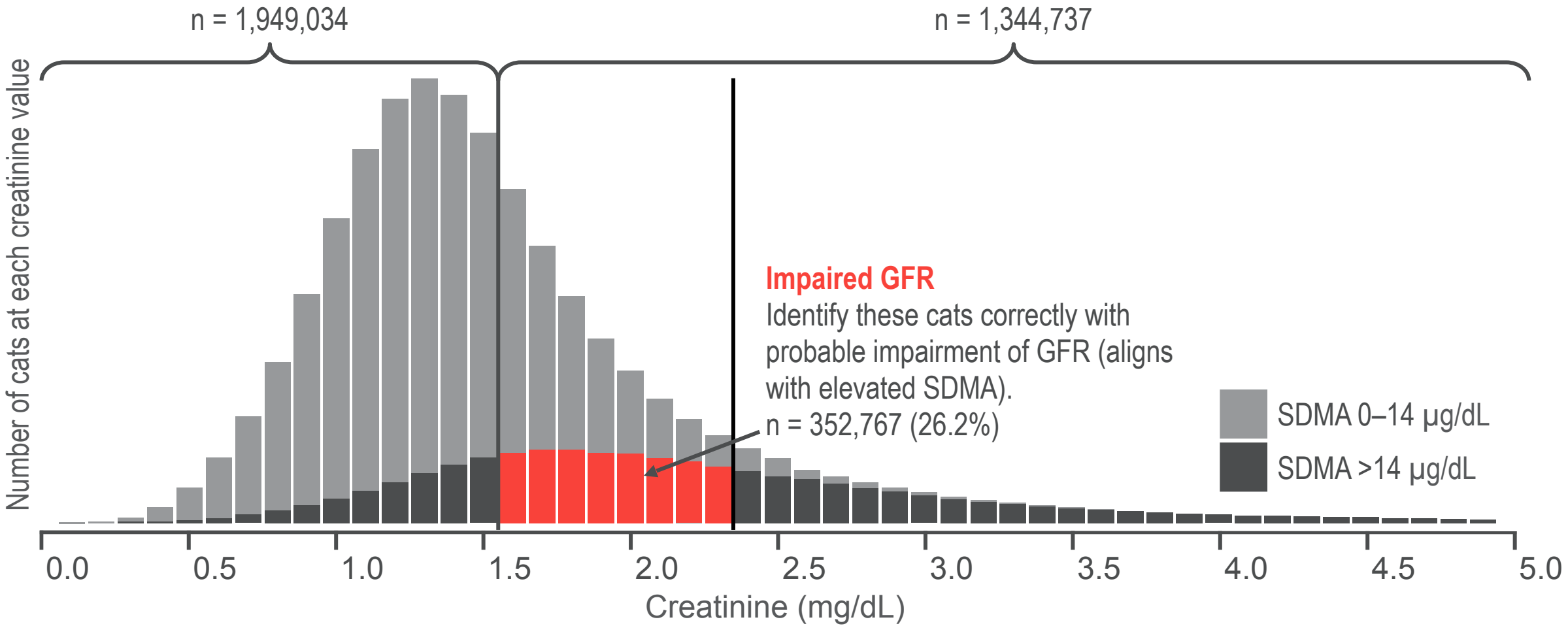
The importance of understanding kidney function and detecting early disease



Source:

1. Population: Distribution of creatinine results for all-cause testing from 3,293,771 cats at U.S. IDEXX Reference Laboratories (upper limit of evaluation creatinine 5 mg/dL); July 14, 2015–January 1, 2018. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.

