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Development and analytical validation of a radioimmunoassay for the quantification of α_1 -proteinase inhibitor in serum and feces from the common marmoset (*Callithrix jacchus*)

DR. Joseph Cyrus Parambeth*, Jonathan A. Lidbury, Jan S. Suchodolski, and Jörg. M. Steiner

Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary and Biomedical Sciences, Texas A&M University, College Station, Texas 77843-4474

Abstract

Background: The objective of this study was to develop and analytically validate a radioimmunoassay (RIA) for the measurement of α_1 -proteinase inhibitor (α_1 -PI) concentrations in serum and feces from the common marmoset.

Methods: Serum samples ($n = 30$) and 3-day fecal samples ($n = 30$) were obtained from healthy marmosets. An RIA was established and validated by determination of sensitivity, working range, dilutional parallelism, spiking recovery, and intra- and interassay variability. A reference interval for α_1 -PI in serum and feces was established.

Results: Sensitivity and upper limit of the working range were 0.75 and 100.62 $\mu\text{g/L}$ respectively. Observed-to-expected (O/E) ratios for serial dilutions ranged from 89.9 – 123.0% (mean $\pm$ SD: 106.0 $\pm$ 11.5%) for 8 serum samples, and from 90.6 – 132.7% (mean $\pm$ SD: 107.6 $\pm$ 19.2%) for 4 fecal samples. O/E ratios for spiking recovery ranged from 97.6 – 104.4% (mean $\pm$ SD: 101.3 $\pm$ 3%) for 4 serum samples, and from 97.5 – 101.4% (mean $\pm$ SD: 99.2 $\pm$ 1.8%) for 4 fecal samples and 3 different spiking concentrations. Coefficients of variation (CV) for intra-assay variability for 8 serum samples ranged from 1.7 to 10.6%, and 2.2 to 5.1% in the 8 fecal samples. The interassay CV for eight serum samples ranged from 1.3 to 9.9%, and from 1.0 to 6.7% in the 8 fecal samples. The reference interval in serum was determined to be 1,047 – 1,484 $\mu\text{g/L}$. The reference interval in serum was determined to be 1,047 – 1,484 $\mu\text{g/L}$. The reference interval for the 3-day mean fecal concentration, and 3-day maximum fecal concentration were determined to be 32.4–124.4 $\mu\text{g/g}$ and 39.1–158.7 $\mu\text{g/g}$ of feces respectively.

Conclusion: The developed assay is sensitive, linear, accurate, precise, and reproducible. Further studies are needed to determine the clinical utility of this assay.

Keywords

New World primate; protein losing enteropathy; immunoassay

*Corresponding author: Tel +1 979 862 2986, JCyrus@cvm.tamu.edu (Joseph Cyrus Parambeth).

Introduction

The common marmoset (*Callithrix jacchus*) is a New World primate that is used in biomedical research.¹ The popularity of the marmoset is related to its small size, relatively easy maintenance, absence of certain zoonotic diseases, multiple births at each parturition, and relatively short life span.² However, inflammation of the gastrointestinal tract has been commonly reported in common marmosets kept in captivity. The disease has been deemed to be endemic, with the reported prevalence ranging from 28–60 % in the laboratory setting.³ Chronic lymphocytic enteritis (CLE) is associated with chronic progressive weight loss in adults despite a good appetite, failure to thrive in juveniles. Diarrhea may or may not be present. The only consistent clinical pathological abnormalities are a mild anemia and hypoproteinemia.^{3,4} The disease is often terminal with affected marmosets either dying of being euthanized. The current gold standard for diagnosis is necropsy.

The development of a non-invasive test that may allow early ante-mortem diagnosis would facilitate screening and removal of affected marmosets before they are enrolled in research trials or breeding programs, thereby reducing the impact of this disease. Given that hypoproteinemia, particularly hypoalbuminemia, has been reported consistently in marmosets with chronic inflammation of the intestinal tract, we hypothesize this hypoproteinemia may be due an intestinal loss of protein as seen in other species with inflammatory bowel disease. The gold standard for detecting gastrointestinal protein loss is the excretion of ⁵¹Cr-albumin in feces, which is not feasible as a routine diagnostic test given the radiation hazards involved.

