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Dried blood spots for the enzymatic diagnosis of lysosomal storage diseases in dogs and cats

Adrian C. Sewell¹, Mark E. Haskins², and Urs Giger³

¹Department of Pediatrics, University Children's Hospital, Frankfurt am Main, Germany

²Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

³Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Background—In people lysosomal storage diseases (LSD) can be diagnosed by assaying enzyme activities in dried blood spots (DBS).

Objective—The aim of this study was to evaluate the feasibility of using DBS samples from dogs and cats to measure lysosomal enzymatic activities and diagnose LSD.

Methods—Drops of fresh whole blood collected in EDTA from dogs and cats with known or suspected LSD and from clinically healthy dogs and cats were placed on neonatal screening cards, dried, and mailed to the Metabolic Laboratory, University Children's Hospital, Frankfurt, Germany. Activities of selected lysosomal enzymes were measured using fluorescent substrates in a 2-mm diameter disk (~2.6 μ L blood) punched from the DBS. Results were expressed as nmol substrate hydrolyzed per mL of blood per minute or hour.

Results—Reference values were established for several lysosomal enzyme activities in DBS from dogs and cats; for most enzymes, activities were higher than those published for human samples. Activities of β -glucuronidase, *N*-acetylglucosamine-4-sulfatase (arylsulfatase B), α -mannosidase, α -galactosidase, α -fucosidase, and hexosaminidase A were measureable in DBS from healthy cats and dogs; α -iduronidase activity was measureable only in cats. In samples from animals with LSD, markedly reduced activity of a specific enzyme was found. In contrast, in samples from cats affected with mucopolidosis II activities of lysosomal enzymes were markedly increased.

Conclusions—Measurement of lysosomal enzyme activities in DBS provides an inexpensive, simple, and convenient method to screen animals for suspected LSD and requires only a small sample volume. For diseases in which the relevant enzyme activity can be measured in DBS, a specific diagnosis can be made.

Keywords

DBS; enzyme assay; genetic disease; laboratory test; screening

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Correspondence A.C. Sewell, Department of Pediatrics, University Children's Hospital, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany adrian.sewell@kgu.de.

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Introduction

Lysosomal storage diseases (LSD) are a group of individually rare genetic disorders of cellular catabolism classified based upon clinical phenotype, primary substrates that accumulate in lysosomes, and the specific enzyme that is deficient in activity.¹ Although specific LSD occur rarely, some may be more frequent in certain ethnic groups in humans, eg, Ashkenazi Jews, and breeds of dogs and cats, eg, Miniature Pinschers and Siamese cats. More than 40 different diseases have been characterized in people and may be subgrouped into glycoproteinoses, neurolipidoses, and mucopolysaccharidoses (MPS) according to the type of storage product that accumulates.² These LSD have been extensively characterized in human patients by their clinical, biochemical, histopathologic, and molecular genetic features. Furthermore, many LSD have been identified in animals, mostly in dogs and cats, and have served as naturally occurring animal models as the clinicopathologic features are similar to those seen in children with the respective LSD. Thus, these animal models have enhanced our understanding of pathogenesis and, together with knockout murine models, have been important in the development and assessment of novel therapeutic strategies.^{1,3-4}

The MPS family of LSD is caused by severely impaired function of one of 11 lysosomal enzymes required for normal degradation of mucopolysaccharides or glycosaminoglycans (GAG). The result is lysosomal accumulation of GAG, which are long chains of repeating subunits that are components of proteoglycans, the ground substance of extracellular matrix. In 1976 the first MPS reported in an animal was in a Siamese cat, and subsequently many other MPS have been reported in cats and dogs (Table 1). In contrast to the severe skeletal and ophthalmologic malformations observed in most MPS types in animals, the MPS III disorders are associated with major neurologic deficits as reported recently with MPS IIIA in Dachshunds⁵ and New Zealand Huntaway dogs⁶ and with MPS IIIB in Schipperkes.^{1,7}

