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News from the Gastrointestinal Laboratory

We had another exciting year at the GI Lab. Your submissions keep coming in, and we enjoy interacting with all of you on the phone. Thank you so much for your patronage. Without it, none of the work we do would be possible.

We continue to be involved in more than 100 research projects, about half of which originate at our lab. The other half are collaborative projects with a wide range of investigators throughout the world: some at academic institutions, some in industry, and some from individuals who work for governmental agencies. Many of these projects would not be possible without you enrolling patients. We offer a heartfelt "thank you" to all of you who graciously do so and to your clients and their pets. Our group of 13 highly capable graduate students makes sure all these

projects are moving forward. While it may at times seem that the progress in the field of veterinary gastroenterology is going at a snail's pace, in reality, a lot of research is being done to improve the lives of your canine, feline, and sometimes even patients of other species. For example, we were and continue to be involved in clinical trials with ISK, a Japanese company based in Osaka, to bring to market in the US the first drug for the treatment of pancreatitis in any species. Recently, this drug, fuzpladib sodium, an LFA-1 antagonist, achieved conditional approval by the FDA. The product will be distributed in the US by Ceva Animal Health, LLC under the brand name Panoquell®-CA1 and should be available in 2023.

We are also very excited to announce that Dr. Kate Aicher (pictured at right) will be moving to the GI Lab as a tenure-track small animal internist, with an emphasis on canine hepatology. Dr. Aicher, who went through veterinary school here at Texas A&M University after serving as our laboratory supervisor for a few years, did her internal medicine residency training at North Carolina State University. She spent several years in private practice as an internist as well as a hospital administrator and then came back to our Canyon campus as a veterinary educator a couple of years ago. Effective January 1, 2023, Dr. Aicher will be returning to College Station as an internist with the GI Lab. Kate is excited about the opportunities of this new role and is poised to start a wide range of research projects. She will also serve as a senior clinician in the Texas A&M University Veterinary Medical Teaching Hospital for about 16 weeks per year and will be answering GI Lab consult calls for 10 to 12 weeks per year. So, the next time you call us, there is a good chance that you will be talking to her about your complex gastrointestinal cases. (Jörg M. Steiner)



Nutramax Laboratories Veterinary Sciences Chair in Canine Hepatology

It brings me great pleasure to announce that the GI Lab at Texas A&M University has recently partnered with Nutramax Laboratories Veterinary Sciences, Inc. to establish the Nutramax Laboratories Veterinary Sciences Chair in Canine Hepatology. This further enhances our veterinary hepatology group: headed by Dr. Jonathan Lidbury, Associate Professor of Small Animal Internal

Medicine and the Rob and Roxann Bilger Chair in Feline Hepatology; and including Dr. Joao Cavin, a board-certified anatomic pathologist, and Dr. John Cullen, also a board-certified



anatomic pathologist and current Hagler Fellow (see the announcement later in this newsletter). The individual appointed as the new Nutramax Laboratories Chair in Canine Hepatology will be a tremendous addition to the group. While all nine faculty members of the GI Lab are committed to our scientific efforts in hepatology, these four individuals will make up the core faculty group in this area.

Recently, Dr. Todd Henderson (President and CEO), David Griffin (Vice President of Research and Technical Services), and several other members of the senior management team of Nutramax Laboratories Veterinary Sciences, Inc. visited the GI Lab to envision future directions for research in small animal hepatology. Dr. Henderson (pictured at left), a veterinarian himself, expressed his excitement about the scientific potential of this long-term collaboration. A great big Thank You! to Nutramax Laboratories Veterinary Sciences, Inc. for making this Chair a reality! (Jörg M. Steiner)

Diagnostic Toolbox:

E. coli-associated Granulomatous Colitis

Fluorescence in situ hybridization (FISH) is a molecular tool that allows for the visualization of specific DNA or RNA sequences in tissues. In veterinary medicine, FISH is used mostly for the detection of bacteria. This technique has the unique advantage of providing information about the spatial localization of bacteria in a sample, which is not possible with bacterial culture.