Alpha₁-proteinase inhibitor (α_1 -PI) is a serum protein produced by the liver that is only lost in minimal quantities into the GI tract during health. Given the similar molecular weight to albumin, it is lost into the intestinal tract at a similar rate as is albumin. However, unlike albumin, it is resistant to degradation by digestive enzymes and bacteria within the intestinal lumen. Intact fecal α_1 -PI can be detected by immunoassays and have been employed to detect increased enteric protein loss in humans,⁵ dogs,⁶ and cats.⁷ In dogs with protein losing enteropathy, increased fecal α_1 -PI concentrations are thought to precede hypoalbuminemia. Assays are species specific and do not cross react.⁸ Recently, we described the purification and partial characterization of marmoset α_1 -PI from marmoset serum.⁹

The aims of this study were to develop and analytically validate an RIA for the quantification of α_1 -PI in serum samples and fecal extracts from marmosets and to establish reference intervals for serum and fecal α_1 -PI concentrations in healthy marmosets.

Materials and methods

Marmoset serum & fecal samples

Serum and fecal samples previously submitted to the Gastrointestinal Laboratory for research testing, and serum harvested from common marmosets euthanized as part of routine colony management procedures stored at –80°C and shipped on dry ice were used for assay development and analytical validation.

Animals

A total of 30 marmosets from two research colonies of common marmosets accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (Southwest National Primate Research Center [SNPRC], Texas Biomedical Research Institute, San 307 Antonio, Texas and the Barshop Institute for Longevity & Aging Studies [BMAC] University of Texas Health Science Center at San Antonio) were used to establish reference intervals for serum and fecal α_1 -PI concentrations in healthy marmosets. These procedures were approved by the animal care and use committee at the respective institutions (AUP 1259CJ & AUP 06120X).

Marmosets at the BMAC were fed a purified diet (#TD.07148 Marmoset Diet, Harlan Teklad Madison, WI), and a primate enrichment mixture consisting of nuts, seeds, and dried fruit from the same manufacturer, both of which were irradiated at the manufacturing plant. Marmosets at the SNPRC were on two diets, a commercial New World primate diet (Mazuri diet (AVP Callitrichid 5LK6) and also on the same purified diet (without irradiation) used at the BMAC. Diet for marmosets at the SNPRC also included daily food enrichment, such as yogurt, fruits, cheerios, etc.

Blood was collected from femoral bleeds from non-anesthetized marmosets held in a restraint device to which they were habituated. Paired serum and naturally passed fecal samples were collected from each animal. Since it has been demonstrated that fecal α_1 -PI concentrations can have day-to-day variations in dogs, and no data exists for the common marmoset, fecal samples were collected for three consecutive days from each animal. Fecal samples were collected as soon after defecation as possible in pre-weighed polypropylene tubes, and stored at -80°C until they were shipped on dry ice.

Extraction of fecal samples

Fecal samples were thawed and then the extraction was done using phosphate buffered saline supplemented with 5% newborn calf serum, 1% Triton X-100, and 0.25 mM thimerosal in a 1:5 dilution using approximately 1.0 g wet weight of feces. The fecal samples were homogenized by vortexing for 20 minutes, then centrifuged for 20 min at $2,100 \times g$ and 5°C , and then the supernatants [fecal extract] were collected using serum filters, and were stored frozen at -80°C until analysis. This extraction is similar to the extraction described for the canine assay ⁸.

Purification of marmoset α_1 -PI

Marmoset α_1 -proteinase inhibitor was purified from marmoset serum by affinity chromatography, and ceramic hydroxyapatite chromatography as described previously ⁹.

Antibody production

Polyclonal antibodies were raised in New Zealand white rabbits using a commercial antibody production service (Express-Line PLUS protocol; Lampire Biological Laboratories, Pipersville, Pennsylvania, USA). Purified marmoset α_1 -PI in phosphate buffered saline was used as the antigen and antiserum specificity was tested as previously

published⁹. Antiserum obtained after the fourth booster injection was selected for the RIA procedure with a final dilution, of 1:5,000.