The importance of understanding kidney function and detecting early disease



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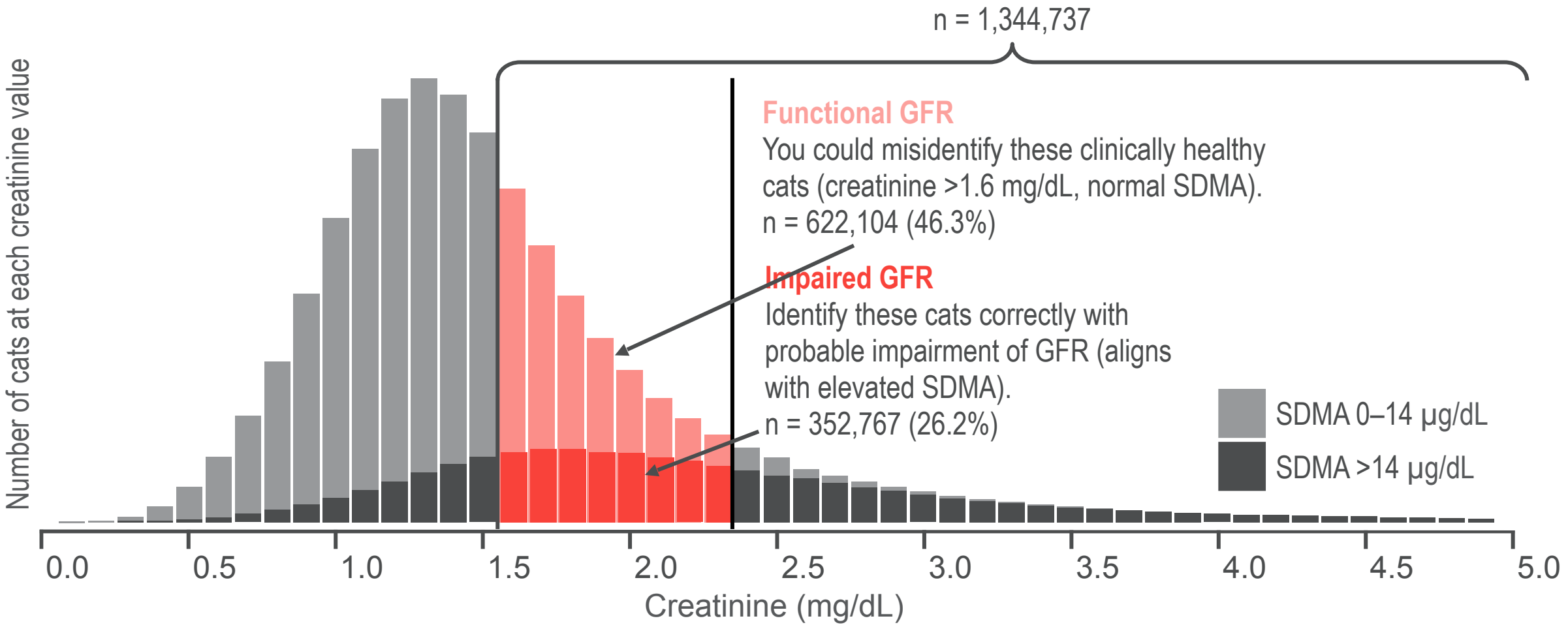

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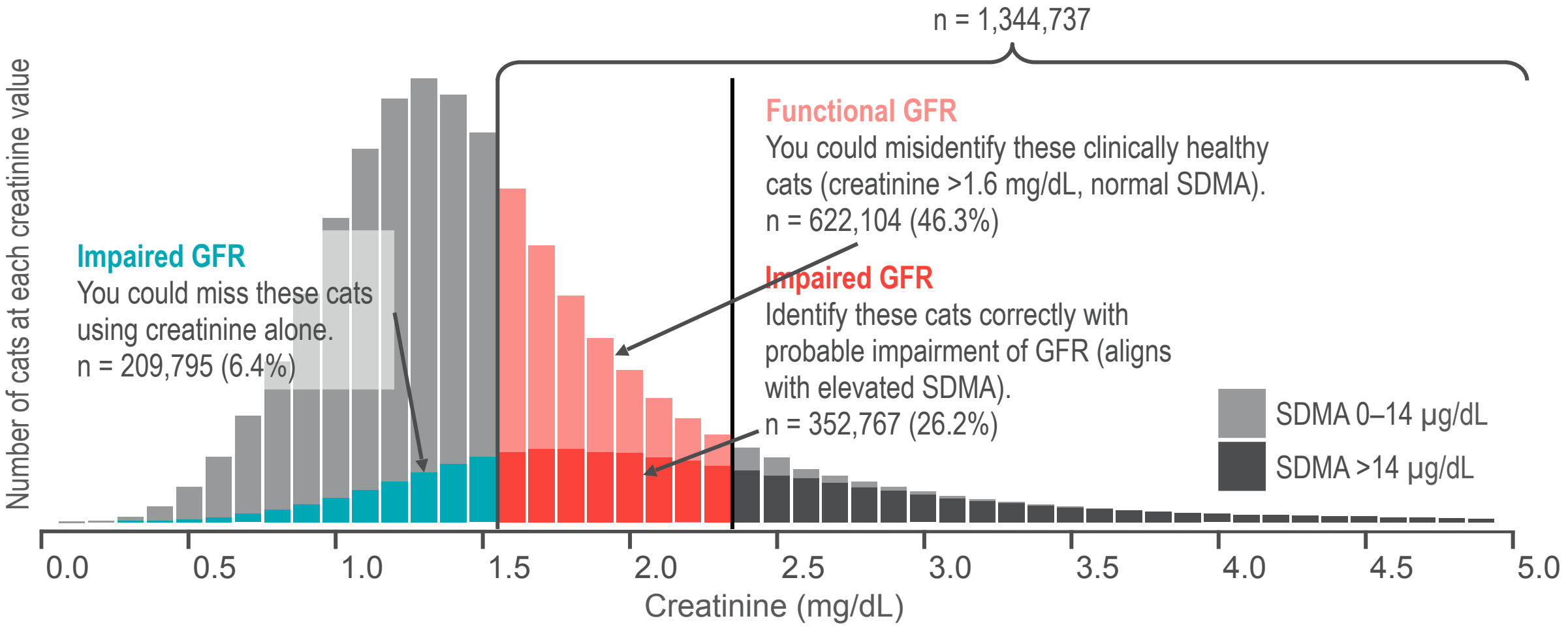