Specialized laboratory testing is required in people and animals suspected of having an LSD.⁸ In most affected animals, circulating WBCs contain inclusions or granules that can be visualized if blood smears are appropriately stained and carefully examined. In those with an MPS or glycoproteinosis, abnormal amounts of GAG or oligosaccharides are excreted in urine; simple tests, such as the Berry MPS spot test⁹ or thin-layer chromatography¹⁰ will detect the excreted products in urine. In individuals with neurolipidosis, diagnosis is more challenging, as storage products are not excreted in urine and clinical signs may be subtle. Confirmation of the diagnosis requires assay of the relevant enzyme activity in suitable material, usually serum, leukocytes, or cultured skin fibroblasts^{1,11}; currently available methods use fluorimetric, colorimetric, or radioisotopic substrates, when commercially available. For certain LSD and breeds for which a specific gene mutation is known, DNA testing is the preferred method for confirmation or for screening the population at risk, including detecting carriers in the breed.¹

For the veterinary clinician, collection of samples for enzyme analysis may be problematic, as serum cannot be used for all the LSD, relatively large amounts of blood are required for isolation of leukocytes, and a skin biopsy is needed to culture fibroblasts. In addition, a specimen from a healthy control animal typically must be assayed simultaneously for enzyme activities. Furthermore, special shipping requirements, such as overnight shipment with ice packs, are required and specimens have to be processed and kept chilled or frozen until analysis. In addition, enzymatic analysis is labor-intensive and costly and is limited to few veterinary laboratories, eg, PennGen at the University of Pennsylvania, or human laboratories willing and able to analyze animal samples. Therefore, an alternative and simpler approach to screening animals would be advantageous.

In human medicine, newborn screening has been performed routinely to detect metabolic and endocrine diseases that are severe, frequent, and treatable.¹² Screening programs use dried blood spots (DBS) made from blood collected between days 3 and 5 of birth and placed on screening cards to detect metabolites characteristic of inherited metabolic diseases; screening cards can be stored at 4°C for years without appreciable loss of metabolites. As treatment modalities are becoming available, there is interest in making a diagnosis of LSD in the neonatal period.¹³⁻¹⁶ In contrast to many other enzymes, most lysosomal enzymes are highly stable¹⁷ and assays have been adapted for use with DBS with promising results. Although not all LSD can be detected using this method, we hypothesized that this strategy could be used for screening dogs and cats with and without LSD for activities of selected lysosomal enzymes in DBS.

Materials and Methods

Populations tested

Dogs and cats with a variety of LSD housed in a research colony at the School of Veterinary Medicine, University of Pennsylvania under NIH and USDA guidelines for the care and use of animals in research, or that were privately owned and evaluated for suspected LSD, were included in this study with approval of the University of Pennsylvania Institutional Animal Care and Use Committee. To establish reference values for lysosomal enzyme activities in DBS, residual samples, ie, blood samples remaining after routine testing, from 30 dogs and 20 cats were also used. Specimens were received from the Department of Veterinary Medicine, University of Hannover and the Department of Veterinary Medicine, University of Munich according to institutional animal care and use guidelines, and specimens received from a private veterinary practice (Small Animal Clinic, Trier, Germany) were obtained with informed written consent of the owners. These control animals ranged from being young adults to <10 years of age and were clinically healthy based on history, physical examination, and routine hematologic and biochemical testing. Animals were not selected based on breed and sex.

Preparation of dried blood spots

Blood was collected from the jugular, cephalic, or saphenus vein into EDTA tubes (sodium EDTA, Becton, Dickinson and Company, Franklin Lakes NJ, USA and Sarstedt AG, Nümbrecht, Germany). Drops of blood were placed on a neonatal screening card (Munktel TFN 06-079, Binzer & Munktel, Battenberg, Germany) so that marked circles were fully saturated but not over-filled (Figure 1). Blood spots were dried at ambient room temperature (20-22°C), and each card was placed in an air-tight sealed bag (Ziploc, SC Johnson, Racine, WI, USA and Neolab GmbH, Heidelberg, Germany) and shipped in an envelope by regular mail or airmail to the Metabolic Laboratory, University Children's Hospital, Frankfurt, Germany. Transit time was typically less than 1 week. On receipt, cards were stored at 4°C for less than 1 month prior to analysis. Specimens were coded so that the analyzing laboratory was unaware if the animal had an LSD or was a healthy control.