Dilution of serum samples

Serum samples were diluted 1:32,000 in radioimmunoassay buffer (RIAB, 0.05 M sodium phosphate, with 0.02% (w/v) sodium azide and 0.5% (w/v) bovine serum albumin (pH 7.5)) for the RIA.

Dilution of fecal samples

Previously stored fecal extracts were thawed and diluted 1:200 in RIAB for the RIA.

Tracer Radioiodination

The tracer was produced by iodination of pure marmoset α_1 -PI with ^{125}I , using the chloramine T method.¹⁰ A stir bar [8 mm $\times$ 1.5 mm; VWR Scientific, West Chester, Pennsylvania, USA] was placed in a polypropylene test tube (75 mm $\times$ 12 mm; VWR Scientific) and 10 μL of 0.25 M sodium phosphate buffer [Sigma-Aldrich, St. Louis, MO] was pipetted into it, which was then placed over a stir plate. With the use of a Hamilton syringe (VWR Scientific) 10 μL of carrier free I^{125} [NaI, 1.0 mCi at time of production] (Perkin Elmer, Waltham, MA) was dispensed into the tube. Then the following solutions were added in succession: 10 μg of the marmoset α_1 -PI, 10 μL of the chloramine T solution [2 mg/mL], 100 μL of a sodium metabisulfite solution (0.4 mg/mL), and 860 μL of a potassium iodide solution (2 mg/mL). All the solutions used were dissolved in 0.05 M sodium phosphate (pH, 7.5). The iodinated $m\alpha_1$ -PI fraction was separated from the free iodide by size-exclusion chromatography on a disposable column equilibrated with 10% (wt/vol) bovine serum albumin (PD-10; GE Healthcare, Piscataway, New Jersey, USA) following the manufacturer's directions. The RIA buffer (RIAB) was used as the mobile phase, and 1-mL fractions were collected. The fraction containing the peak protein concentration was used as the tracer. The tracer was adjusted with RIA buffer to approximately 45,000 counts/minute/100 μL tracer and kept at 4°C until further use.

Marmoset α_1 -proteinase inhibitor- radioimmunoassay procedure

All polypropylene reaction tubes (VWR Scientific) were set up in a duplicate fashion. The tubes were labelled total count (TC), nonspecific binding (NB), reference blank (B0), and standards labelled 100, 50, 20, 10, 5, 2, and 1 ng/mL. The tubes were filled accordingly: TC received 100 μL tracer only, NB received 100 μL tracer and 200 μL RIAB, B0 received 100 μL tracer, 100 μL antibody solution, and 100 μL RIAB. The standards received 100 μL tracer, 100 μL antibody solution, and 100 μL standard solution of 100, 50, 20, 10, 5, 2, and 1 ng/mL $m\alpha_1$ -PI in RIAB, respectively. Unknown samples received 100 μL tracer, 100 μL antibody solution, and 100 μL of the unknown sample. Tubes were vortexed and then incubated for 4 hours at room temperature. At the end of the incubation period, all, except the tubes labeled TC, received 100 μL of a rabbit carrier serum (1 mL normal rabbit serum mixed with 99 mL RIAB) and 1 mL of a commercially available precipitation solution (N6; Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). All tubes except the TC tubes were vortexed and then centrifuged at 3,360 $\times$ g and 10°C for 30 min. The supernatant

of all tubes, were carefully decanted. The tubes were visually inspected to ensure that the pellets were present. The tubes were placed in a gamma counter (Riastar; Packard Instrument Company, Meriden, Connecticut, USA) and counted for 1 minute. A standard curve was generated using a log/logit curve fit. $\text{Ma}_1\text{-PI}$ concentrations were marked on the x-axis in a logarithmic fashion and values on the y-axis were calculated from $y = \log_e\left(\left(B_{\text{standard}} / B_0\right) / \left(1 - \left(B_{\text{standard}} / B_0\right)\right)\right)$ with B_{standard} being the counts per minute for each standard and B_0 being the counts per minute for the reference blank. Test samples containing $\text{ma}_1\text{-PI}$ in concentrations greater than the working range of the assay were further diluted by a factor of 2 and re-assayed.