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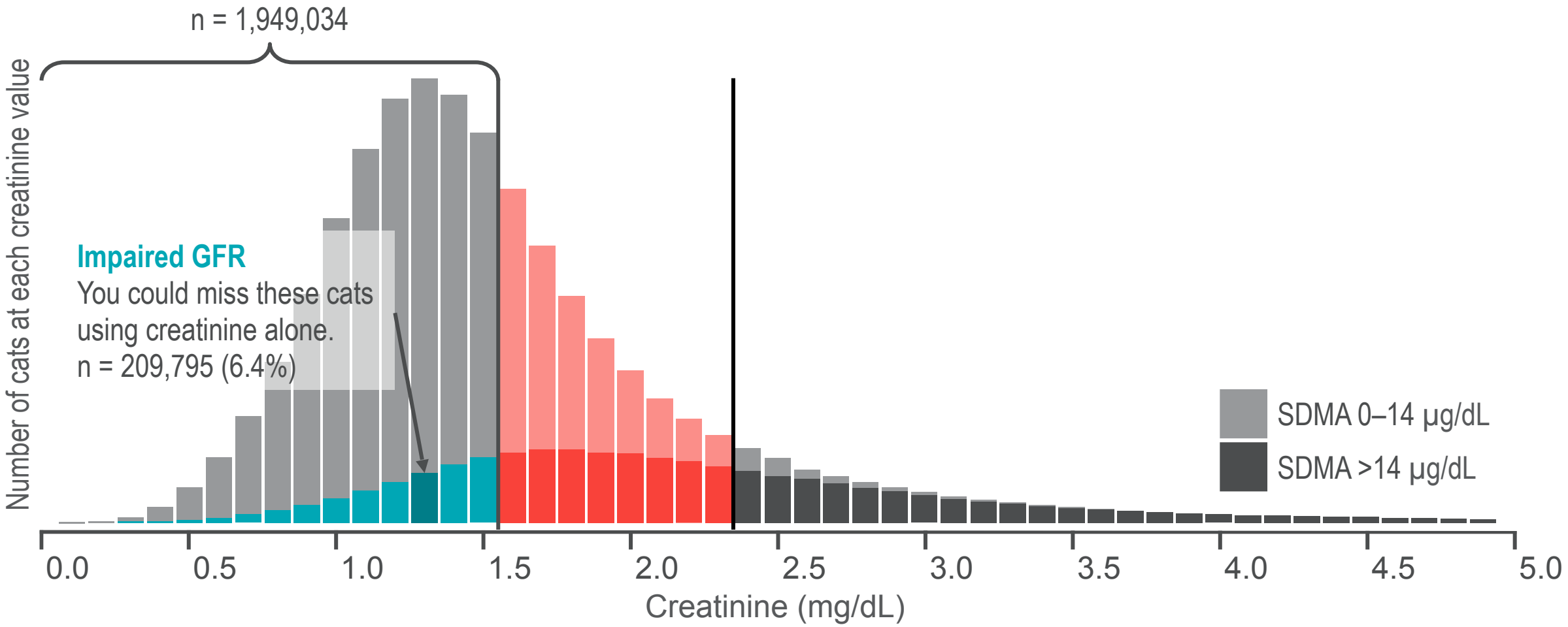
Using a different test is better than arbitrarily changing a RI