FISH is considered the gold-standard method to detect intramucosal and/or intracellular *Escherichia coli* in dogs with *E. coli*-associated granulomatous colitis (GC). The condition was formerly known as histiocytic ulcerative colitis, and it is more common in young Boxer dogs and French bulldogs. Rarely, this disease occurs in other dog breeds, and there is a single case report of a cat. Clinically, dogs with *E. coli*-associated GC present with chronic gastrointestinal signs consistent with large bowel diarrhea, such as increased frequency of defecation, mucoid stools, hematochezia, and tenesmus. Weight loss and hypoalbuminemia are often reported in dogs with severe forms of this disease. Diagnosis is achieved by ruling out other causes of gastrointestinal signs, using fecal parasitological examination, CBC, chemistry panel, other specific diagnostics to rule out infectious or endocrine disorders, and diagnostic imaging, before proceeding to endoscopic colonic biopsy. Histopathology of colonic biopsies reveals characteristic infiltration of the colonic lamina propria by large macrophages with abundant cytoplasm, which is positive on Periodic-acid Schiff stain. However, a definitive diagnosis can only be reached by utilizing FISH probes that are specific for *E. coli*. These are applied to formalin-fixed biopsy samples to confirm invasive bacteria in the colonic lamina propria and in the cytoplasm of macrophages (Figure 1).

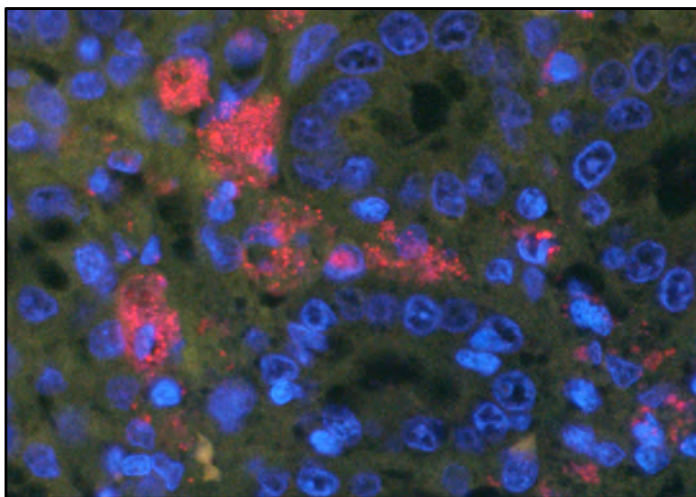


Figure 1. Colon from a French bulldog with *E. coli*-associated granulomatous colitis. Fluorescence in situ hybridization for *E. coli* showing numerous invasive bacteria (red) in the cytoplasm of macrophages. Mucosal autofluorescence appears in green and nuclei are blue (DAPI)

An 8-10 week course of fluoroquinolone antimicrobials is the recommended treatment for GC in dogs. However, antimicrobial resistance is an emerging concern, resulting in treatment failure in some dogs. FISH is utilized to identify intramucosal *E. coli* in formalin-fixed colonic biopsy specimens, but it cannot predict antimicrobial resistance or the antimicrobial susceptibility profile.

Bacterial culture and antimicrobial susceptibility profiling can be performed on fresh (non-formalin-fixed) colonic biopsies (not currently offered by the GI Lab). Treatment of GC guided by susceptibility profiling has been associated with positive long-term outcomes.

Recently, immunohistochemistry (IHC) was shown to be an economical and sensitive assay for the detection of invasive *E. coli* in dogs with GC with a relatively quick turnaround time (Figure 2). However, this technique should be used only in cases where Periodic-acid Schiff positive macrophages are identified in the colonic mucosa. Immunohistochemistry should not be used to diagnose other types of *E. coli* infections since the antibody can cross-react with other gram-negative bacteria.