Marmoset α_1 -proteinase inhibitor radioimmunoassay validation

The $\text{ma}_1\text{-PI}$ RIA was analytically validated by determining the lower limit of the assay, the linearity, accuracy, precision, and reproducibility by evaluating assay sensitivity, dilutional parallelism, spiking recovery, intra-assay variability, and inter-assay variability. Serum and fecal samples were selected at random from the available samples and efforts were made to ensure that they fell into different areas of the working range of the assay. Serum samples used for validation were generated from a pool of several samples because marmosets are significantly smaller than companion animals and serum volumes greater than 0.5 mL were not available for most animals. The lower limit of the assay was determined by analyzing 10 duplicates of the B_0 tubes and calculating the mean and the standard deviation of the raw counts for the zero $\text{ma}_1\text{-PI}$ concentration. The $\text{ma}_1\text{-PI}$ concentration that corresponded to the mean count minus three standard deviations was estimated on the standard curve and defined as analytic sensitivity (lower limit of the working range) of the RIA. The upper limit of the working range was taken as the highest standard. Linearity of the assay was determined by use of 8 serum samples in two fold dilutions from 1:32,000 to 1:512,000, and 4 fecal samples in two fold dilutions from 1:1 to 1:16. Accuracy of the assay was tested by spiking 4 sera and 4 fecal extracts with known concentrations of pure $\text{ma}_1\text{-PI}$ (20, 50, and 100 $\mu\text{g/L}$ for serum samples and 20, 50, and 100 $\mu\text{g/g}$ for fecal extracts). Recovery was determined by calculating the ratio of the observed/expected $\times 100$. Intra-assay variability or precision of the assay was evaluated by assaying 8 serum samples, and 8 fecal extracts 4 times within the same assay, followed by calculating the coefficient of variation: $\text{CV} \% = (\text{SD}/\text{mean}) \times 100$. Inter-assay variability or reproducibility of the assay was determined by analyzing 8 sera and 8 fecal extracts in 3 consecutive assays and calculating inter-assay coefficient of variation; $\text{CV} \% = (\text{SD}/\text{mean}) \times 100$. To evaluate if there was a position effect within the assay (end of-run effect) two samples were tested in the same run by analyzing each sample 24 times in the same assay, with 8 duplicates placed in the beginning, middle, and at the end of the same assay. Mean $\text{ma}_1\text{-PI}$ concentration calculated from these positions on the assay were compared using a Kruskal-Wallis test (rank sum) test.

Reference interval determination

The reference interval for serum concentrations of marmoset $\alpha_1\text{-PI}$ were determined by calculating the central 95th percentile of serum concentrations measured in the 30 healthy marmosets from both the colonies. Fecal $\alpha_1\text{-PI}$ concentrations were measured in the same 30 marmosets, and the reference interval was established calculating the 95th central

percentiles of both, the mean and the maximum fecal α_1 -PI concentrations in the fecal samples from 3 consecutive days. The coefficient of variation was also calculated between the three separate fecal samples collected. None of the marmosets had any clinical signs of wasting or lymphocytic enteritis and therefore at the time of collection of serum or feces and were deemed to be healthy. Normality was tested using

Shapiro–Wilk test.

Statistical analyses were performed using a statistical software program [Graph Pad prism software, GraphPad Software, Inc. La Jolla, CA 92037 USA) and statistical significance was set at $p < 0.05$.