Enzymatic methods

The reagents 4-methylumbelliferone, 4-methylumbelliferyl (4MU)- α -L-iduronide, D-saccharic acid 1,4-lactone, 4MU-sulfate, 4-MU- α -D-mannopyranoside, 4-MU- α -L-fucopyranoside, 4-MU-6-sulfo-*N*-acetyl- β -D-glucosaminide, and *N*-acetylgalactosamine (Sigma-Aldrich, Munich, Germany) and 4MU-2-acetamido-2-deoxy- α -D-glucosaminide, 4MU- α -D-galactopyranoside, and 4-MU- β -D-glucuronide (Calbiochem-Merck-Biosciences, Darmstadt, Germany) were purchased. All other chemicals were of reagent grade or better. For each substrate solution (Table 2), aliquots of 1 mL were stored at -20°C for a maximum of 4 months and thawed immediately prior to use.

Enzyme assays were performed using fluorescent substrates and measuring the released 4MU fluorochrome as previously described for human samples.¹⁵ Briefly, for each assay a 2-mm diameter disk (~2.6 μ L blood) was punched from the DBS using a Harris punch (Whatman FTA Harris Uni-Core, Carl Roth GmbH, Karlsruhe, Germany) and placed in a 2-mL microcentrifuge tube (VWR 211-0034, VWR International GmbH, Darmstadt, Germany). Substrate solution was added in the appropriate volume, and the tubes were capped and incubated for the appropriate time at 37°C (Table 2). The reaction was stopped by the addition of 0.5 mL glycine/sodium carbonate buffer (0.17 M, pH 12.0; stop buffer), the tubes were centrifuged at 1000g for 5 minutes, and fluorescence (excitation 365 nm, emission 450 nm) was measured with a spectral fluorimeter (SP25, Kontron, Zurich, Switzerland). Assays were performed in duplicate for each sample and 2-mm punches from plain cards served as blanks. Fluorescence readings were corrected for blanks and results were compared with those of a 4MU calibrator prepared in stop buffer. Enzyme activity was expressed as nmol substrate hydrolyzed per mL of blood per minute (nmol/mL/min) or hour (nmol/mL/hour).

The effect of variable incubation times was studied for each enzyme by incubating DBS for 0-6 hours at 37°C (Figure 2) and for 24 hours at 37°C (data not shown), and an incubation time was chosen within the linear part of the curve for each enzyme. To investigate the effect of storage time on enzyme activity, samples from 5 control dogs and 5 control cats were stored at 4°C for < 5 days, at 4°C for 6 months, and at 20°C for 12 months. .

Intra-assay precision (reported as the coefficient of variation [CV]) for each enzyme assay in DBS from dogs and cats was determined by measuring enzyme activity 10 times in 10 dogs and 10 cats. Interassay precision (reported as CV) for each enzyme assay was determined by measuring the activity 5 times within a period of 2 months.

Statistical analysis

Normality of the data was tested with the Shapiro-Wilk test for sample sizes > 10. As the results were not normally distributed, the reference values for each enzyme are reported as median and minimum-maximum. Linear regression analysis was performed for enzyme activity as a function of time.¹⁸ Statistical analysis of storage conditions (duration and temperature) was not performed owing to the low numbers of samples compared.

Results

All tested lysosomal enzyme activities could be readily measured in DBS with the exception of α -iduronidase in dogs and *N*-acetyl- α -glucosaminidase in dogs and cats even after 48 hours incubation (Table 3). Although enzyme activities varied among healthy animals, activities were never < 33% of the median value. Activities of all tested enzymes were higher in DBS samples from dogs and cats than those published for human adults; incubation times were comparable to those reported for human DBS (Table 3). Based on linear regression analysis, r^2 values were .96 or better. Intra-assay CVs for each enzyme activity from healthy control dogs and cats were 7%, and inter-assay CVs for each enzyme activity were 12% (Table 3).

Storage of DBS at 4°C for 6 months did not result in significant changes in enzyme activities; however, storage for one year at ambient room temperature (20°C) caused a mean reduction in enzyme activity of approximately 20% for all enzymes tested except β -glucuronidase, for which activity was not reduced (Table 4). However, these reductions in enzyme activities never compromised the differentiation between affected animals and controls, as affected animals had a marked reduction in activity limited to only one enzyme.