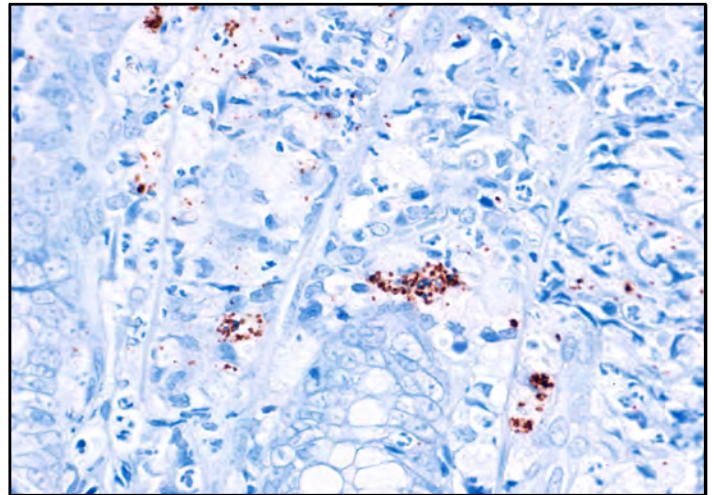


Figure 2. Colon from a French bulldog with *E. coli*-associated granulomatous colitis. Immunohistochemistry for *E. coli* showing abundant immunolabeling (red) in the cytoplasm of macrophages and in the lamina propria.

Both FISH and IHC are ancillary diagnostic methods and should be preceded by histopathologic evaluation of colonic tissue and histochemistry stains for infectious diseases. The GI Lab offers a histopathology specialty service that provides high-quality interpretation and reporting of gastrointestinal, pancreatic, and liver biopsy specimens. Pathologists with expertise in these areas may recommend ancillary testing and request authorization for tests that incur additional fees. The GI Lab currently offers IHC for *E. coli* and FISH for bacteria in general (EUB338 probe) and for *E. coli* (*E. coli/Shigella* probe). Both FISH and IHC can be performed on formalin-fixed paraffin-embedded tissues routinely submitted to the histopathology service. If histopathology was previously performed by another laboratory, five unstained slides (on charged slides) for each assay are also acceptable. (Paula R. Giarretta)

References

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Consultants' Corner:

Frequently Asked Questions

As many of you know the GI Lab offers a complimentary consultation service to the veterinarians who use our laboratory. Our team of board-certified internists is comprised of Jonathan Lidbury, Jörg Steiner, David Williams, Katie Tolbert, Emily Gould, Kate Aicher, and Chee-Hoon Chang. We all enjoy discussing challenging gastrointestinal cases with you. To set up a consultation, just call the lab at (979) 862-2861 and talk to one of our customer service representatives. As we all take consultations in addition to our other clinical, research, and teaching responsibilities, if we are not available to talk to you immediately, our staff will take a message, and the consultant on duty that week will call you back. To increase the chances of us connecting with you promptly, please give us a 4-hour window during which to return your call. It can also really help to let your reception staff

know that you are expecting our call and to let them know if it is okay to pull you out of an exam room.

When more than just an interpretation of results is needed, it is also really helpful if you have the following patient information available at the time of the consult call:

1. Signalment
2. Presenting complaint and chronicity of clinical sign(s)
3. If diarrhea is present, can it be categorized as small, large, or mixed bowel
4. Other pertinent diagnostic test results (e.g., CBC, chemistry panel, urinalysis, etc.)
5. Dietary history
6. Response to past interventions (including diet)

Below, please see answers to some of the most frequently asked questions that we are asked.

If you have any questions about our consultation service, please feel free to contact us. (Jonathan A. Lidbury and Emily N. Gould)

How do I use a baseline cortisol measurement to rule out hypoadrenocorticism (Addison's disease)?

Hypoadrenocorticism in dogs can be reliably and cost-effectively ruled out using a baseline serum cortisol measurement. When the baseline cortisol is ≥ 2.0 $\mu\text{g/dL}$, hypoadrenocorticism is ruled out. For dogs in which the resting cortisol is < 2.0 $\mu\text{g/dL}$, an ACTH stimulation test must be performed to definitively confirm or exclude a diagnosis. We now offer an extended GI panel that adds a baseline cortisol measurement to our standard panel (cPLI, cTLI, cobalamin, and folate). Please note that this extended panel is NOT available for cats. As we save leftover serum for several weeks, it may be possible to add baseline cortisol to testing at a later date – just call to ask about this.