Results

The lower limit of the assay was calculated to be 0.8 $\mu\text{g/L}$. The upper limit of the working range was calculated to be 100.6 $\mu\text{g/L}$. Eight serum samples in two fold dilutions from 1:32,000 to 1:512,000 and 4 fecal samples in two fold dilutions from 1:1 to 1:16 were assayed. The observed to expected ratios (O/E) for serial dilutions were (minimum-maximum (mean $\pm$ SD)) 89.9 – 123.0 % (106.0 $\pm$ 11.5%) for serum, and 90.6 – 132.7% (107.6 $\pm$ 19.2%) for fecal extracts [Table 1a, 1b]. The O/E for spiking recovery for 3 different spiking concentrations were (minimum-maximum (mean $\pm$ SD)) 97.6 – 104.4% (101.3 $\pm$ 3%) for serum and 97.5 – 101.4% (99.2 $\pm$ 1.8%) for serum [Table 2a, 2b]. Intra-assay coefficients of variation (CV %) (n=8) were 1.7, 2.8, 2.8, 3.5, 4.6, 4.6, 5.6, and 10.6% for serum samples and 2.2, 2.3, 2.7, 3.1, 4.0, 4.3, 4.3, and 5.1% for fecal extracts (n=8) [Table 3a, 3b]. Also, inter-assay coefficients of variation (CV %) (n=8) were 1.3, 5.2, 5.6, 6.3, 8.0, 8.2, 8.4, and 9.9% for serum samples and were 1.0, 1.1, 2.6, 3.2, 3.3, 3.8, 4.8, and 6.7% for fecal extracts (n=8) [Table 4a, 4b]. No significant differences in mean α_1 -PI concentrations depending on position of samples in the assay were observed ($p=0.4128$).

A reference interval for serum and fecal α_1 -proteinase inhibitor concentrations (three day mean, three day maximum) in feces was calculated using the central 95th percentile using serum and fecal samples from healthy control marmosets [Table 5, Figures 1–3]. The median coefficient of variation for fecal α_1 -proteinase inhibitor concentrations between the three-day fecal samples was 21.4% (range: 2.9 – 74.3%).

Discussion

An RIA for the measurement of α_1 -PI in serum samples and fecal extracts from marmosets was successfully developed. The analytical sensitivity of the assay was 0.8 $\mu\text{g/L}$. This value is adequate, considering the reference interval of 1,047 – 1,484 mg/L in serum and 32 – 124 ($\mu\text{g/g}$) in feces. The O/E values for the dilutional parallelism ranged from 89.9 – 123.0%, 90.6 – 132.7% and spiking recovery were 97.6 – 104.4%, 97.5 – 101.4% for serum and feces, respectively. These values indicate that the assay is linear and accurate and is close to what has been reported to be acceptable of a range of 80–120%.^{11,12} Two samples, one serum sample with an O/E of 123.0% and one fecal sample with an O/E of 132.7% were outside the “acceptable” limits.

One limitation of the study was that while the lower and upper limits of detection were established, a quantifiable measure of the upper and lower limits of quantitation (ULOQ/ LLOQ) of the assay were not. The LLOQ may have explained the minor failures in the dilutional linearity test (123.0% for serum and 132.7% for fecal extracts).

The coefficients of variability were relatively low for both intra- and inter-assay variability the maximum being 10.6%, indicating that the assay is precise and reproducible, and is well within the acceptable ranges of <15%.^{11,12} No effect of position of samples on the run was detected in the assay.

The reference interval for serum marmoset α_1 -PI values are comparable with the reference intervals reported for canine, feline, and human α_1 -PI in plasma/serum which are 732–1,802 mg/L,⁸ 250–600 mg/L,¹³ and 900–1,200 mg/L,¹⁴ respectively.

However, the fecal α_1 -PI concentrations in healthy cats ranged from 0.04 (i.e., undetectable) to 1.72 $\mu\text{g/g}$ (median: 0.51 $\mu\text{g/g}$). In adult dogs, fecal α_1 -PI concentrations ranged from <2.2 to 26.8 $\mu\text{g/g}$ (median: 4.7 $\mu\text{g/g}$), whereas in the 30 marmosets in this study the concentrations ranged from 30.8–134.0 $\mu\text{g/g}$ (median: 63.5 $\mu\text{g/g}$). Most human fecal α_1 -PI assays are performed on dried / lyophilized feces. In one study using wet weight of feces, in healthy human individuals fecal α_1 -PI concentrations measure using an ELISA ranged from 1–964 $\mu\text{g/g}$ and a concentration of <545 $\mu\text{g/g}$ was considered normal.⁵ Possible explanations for this finding are subclinical disease in some of the marmosets in this study or possibly that marmosets lose more α_1 -PI into their gastrointestinal tract than carnivores.