Analysis of DBS samples from affected animals demonstrated marked reduction of activity of a single enzyme; for most enzymes activity was reduced to 0% of normal activity with β -glucuronidase in one cat (ACS/11/07) reaching 22% of median activity and arylsulfatase B activity in another cat (7644) reaching 20.5% of median activity (Table 5). In all cases, the correct diagnosis was made when compared with standard enzyme testing or specific mutation analysis. Although the activity of one specific lysosomal enzyme was markedly reduced for each LSD, as expected, other enzyme activities were either within the range of reference values normal or even increased compared with reference values. In DBS samples from cats affected with mucopolipidosis II, all enzyme activities were markedly higher than those of samples from healthy control animals.

Discussion

In this study we have shown that DBS can be used for the enzymatic diagnosis of selected LSD in dogs and cats, and samples obtained as part of routine laboratory testing are sufficiently stable to allow shipping at ambient temperature. Similar to what has been found for human samples, storage of DBS did not result appreciable loss of activity for the enzymes assayed.¹³ Enzymatic activities can therefore be measured reliably for arylsulfatase B, β -glucuronidase, α -mannosidase, hexosaminidase A, α -fucosidase, and α -galactosidase in DBS from both dogs and cats; α -iduronidase activity could be measured in DBS only from cats. The reason for this discrepancy remains unknown. No measurable activity of N-acetyl- α -glucosaminidase was found in DBS from control dogs and cats; thus, conventional leukocyte assays are still required for activities of α -iduronidase in dogs, N-acetyl- α -glucosaminidase, and other enzymes not evaluated in this study.¹ To our knowledge, there have been no studies to date that have shown that N-acetyl- α -glucosaminidase can be assayed in DBS from people. Furthermore, we chose not to assay heparan sulfamidase activity owing to the cost of the substrate and lack of studies on the assay of this enzyme in human DBS. A recent report, however, has indicated that new substrates are available for heparan sulfamidase and other enzymes and soon will be tested in DBS.¹⁹ The activity of iduronate sulfatase was not measured because of cost, and sphingomyelinase and galactocerebrosidase both require radioactively labeled substrates and thus were not evaluated. Not all lysosomal enzyme activities can be measured by fluorimetry, and sophisticated tandem mass-spectrometric multiplex platforms to measure products of a wide variety of lysosomal enzymes are becoming available for human samples.^{20,21}

Results of the DBS method matched the specific diagnosis in animals with known distinct enzyme deficiencies. Classical assays for α -iduronidase and arylsulfatase B activities usually require isolated leukocytes, and the DBS method offers a distinct advantage in diagnosing cats with suspected MPS I or VI. For dogs, DBS can be used for MPS VI, but the diagnosis of MPS I requires serum or leukocytes. The same applies to the diagnosis of MPS IIIA and MPS IIIB in dogs where isolated leukocytes are the preferred specimen. For MPS VII, assay of β -glucuronidase activity in DBS provides an easy alternative to using serum or leukocytes; this is also true for feline α -mannosidosis activity. Hexosaminidase A activity could be readily measured, but we did not have samples from any companion animal with gangliosidosis. Similarly, a deficiency of α -galactosidase should be detectable in DBS and has been demonstrated in people^{22,23}; however, to date Fabry disease has not been described in companion animals, probably due to the difficulty in detecting clinical signs.^{3,24}

Interestingly, all enzyme activities measured in DBS from dogs and cats were higher than those reported for human samples. Although WBC counts were not determined in this study, cats and dogs have higher WBC counts than people, which may explain the higher enzyme activities. A previous report noted a positive correlation between WBC counts and

magnitude of enzyme activity in human DBS.¹³ The higher activities in dogs and cats permitted omission of a hemoglobin precipitation step, as recommended for human DBS samples in a study in which the authors suggested that no clear distinction could be made between patients and controls, because the fluorescence emitted from human samples is very low.²⁵ In our study, except for α -iduronidase in dogs enzyme activities measured in samples from animals with LSD could clearly be distinguished from the reference values.

Cats with mucopolidosis II (ML II) had increased activities of arylsulfatase B, β -glucuronidase, and α -iduronidase in DBS samples. This unique LSD is caused by lack of phosphotransferase activity, an enzyme in the Golgi apparatus required for post-translational phosphorylation of the mannose moiety of most lysosomal hydrolases.²⁶ Enzymes lacking the mannose-6-phosphate signal are not directed to the lysosomes and are secreted into the extracellular fluid. The finding of high activities of lysosomal enzymes in plasma or serum from affected individuals has been the mainstay for the clinical diagnosis of ML II,²⁷ and enzyme activities in DBS from colony-bred cats homozygous for ML II confirmed this diagnostic hallmark and can be used to screen for this disease.