How long do patients need to be held off food before measurement of serum TLI concentration?

Serum TLI may be slightly increased after feeding in healthy dogs and cats. Therefore, both dogs and cats should not be fed for at least 8 hours before blood sample collection. As these increases are small, if a dog or cat is inadvertently fed before sample collection and the result is well within the normal range, exocrine pancreatic insufficiency is unlikely. However, if the result is borderline for the diagnosis of exocrine pancreatic insufficiency, repeat sampling after withholding food for at least 8 hours is required.

My canine/feline patient has a high serum TLI concentration but a normal serum PLI (Spec cPL/Spec fPL) concentration. Does it have pancreatitis?

We see this seemingly contradictory pattern of results in some patients. However, since the PLI is a more sensitive test for pancreatitis than the TLI assay it does NOT suggest that the patient has pancreatitis. If you have a patient with a high TLI, or you are concerned about

pancreatitis and you have not run a serum PLI, you can add this test to help determine if the patient has pancreatitis or not. We save any excess serum you send to us, so just call the lab and see if we have enough left over to run a PLI.

How long should a diet trial for a dog/cat with gastrointestinal disease last?

For most dogs and cats with gastrointestinal disease, if there has been no response to a strict diet trial within 2 to 3 weeks, they are unlikely to respond. Animals that do respond can continue to improve with time. Many dogs and cats with chronic gastrointestinal disease respond to a change of diet. Therefore, in stable appetent patients, often more than one diet trial is recommended.

What is the canine/feline fecal dysbiosis index, and when should I order them?

The canine/feline microbiota dysbiosis index (DI) is a PCR-based assay that quantifies the abundance of key bacterial groups from a fecal sample. The DI gives us the ability to consolidate the quantitative PCR results for each bacterial group into one single number. Thus, it allows us to assess whether or not the fecal microbiota of an individual dog/cat is broadly similar to that of the majority of healthy dogs/cats. A DI above 2 (dogs) or 1 (cats) indicates a major shift and dysbiosis with high specificity, while a DI between 0 and 2 (dogs) and 0 and 1 (cats) indicates a moderate shift in the fecal microbiome. It is important to note that dysbiosis often occurs secondary to other conditions, such as diet-responsive enteropathy, IBD, or EPI, and that an increased (abnormal) DI is not an indication to start any specific therapy such as antimicrobials. (Tylosin and metronidazole have been shown to increase DI in dogs and cats.)

Indications for running a DI include:

- 1) screening donor dogs/cats before FMT,
- 2) monitoring changes in the microbiota of

FMT recipients, and 3) monitoring the recovery of GI dysbiosis after antibiotic therapy. For more information on the DI, please refer to our website for more information.

What is the best way to submit specimens for fecal PCR/dysbiosis index testing?

When possible, we recommend that antimicrobial (and anthelmintic) therapies for protozoal agents) be discontinued at least 14 days (ideally 28 days for the canine/feline fecal dysbiosis index) before the fecal sample is collected. Approximately 1 gram of feces (a piece the size of a grape) is needed for these tests, and the sample must be free from cat litter, as some components of certain types of cat litter may inhibit PCR reactions. Samples must remain cold until receipt in the lab and, therefore, should be shipped by overnight courier service with frozen gel ice packs. Samples can be stored in the refrigerator over the weekend if you cannot ship by Thursday. (Our lab personnel are not here on weekends to receive samples.)

Are you able to measure canine fecal alpha 1-protease inhibitor?