A relatively high coefficient of variation for fecal α_1 -proteinase inhibitor concentrations between the three-day fecal samples were seen in the marmosets (median 21.4%, Range 2.9 – 74.3%). The reason for this is unknown, however a similarly high CV has also been reported for dogs (median: 29.2%, range 0.0% to 102.0%).¹⁵ One possible explanation for this could be the effect of water content, as the fecal samples were normalized to volume. This perhaps could have potentially been reduced by normalization to an independent variable like dry weight and this was not further explored in this study.

As serum and fecal samples were not readily available, stability of samples at different storage conditions were not studied. Sample collection and storage conditions used in dogs^{15,16} and cats⁷ were presumed to be appropriate. As the samples were collected, stored, extracted, and assayed around the same time from the two marmoset colonies, we do not expect this to affect our reference interval, however, changes in serum concentrations have been reported in dogs where samples were stored over a period of one year.⁸

A limited number of samples were available to establish the serum and fecal reference intervals for m α_1 -PI. Given the small number of animals in the study, no attempts were made to make age specific reference intervals, which may have been more appropriate as younger dogs are reported to have higher concentrations of fecal α_1 -PI.¹⁵ While it would have been ideal to have 120 marmoset samples to establish the reference interval, this would not have been practical. The newly revised Clinical and Laboratory Standards Institute's (CLSI) document, does describe establishing a reference interval for an analyte using a samples size of 20, however it only permits two individuals to be outside the established

reference interval. In our study, 4,2, and 2, individuals were outside the established reference intervals for serum, three day mean fecal, and three day maximum fecal α_1 -PI concentrations respectively. Hence, additional work may be needed to establish a more robust reference interval for serum α_1 -PI concentrations in the common marmoset using this assay.

Another limitation of this study was that the animals that were used to establish the reference interval were presumed to be healthy based on absence of clinical signs, however, given how little is known about the disease, subclinical chronic lymphocytic enteritis cannot be ruled out as gastrointestinal biopsies were not available.

The RIA for the measurement of serum and fecal α_1 -PI concentrations in the common marmoset described here is sensitive, linear, accurate, precise, and reproducible. Further studies are needed to determine the utility of this test for diagnosing gastrointestinal disease, particularly CLE in the marmoset.

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Abbreviations

α_1-PI	Alpha ₁ -proteinase inhibitor
CLE	Chronic lymphocytic enteritis
CV	Coefficients of variation
O/E	Observed-to-expected
RIA	radioimmunoassay

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Figure 1.
Histogram showing the normal distribution of the 95% confidence interval of the serum α1-PI concentration (mg/L).

Figure 2. Histogram showing the normal distribution of the 95% confidence interval of the three day mean fecal α_1 -PI concentration ($\mu\text{g/g}$).

Figure 3.

Histogram showing the normal distribution of the 95% confidence interval of the three day maximum fecal α_1 -PI concentration ($\mu\text{g/g}$).

Table 1.

Dilutional parallelism of a radioimmunoassay (RIA) for the measurement of serum alpha₁-proteinase inhibitor (α₁-PI) concentrations in the common marmoset

Serum Sample ID	Dilution	Observed α ₁ -PI Concentration (ug/L)	Expected α ₁ -PI Concentration (ug/L)	O/E %	Average O/E %
S1	1:32000	28.7	N/A	N/A	99.9
	1:64000	16.2	14.3	113.3	
	1:128000	7.1	7.2	98.8	
	1:256000	4.0	3.6	111.6	
	1:512000	1.4	1.8	76.1	
S2	1:32000	31.4	N/A	N/A	118.6
	1:64000	19.1	15.7	121.5	
	1:128000	7.7	7.9	98.6	
	1:256000	5.7	3.9	144.9	
	1:512000	2.1	2.0	109.3	
S3	1:32000	29.0	N/A	N/A	123.0
	1:64000	17.7	14.5	122.4	
	1:128000	7.5	7.2	103.7	
	1:256000	4.4	3.6	120.4	
	1:512000	2.6	1.8	145.5	
S4	1:32000	30.1	N/A	N/A	114.5
	1:64000	15.2	15.1	101.2	
	1:128000	8.7	7.5	116.2	
	1:256000	4.7	3.8	125.0	
	1:512000	2.2	1.9	115.4	
S5	1:32000	28.2	N/A	N/A	89.9
	1:64000	17.0	14.1	120.5	
	1:128000	4.6	7.1	65.8	
	1:256000	2.5	3.5	71.1	
	1:512000	1.8	1.8	102.4	
S6	1:32000	28.1	N/A	N/A	102.9
	1:64000	15.2	14.0	108.3	
	1:128000	5.8	7.0	81.9	
	1:256000	3.6	3.5	103.2	
	1:512000	2.1	1.8	118.2	
S7	1:32000	34.1	N/A	N/A	102.2
	1:64000	20.3	17.0	118.8	
	1:128000	10.0	8.5	117.8	
	1:256000	3.9	4.3	90.4	