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Commercially available renal biomarkers

1

SDMA
Creatinine
BUN

2

Phosphorus
Hematocrit
Potassium
Magnesium

3

Urinalysis
USG
Proteinuria
Urine protein: creatinine ratio

SDMA and Feline Concurrent Disease

What are their kidneys telling you?

Listen closer with IDEXX SDMA®

Detects

diseases of the kidney sooner[1–3]

Chronic kidney disease
Acute kidney injury
Pyelonephritis
Upper urinary obstruction
Kidney stones
Glomerulonephritis
Congenital disease

Reflects

other disease processes affecting the kidneys[4]

Hyperthyroidism
Vector-borne disease
Systemic hypertension
Cardiorenal syndrome
Lower urinary obstruction
Sepsis
Cancer
Drug toxicity

Sources:
1. Nabity MB, Lees GE, Boggess M, et al. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for early detection of chronic kidney disease in dogs. *J Vet Intern Med*. 2015;29(4):1036–1044.
2. Hall JA, Yerramilli M, Obare E, Yerramilli M, Jewell DE. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. *J Vet Intern Med*. 2014;28(6):1676–1683.
3. Hall JA, Yerramilli M, Obare E, Yerramilli M, Almes K, Jewell DE. Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidney disease. *J Vet Intern Med*. 2016;30(3):794–802.
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Chronic kidney disease
Acute kidney injury
Pyelonephritis
Upper urinary obstruction
Kidney stones
Congenital disease

Reflects

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Hyperthyroidism

Systemic hypertension

Lower urinary obstruction

Drug toxicity

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IDEXX

What are their kidneys telling you?

Reflects

other disease processes affecting the kidneys[4]

- Hyperthyroidism
- Systemic hypertension
- Lower urinary obstruction
- Drug toxicity

Sources:
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IDEXX

Feline Hyperthyroidism

- Prevalence varies based on geography with senior cats at diagnosed at approximately 10-12% and geriatric cats closer to 25%
- 15-49% of cats with hyperthyroidism have renal dysfunction
- Reduced muscle mass is a hallmark of feline hyperthyroidism (FHT).

SDMA is less affected by extrarenal factors – more consistent marker than creatinine in FHT.

Elevated SDMA with appropriate clinical picture should elicit concern for underlying renal disease – should encourage action

1) Peterson ME. Hyperthyroidism in cats: what's causing this epidemic of thyroid disease and can we prevent it? J Feline Med Surg 2012; 14: 804–818.
2) Wakeling J, Melian C, Font A, et al. Evidence for differing incidences of feline hyperthyroidism in London UK and Spain. Proceedings of the 15th ECVIM-CA congress; 2005 Sept 1–3; Glasgow, p 220.
3) Sassnau R. Epidemiologic investigation on the prevalence of feline hyperthyroidism in an urban population in Germany. Tierarztl Prax Ausg K Kleintiere Heimtiere 2006; 34: 450–457.
4) Miyamoto T, Miyata I, Kurobane K, et al. Prevalence of feline hyperthyroidism in Osak and the Chugoku Region. J Jpn Vet Med Assoc 2002; 55: 289–292.
5) de Wet CS, Mooney CT, Thompson PN, et al. Prevalence of and risk factors for feline hyperthyroidism in Hong Kong. J Feline Med Surg 2009; 11: 315–321.
6) Williams TL, Elliott J, Syme HM. Association of iatrogenic hypothyroidism with azotemia and reduced survival time in cats treated for hyperthyroidism. J Vet Intern Med. 2010;24:1086-1092.