We are pleased to say that we are offering the canine fecal alpha 1-protease inhibitor test again. Alpha 1-protease inhibitor is a marker of excess fecal protein loss in dogs. Samples must be submitted in special pre-weighed fecal tubes. Fresh fecal samples (one gram each) from naturally-passed (i.e. no fecal loop) defecations from 3 consecutive days should be collected as soon as possible after defecation. Each sample tube must be immediately frozen after collection. All 3 samples should then be shipped frozen by overnight courier. To order the pre-weighed fecal tubes, please email the lab at gilab@cvm.tamu.edu. This test is NOT available for cats.

Dr. Jonathan Lidbury Appointed Asst. Dept. Head of Research & Graduate Studies

Another exciting development this year has been the appointment of our own Dr. Jonathan Lidbury as Assistant Department Head of Research and Graduate Studies in the Department of Veterinary Small Animal Clinical Sciences here at Texas A&M University. Jonathan has been one of the faculty members at the GI Lab since 2015 and has chaired four doctoral student committees as well as served as co-chair to several other

graduate students during this time. In his new role, Jonathan will play an important part in advancing the research mission of the department. This will include working with the Associate Dean of Research and Graduate Studies to support graduate education in the department, the administration of intramural grants, and mentoring junior faculty members. Congratulations, Jonathan, on this appointment! (Emily N. Gould)



Dr. John Cullen Named 2022 Hagler Fellow

The Hagler Institute for Advanced Study at Texas A&M University (<https://hias.tamu.edu>) aims to bring the most notable scientists in any field to our campus for a limited time. Every year, a scientific advisory panel picks individuals who have made a big impact in their field from those nominated to become a Hagler Fellow. It is our great pleasure to announce that Dr. John Cullen (pictured at right) was recently inducted as a Hagler Fellow of the class of 2022-23. Dr. Cullen is one of less than 40 Distinguished Diplomates of the American College of Veterinary Pathology and is widely renowned as both a toxicologic pathologist and as a veterinary hepatopathologist. Notably, while the Hagler Institute for Advanced Study

has brought several Noble Laureates to Texas A&M over the last decade, Dr. Cullen is the first veterinarian ever to have been selected as a Hagler Fellow. Congratulations John! What an accomplishment! John has already been here at the GI Lab for two visits, and he will be here one week every month for the next 2-3 years. What makes this so exciting for us is that he is not just visiting, but he also will be playing an active role in our emerging veterinary hepatology group, helping us with planning and conducting highly impactful clinical trials, as well as mentoring graduate students in that area. A great big welcome to John Cullen – or as we like to say in Texas – Howdy! (Jörg M. Steiner)



Dr. Jörg Steiner Named Regents Professor

Dr. Jörg Steiner, University Distinguished Professor and Director of the GI Lab, was recently named Regents Professor with the Texas A&M University System. Texas A&M University, the home of the GI Lab, is the largest university in the Texas A&M University System with more than 70,000 students at the flagship College Station campus and at several other locations within Texas and beyond. In addition, the Texas A&M University System is comprised of 10 other Universities including

Prairie View A&M University, Tarleton State University, and Texas A&M University – San Antonio, just to name a few. Every year, a select group of faculty is honored not only for their achievements in research and teaching, but also for their service to the University System and their profession. Dr. Steiner (pictured at right) is currently the only veterinarian to serve as a Regents Professor for the Texas A&M University System. (Jonathan A. Lidbury)



Vet Ku - Texas A&M 2023 Int. Med. Conference

Focus: Nephrology and Urology

In 2018 and 2019, we held the Vet KU – Texas A&M University Internal Medicine Conference in Thailand. Both years we were joined by around 100 veterinarians from the US and from across Asia. In 2018 our focus was gastroenterology, and in 2019 it was endocrinology. The lectures were both interesting and informative, the panel discussions were lively and thought-provoking, and I can certainly say that I learned a lot. It was also a great experience to spend time getting to know colleagues from all over the world. The conferences were made possible by generous support from IDEXX Laboratories, Nestlé Purina, Nutramax Laboratories, Royal Canin, Hill's Pet Nutrition, Dechra, and Haemaru Animal Referral Hospital.