For some LSD, the genetic mutation is known for a particular breed, eg, MPS VI in Siamese cats²⁸ and Miniature Pinschers and Schnauzers,²⁹ MPS VII in German Shepherds,³⁰ MPS IIIA in Dachshunds,³¹ and MPS IIIB in Schipperkes.¹ In people, DBS samples from newborns may be used directly for mutation testing, eg, for cystic fibrosis,³² or for retrospective mutation testing in the case of positive metabolite screening results.³³ DBS samples have also proven useful for mutation testing in dogs and cats at PennGen (author's experience). Thus, use of DBS to assay lysosomal enzyme activities and identify genetic mutations provides an inexpensive and convenient method to screen animal patients suspected of having an LSD. For example, in our study 2 new cases of feline β -glucuronidase deficiency (MPS VII) and a case of feline α -mannosidosis were identified using the DBS method, with the diagnosis subsequently confirmed by conventional methods. However, specific genetic mutations for these LSD remain undetermined.

In conclusion, DBS samples can be obtained easily, safely transported by regular mail at ambient temperature without altering enzyme activity, and offer a suitable alternative sample source for measuring activities of some lysosomal enzymes in dogs and cats, which is the basis of diagnosing a specific LSD unless the gene and relevant mutation are known. DBS samples minimize biological hazards, as filter paper impregnated with blood is contained in a sealed plastic bag, avoiding tube breakage and leakage during shipment. Furthermore, the small sample volume required permits testing of neonatal kittens and puppies. The assays are also less expensive to perform than conventional serum or leukocyte tests. Thus, use of the DBS method should enhance identification of LSD in cats and dogs.

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Figure 1.
A newborn screening card (Munktell TFN 06-079, Binzer & Munktell, Battenberg, Germany) showing dried blood spots from a dog.

Figure 2. Effect of incubation time in substrate solution on the activity of 3 selected lysosomal enzymes at 37°C in dried blood spots from a healthy control dog. Diamonds, β -glucuronidase; circles, hexosaminidase A; triangles, α -mannosidase.

Table 1 Selected naturally occurring lysosomal storage diseases in dogs and cats.^{1,16}

Disease	Enzyme deficiency	Breed
Mucopolysaccharidoses (MPS)		
MPS I (Hurler)	α -iduronidase	Domestic Short-Haired cat, ³⁴ Plott ³⁵
MPS IIIA (Sanfilippo A)	Heparan-sulfamidase	Wirehaired Dachshund, ⁵ New Zealand Huntaway ⁶
MPS IIIB (Sanfilippo B)	<i>N</i> -acetyl- α -glucosaminidase	Schipperke ⁷
MPS VI (Maroteaux-Lamy)	<i>N</i> -acetylglucosamine 4-sulfatase (arylsulfatase B)	Siamese and Domestic Short-Haired cat, ^{36,37} Miniature Pinscher, ³⁸ Miniature Schnauzer, ²⁹ Chesapeake Bay Retriever, ¹ Miniature/Toy Poodle ³⁹
MPS VII (Sly disease)	β -glucuronidase	Mixed-breed dog, ⁴⁰ German Shepherd, ⁴¹ Domestic Short-Haired cat ^{42,43}
Glycoproteinoses		
Alpha-mannosidosis	α -mannosidase	Persian and Domestic Short-Haired cat ⁴⁴
Alpha-fucosidosis	α -fucosidase	English Springer Spaniel, ^{45,46} Domestic Short-Haired cat ⁴⁷
Neurolipidoses		
Mucopolipidosis II	<i>N</i> -acetylglucosamine-1-phosphotransferase	Domestic Short-Haired cat ⁴⁸
GM2-gangliosidosis type Sandhoff	Hexosaminidase A + B	Golden Retriever ⁴⁹ ; Domestic Short-Haired cat ⁵⁰

Table 2

Assay conditions for detecting selected lysosomal enzyme activities in dried blood spots from dogs and cats.