Feline Hyperthyroidism and Renal Disease

- Hyperthyroidism is the most common endocrine disease of older cats.
- CKD is estimated to affect 1-3% of all cats, and > 30% of cats older than 15 years of age.[1-5]
- Prevalence of hyperthyroidism and renal disease occurring together reported at approximately 14%.[5,6]
  - Masking of underlying kidney disease is common due to increased GFR during disease state and reduction of GFR post- therapy[7]

1)Peterson ME. Hyperthyroidism in cats: what's causing this epidemic of thyroid disease and can we prevent it? J Feline Med Surg 2012; 14: 804–818.
2)Wakeling J, Melian C, Font A, et al. Evidence for differing incidences of feline hyperthyroidism in London UK and Spain. Proceedings of the 15th ECVIM-CA congress; 2005 Sept 1–3; Glasgow, p 220. 9 Sassnau R.
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5)Prevalence of and risk factors for feline hyperthyroidism in Hong Kong. J Feline Med Surg 2009; 11: 315–321.
6) Williams TL, Peak Kjbrodbelt d, et al. Survival and the Development of Azotemia after Treatment of Hyperthyroid Cats. *J Vet Intern Med* 2010;24:863-869
7) Vaske HH, Schermerhorn T, Grauer GF. Effects of feline hyperthyroidism on the kidney, a review. *J Fel Med Surg*; 2016:18(2) 55-59

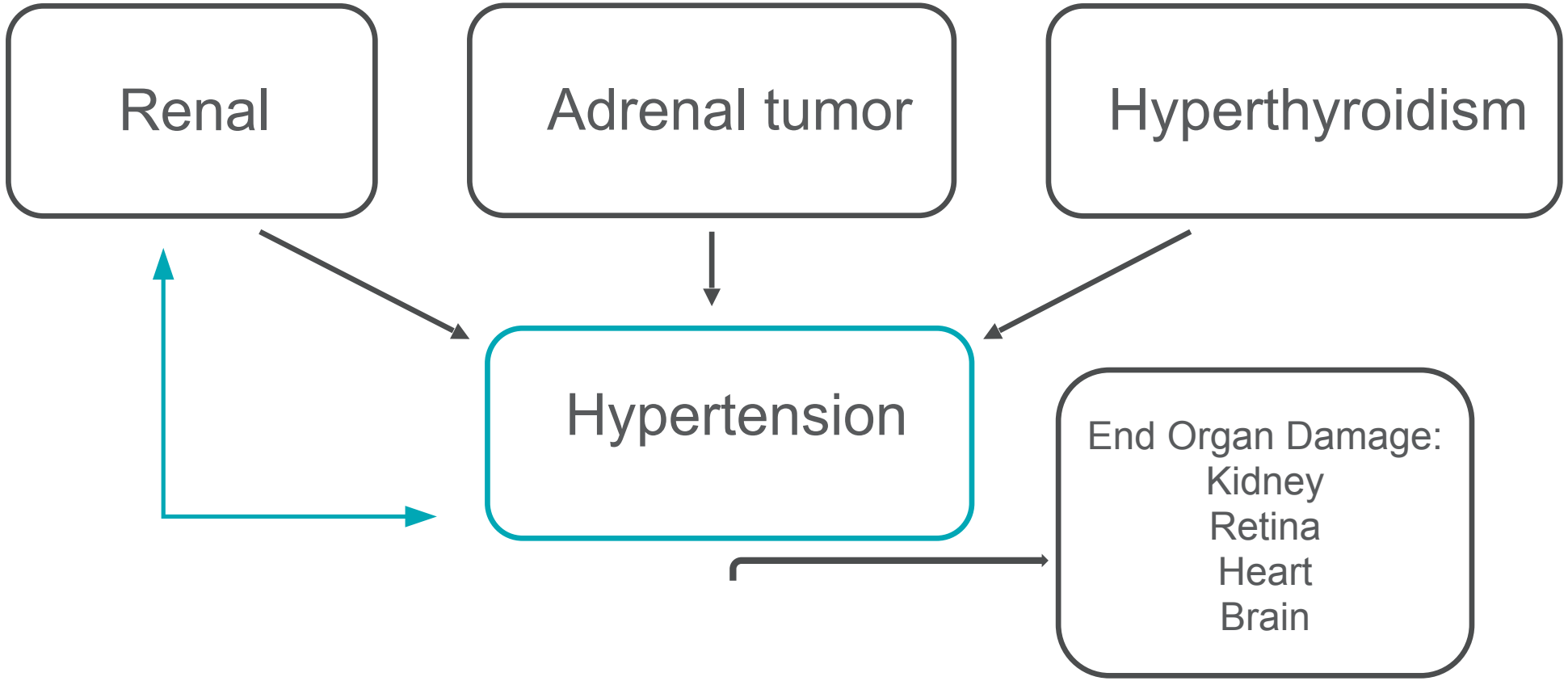
Iatrogenic hypothyroidism is associated with azotemia