We had planned to hold our conference annually, but unfortunately, we were not able to have a conference in 2020, 2021, or 2022 for obvious reasons. Therefore, we are very excited to announce the 2023 Internal Medicine Conference in partnership with the Faculty of Veterinary Medicine at Kasetsart University in Bangkok, Thailand. The conference will be held at the family-friendly five-star Hilton, Pattaya, Thailand, between Monday, October 9, 2023, and Friday, October 13, 2023. The focus of this forthcoming conference will be nephrology and urology. A panel of internationally renowned experts will deliver 25 hours of top-quality continuing education.



Our in-depth program focuses on providing you with the latest practically relevant information on renal and lower urinary tract disease in dogs and cats. To this end, several sessions will outline a logical diagnostic approach to challenging but common problems, while others will help you formulate better treatment plans. The final hour of each day will be an interactive session, covering complex and controversial topics. We have been fortunate to recruit four fantastic speakers: Jonathan Elliott from the Royal Veterinary College in London (UK), JD Foster from Friendship Hospital for Animals in Washington, DC (USA), Jody Lulich from the University of Minnesota in St. Paul (USA), and Sherri Ross from the Atlantic Veterinary College in Charlottetown (Canada). Lectures will run between 8:00 am and 1:10 pm, allowing you free afternoons to enjoy the beautiful venue with your family. Thanks to our generous sponsors, a social program will be offered in addition to the educational program. This will



provide an excellent opportunity for you to network and mingle with colleagues from the United States and across Asia.

The 34-story Hilton–Pattaya, Thailand, boasts sweeping ocean views and is adjacent to Central Festival Pattaya Beach, southeast Asia's largest beachfront shopping center. It is also just over a kilometer from Pattaya Walking Street. The outdoor infinity pool boasts panoramic views from the 16th floor, and there are three restaurants, a spa, and a rooftop bar to enjoy. We have negotiated a fantastic rate for conference participants with rooms starting at 7,000 THB (about 200 USD) per night including a buffet breakfast. Please book early as this hotel is very popular! The drive to and from the main airport in Bangkok is easy and convenient and takes approximately one and a half hours. Vibrant, multicultural Pattaya lies on the east coast of the Gulf of Thailand and is about 90 miles from Bangkok. The Hilton has a superb beachfront location offering soft white sands and warm water. Pattaya Beach, the most popular in the area, is close by and offers a wide variety of water sports. The bustle of Central Pattaya with its electrifying nightlife is only a walk away. Other famous attractions in the area include the beautiful island of Koh Larn, the Pattaya floating market, and the unique, ornate Sanctuary of Truth.

For more information on how to secure our discounted room rate and to register for the conference, please visit our conference website at <https://texasimconference.tamu.edu>. If you have any other questions please feel free to contact our office manager, Rhonda Rosa [rrosa@cvm.tamu.edu, (979) 458-1662].

We hope that you can join us for what promises to be an unforgettable event! (Jonathan A. Lidbury)



"World-class continuing education in a breathtaking setting."