Enzyme	Elution liquid	Substrate	Incubation time (hours)
<i>N</i> -Acetyl- α -D-glucosaminidase	50 μ L distilled water, 25 μ L 0.2 mol/L sodium acetate buffer pH 4.3	25 μ L 4MU-2-acetamido-2-deoxy- α -D-glucosaminide in distilled water	20
<i>N</i> -acetylglucosamine-4-sulfatase (arylsulfatase B)	45 μ L distilled water, 30 μ L 15 mmol/L lead acetate in 0.05 mol/L sodium acetate buffer pH 5.0	75 μ L 10 mmol/L 4MU sulfate in 0.05 mol/L sodium acetate buffer pH 5.0	20
α -D-fucosidase	50 μ L distilled water	50 μ L 1.0 mmol/L 4-MU- α -L-fucopyranoside in 0.2 mol/L citrate/phosphate buffer pH 5.0	2
α -D-galactosidase	40 μ L 0.25 mol/L <i>N</i> -acetylgalactosamine	100 μ L 5 mmol/L 4-MU- α -D-galactopyranoside in 0.15 mol/L citrate/phosphate buffer pH 4.4	20
β -glucuronidase	50 μ L distilled water	50 μ L 10 mmol/L 4MU- β -glucuronide in 0.1 mol/L sodium acetate buffer pH 4.8	2
Hexosaminidase A	20 μ L distilled water + 30 μ L citrate/phosphate buffer pH 4.2	30 μ L 6 mmol/L 4-MU-6-sulpho- <i>N</i> -acetyl- β -D-glucosaminide in 0.1	2
α -iduronidase	40 μ L 0.05 mol/L sodium formate buffer pH 2.8 containing 0.3 μ g D-saccharic acid-1,4-lactone	20 μ L 2 mmol/L 4MU - α -L-iduronide cyclohexylammonium salt in distilled water	20
α -D-mannosidase	50 μ L distilled water	50 μ L 1.5 mmol/L 4-MU- α -D-mannopyranoside in 0.2 mol/L citrate/phosphate buffer pH 3.8	2

Table 3

Activity of selected lysosomal enzymes in dried blood spots from healthy dogs and cats compared with human adults.

Enzyme and Units	Median and minimum-maximum activity in dogs $n = 30$ (intra-/inter-assay CV as %)*	Median and minimum-maximum activity in cats $n = 20$ (intra-/inter-assay CV as %)*	Minimum-maximum activity in human adults ^{21,35}	Minimum-maximum activity in human adults ²³
<i>N</i> -acetyl- α -glucosaminidase (nmol/mL/min)	Not detectable	Not detectable	Not assayed	0.016–0.097
<i>N</i> -acetylglucosamine 4-sulfatase (arylsulfatase B) (nmol/mL/min)	1.5 1.0–5.2 (6.6/9.4)	2.9 1.5–9.0 (6.0/8.2)	0.12–0.37 ²¹	0.10–0.36
α -D-fucosidase	3.2 1.4–5.7 (5.2/8.0)	3.6 1.8–7.0 (6.0/6.6)	0.16–0.47 ²¹	Not assayed
α -D-galactosidase (nmol/mL/hour)	244.2 117.0–505.2 (6.5/10.2)	222.0 100.8–444.0 (6.9/11.3)	1.37–7.66 ³⁵	2.94–14.36
β -glucuronidase (nmol/mL/min)	2.1 1.0–6.4 (5.2/7.6)	3.9 1.4–7.0 (4.6/6.7)	0.8–3.42 ²¹	0.10–0.36
Hexosaminidase A (nmol/mL/min)	2.3 1.1–5.2 (6.0/8.8)	6.2 3.2–14.7 (6.2/7.7)	0.4–1.21 ²¹	0.40–1.59
α -iduronidase (nmol/mL/hour)	Not detectable	22.6 13.2–64.0 (6.8/10.6)	2.3–10.00 ²¹	1.13–9.50
α -D-mannosidase (nmol/mL/min)	13.3 6.9–50.9 (6.0/8.7)	30.5 16.0–65.0 (5.2/7.4)	0.4–2.5 ²¹	Not assayed

* Intra-assay CVs for each enzyme assay for healthy control dogs ($n = 10$) and cats ($n = 10$) with activity measured 10 times; inter-assay CVs for each enzyme activity measured 5 times within 2 months.