Cats with iatrogenic hypothyroidism have a greater incidence of increased creatinine and SDMA.[1]

- Likely due to decreased GFR
- Worsening underlying kidney disease [2,3,4]
- Development of azotemia negatively impacts survival time.[1]
- Restoring euthyroidism appears to reduce the occurrence of azotemia.[1]

1) Williams TL, Elliott J, Syme HM. Association of Iatrogenic Hypothyroidism with Azotemia and Reduced Survival Time in Cats Treated for Hyperthyroidism. *J Vet Intern Med* 2010;24:1086-1092
2) Covey HL, Chang Y, Elliott J, Syme HM. Changes in thyroid and renal function after bilateral thyroidectomy. *J Vet Intern Med* 2019;33:508-509
3) Riensche MR, Graves TK, Schaeffer DJ. An investigation of predictors of renal insufficiency following treatment of hyperthyroidism in cats. *Jour Fel Med Surg.* 2008;10: 160-166
4) Peterson ME, Nichols R, Rishniw M. Serum thyroxine and thyroid-stimulating hormone concentration in hyperthyroid cats that develop azotemia after radioiodine therapy. J Small Anim Pract. 2017:58(9) 519-530

Systemic Hypertension



Hypertension occurs when there is smooth muscle contraction in the small arterioles

Systemic Hypertension

Defined as[1]:

- Normotensive (minimal TOD risk) SBP <140 mm Hg
- Prehypertensive (low TOD risk) SBP 140-159 mm Hg
- Hypertensive (moderate TOD risk) SBP 160-179 mm Hg
- Severely hypertensive (high TOD risk)SBP ≥180 mm Hg

Since blood pressures > 160 are associated with a moderate risk of pathologic changes treatment is usually recommended." or some such rather than writing usually

- Hypertension can occur with any severity of kidney disease.

- Blood pressure should be part of the routine follow-up for all cats with kidney disease.

1) International Renal Interest Society. IRIS Guidelines. http://www.iris-kidney.com/guidelines/index.html. Updated 2019. Accessed December 10, 2019.

Clinical Case Example

13-year-old, spayed female domestic long hair

Chemistry	5/13/16 2:39 PM	
Glucose	117	72 - 175 mg/dL
IDEXX SDMA (Learn More)	P 17	0 - 14 µg/dL
Creatinine	177	70 – 203 umol/L
BUN	58	16 - 37 mg/dL
BUN: Creatinine Ratio	29.0	
Phosphorus	3.6	2.9 - 6.3 mg/dL
Calcium	10.2	8.2 - 11.2 mg/dL
Sodium	147	147 - 157 mmol/L
Potassium	3.9	3.7 - 5.2 mmol/L
Na: K Ratio	38	29 - 42
Chloride	116	114 - 126 mmol/L
TCO2 (Bicarbonate)	15	12 - 22 mmol/L
Anion Gap	20	12 - 25 mmol/L
Total Protein	6.6	6.3 - 8.8 g/dL
Albumin	2.9	2.6 - 3.9 g/dL
Globulin	3.7	3.0 - 5.9 g/dL
Albumin: Globulin Ratio	0.8	0.5 - 1.2

ALT	252	27 - 158 U/L
AST	138	16 - 67 U/L
ALP	15	12 - 59 U/L
GGT	<1	0 - 6 U/L
Bilirubin - Total	<0.1	0.0 - 0.3 mg/dL
Bilirubin - Unconjugated	0.0	0.0 - 0.2 mg/dL
Bilirubin - Conjugated	<0.1	0.0 - 0.2 mg/dL
Cholesterol	183	91 - 305 mg/dL
Amylase	1,646	623 - 2,239 U/L
Lipase	121	11 - 242 U/L
Creatine Kinase	382	64 - 440 U/L
Hemolysis Index	1+	
Lipemia Index	N	

Differentials:
Primary vs Secondary kidney disease
Hyperthyroidism
Primary liver disease

Questions?