Serum Submissions			Fecal Submissions	
Assay	Vol. req'd	Price	Assay	Price
TLI, PLI, Cobalamin, Folate, Cortisol (dogs only)	2.0 ml fasted	\$85.00	Canine Alpha-1 Proteinase Inhibitor	\$54.00
TLI, PLI, Cobalamin, Folate	2.0 ml fasted	\$76.00	Note: A set of 3 fecal samples must be submitted in pre-weighed tubes for testing. Email gilab@cvm.tamu.edu to order fecal α ₁ PI collection tubes (15 for \$25.00).	
TLI, Cobalamin, Folate	1.0 ml fasted	\$55.00	Dysbiosis Index: Canine or Feline	\$48.00
PLI, Cobalamin, Folate	1.0 ml fasted	\$55.00	Canine Enteropathogen Panel	\$110.00
TLI, PLI	1.0 ml fasted	\$55.00	Canine panel includes PCR testing for: net F toxin gene- <i>C. perfringens</i> , <i>C. difficile</i> , <i>Campylobacter jejuni</i> , canine parvovirus, <i>Salmonella</i> spp., and IFA testing for <i>Giardia</i> and <i>Cryptosporidium</i>	
Cobalamin, Folate	1.0 ml fasted	\$38.00	Feline Enteropathogen Panel	\$120.00
TLI	1.0 ml fasted	\$29.00	Feline panel includes PCR testing for: net F toxin gene- <i>C. perfringens</i> , <i>C. difficile</i> , <i>Campylobacter jejuni</i> , feline panleukopenia virus (FPV), <i>Salmonella</i> spp., <i>Tritrichomonas foetus</i> , and IFA testing for <i>Giardia</i> and <i>Cryptosporidium</i>	
PLI	1.0 ml fasted	\$29.00	Real-time PCR Assays	\$36.00 \$12.00
Note: Spec cPL or Spec fPL test is only offered as part of a panel or alone as a follow-up	Pre-feeding: 1.0 ml fasted	\$18.00	First PCR assay	
	2 hrs post-feeding: 1.0 ml	\$18.00	Each additional PCR assay	
Canine C-reactive Protein	0.5 ml fasted	\$31.00	<i>Tritrichomonas foetus</i> , <i>Campylobacter jejuni</i> and <i>C. coli</i> , <i>Heterobilharzia americana</i> , canine parvovirus (CPV-2), feline panleukopenia virus (FPV), <i>Salmonella</i> spp., net F toxin gene- <i>C. perfringens</i>	
Bile Acids			Immunofluorescence Assay (IFA) for <i>Giardia</i> and <i>Cryptosporidium</i>	\$38.00
Methylmalonic Acid	0.5 ml fasted	\$56.00	Bacterial Toxin Assays (ELISA) for <i>Clostridium difficile</i> Toxin A and B	\$34.00
Gastrin	0.5 ml fasted	\$29.00		
Triglycerides	0.5 ml fasted	\$16.00		



Sample submission forms customized with your clinic's information are available on our website at <https://vetmed.tamu.edu/gilab>. Click the "Clinic Login" button.

For any questions or to set up a new account, please contact us by phone at (979) 862-2861 or by email at gilab@cvm.tamu.edu.



Current studies	Brief project description
Treatment trial for canine chronic pancreatitis Dr. Sue Yee Lim – slim@cvm.tamu.edu	This clinical trial aims to assess the efficacy of cyclosporine or prednisolone for treating chronic pancreatitis in dogs . Patients will receive prednisolone or cyclosporine for the three weeks of the study at no charge as well as GI panels.
Treatment trial for feline chronic pancreatitis Dr. Yu-An (Andy) Wu – yuanwu@cvm.tamu.edu	The aim of this case series is to assess the efficacy of cyclosporine for treating chronic pancreatitis in cats . Costs of the cyclosporine will be reimbursed. The study includes a total of 3 visits (initial appointment, 10th day, and 21st day). Shipping and GI panels are covered.
Evaluation of markers of pancreatic disease in cats before and after switching to a special diet for kidney disease or diabetes mellitus (The CATPAD study) Dr. Yu-An (Andy) Wu – yuanwu@cvm.tamu.edu	The CATPAD study is a project that looks at cats' pancreatic health and the possible association with diet. We are currently enrolling cats that are about to be switched to a commercially available therapeutic diet intended for cats with kidney disease or diabetes mellitus. More information is available at: https://vetmed.tamu.edu/gilab/research/catpad-study/ .
Evaluation of anti-inflammatory and cytotoxic properties of acid suppressants on canine resectable mast cell tumors (MCTs) Dr. Emily Gould – egould@cvm.tamu.edu	Study aims are to evaluate blood and tissue cytokines, MCT viability, and quantifiable histamine (and/or histamine metabolites) before and after acid suppressant or placebo therapy in dogs with surgically resectable MCTs. Study includes a total of 3 visits (initial appointment, surgical resection of tumor, and one post-operative recheck) and will cover \$500 of the patient's surgical bill at Texas A&M.