Table 4

Effect of temperature and storage on selected lysosomal enzyme activities in dried blood spots from healthy control cats and dogs.

Species <i>n</i> = 5	Enzyme	Mean $\pm$ SD Activity (nmol/mL/min or nmol/mL/hour)		
		< 5 days @4°C	6 months @ 4°C	12 months @ 20°C
Cat	<i>N</i> -acetylglucosamine 4-sulfatase (Arylsulfatase B)	2.6 $\pm$ 0.7	2.8 $\pm$ 0.6	2.0 $\pm$ 0.5
Cat	α -L-fucosidase	3.9 $\pm$ 1.3	3.6 $\pm$ 1.5	3.0 $\pm$ 1.1
Cat	α -galactosidase*	236.8 $\pm$ 82.4	227.3 $\pm$ 88.1	194.2 $\pm$ 67.6
Cat	β -glucuronidase	4.9 $\pm$ 2.7	4.9 $\pm$ 3.0	4.5 $\pm$ 2.4
Cat	Hexosaminidase A	6.9 $\pm$ 2.8	6.7 $\pm$ 2.0	5.9 $\pm$ 2.4
Cat	α -iduronidase*	17.6 $\pm$ 4.1	18.2 $\pm$ 6.4	14.4 $\pm$ 3.4
Cat	α -mannosidase	23.3 $\pm$ 5.2	22.6 $\pm$ 6.9	19.1 $\pm$ 4.3
Dog	<i>N</i> -acetylglucosamine 4-sulfatase (Arylsulfatase B)	1.9 $\pm$ 0.9	1.8 $\pm$ 0.7	1.5 $\pm$ 0.7
Dog	α -L-fucosidase	3.6 $\pm$ 1.3	3.1 $\pm$ 2.0	3.0 $\pm$ 1.1
Dog	α -galactosidase*	303.8 $\pm$ 84.7	296.3 $\pm$ 80.6	258.2 $\pm$ 72.0
Dog	β -glucuronidase	3.6 $\pm$ 1.4	3.8 $\pm$ 1.0	3.6 $\pm$ 1.4
Dog	Hexosaminidase A	2.8 $\pm$ 0.6	2.6 $\pm$ 0.3	2.4 $\pm$ 0.5
Dog	α -mannosidase	35.5 $\pm$ 17.2	32.1 $\pm$ 14.4	29.1 $\pm$ 14.1

Table 5

Enzyme activities in dried blood spots from dogs and cats and specific lysosomal storage diseases (LSD).

Case	Species	Enzyme assayed	Activity nmol/mL/min (or nmol/mL/h)	Reference values nmol/mL/min (or nmol/mL/h)	LSD
T67 *	Dog	α -fucosidase	0.0	1.6–5.5	α -fucosidosis
M2447 *	Dog	β -glucuronidase	0.0	1.1–4.9	MPS VII
M2340 *	Dog	β -glucuronidase	0.0	1.1–4.9	MPS VII
7713 *	Cat	β -glucuronidase Arylsulfatase B α -iduronidase	30.6 12.6 94.3	1.4–5.7 1.5–6.0 13.2–53.2	ML II
7715 *	Cat	β -glucuronidase Arylsulfatase B α -iduronidase	21.6 13.6 102.4	1.4–5.7 1.5–6.0 13.2–53.2	ML II
7040 *	Cat	α -iduronidase	0.0	13.2–53.2	MPS I
7660 *	Cat	Arylsulfatase B	0.4	1.5–6.0	MPS VI
7644 *	Cat	Arylsulfatase B	0.6	1.5–6.0	MPS VI
27170 *	Cat	Arylsulfatase B	0.4	1.5–6.0	MPS VI
27121 *	Cat	Arylsulfatase B	0.1	1.5–6.0	MPS VI
27142 †	Cat	α -mannosidase	0.1	16.0–65.0	α -mannosidosis
ACS/11/07 †	Cat	β -glucuronidase	0.9	1.4–5.7	MPS VII
T168 †	Cat	β -glucuronidase	0.0	1.4–5.7	MPS VII

* Colony animals with diagnosis confirmed by molecular and conventional enzyme testing.

† Animal patients with diagnosis confirmed by conventional enzyme testing. MPS indicates mucopolysaccharidosis.