Serum Sample ID	Dilution	Observed α_1 -PI Concentration (ug/L)	Expected α_1 -PI Concentration (ug/L)	O/E %	Average O/E %
	1:512000	1.7	2.1	81.7	
S8	1:32000	31.9	N/A	N/A	97.0
	1:64000	15.4	16.0	96.8	
	1:128000	7.7	8.0	96.9	
	1:256000	3.7	4.0	93.5	
	1:512000	2.0	2.0	100.7	

O/E — observed/expected

Table 1b.

Dilutional parallelism of a radioimmunoassay (RIA) for the measurement of fecal α_1 -proteinase inhibitor (α_1 -PI) concentrations in the common marmoset

Sample	Dilution	Observed α_1 -PI Concentration ($\mu\text{g/g}$)	Expected α_1 -PI Concentration ($\mu\text{g/g}$)	Observed/Expected %	Average O/E%
F1		131.2			94.7
	1:2	61.1	65.6	93.2	
	1:4	30.8	32.8	93.8	
	1:8	15.7	16.4	95.4	
	1:16	7.9	8.2	96.4	
F2		166.8			90.6
	1:2	74.2	83.4	88.9	
	1:4	37.8	41.7	90.5	
	1:8	17.5	20.9	84.0	
	1:16	10.3	10.4	98.8	
F3		90.3			132.7
	1:2	49.1	45.1	108.8	
	1:4	27.8	22.6	123.0	
	1:8	14.9	11.3	132.3	
	1:16	9.4	5.6	166.6	
F4		85.0			112.4
	1:2	45.6	42.5	107.3	
	1:4	21.0	21.2	99.1	
	1:8	10.8	10.6	101.7	
	1:16	7.5	5.3	141.6	

O/E — observed/expected

Table 2a.

Spiking recovery of a radioimmunoassay (RIA) for the measurement of serum alpha₁-proteinase inhibitor (α₁-PI) concentrations in the common marmoset

Sample	Spiked concentration (µg)	Observed α ₁ -PI Concentration (µg/g)	Expected α ₁ -PI Concentration (µg/g)	Observed/Expected %	Average O/E%
S1	-	19.7			102.6
	10	31.7	29.7	106.7	
	25	44.6	44.7	99.8	
	50	70.5	69.7	101.2	
S2	-	21.8			104.4
	10	35.6	31.8	111.9	
	25	46.9	46.8	100.0	
	50	72.6	71.8	101.1	
S3	-	23.1			99.7
	10	32.5	33.1	98.2	
	25	48.6	48.1	101.0	
	50	73.0	73.1	99.9	
S4	-	20.5			97.6
	10	31.0	30.5	101.6	
	25	42.3	45.5	93.2	
	50	69.0	70.5	98.0	

O/E — observed/expected

Table 2b.

Spiking recovery of a radioimmunoassay (RIA) for the measurement of fecal α_1 -proteinase inhibitor (α_1 -PI) concentrations in the common marmoset

Sample	Spiked concentration (µg)	Observed α_1 -PI Concentration (µg/g)	Expected α_1 -PI Concentration (µg/g)	Observed/Expected %	Average O/E%
F1	-	39.1			97.5
	10	48.4	49.1	98.5	
	25	65.0	64.1	101.4	
	50	82.5	89.1	92.6	
F2	-	52.1			101.4
	10	66.8	62.1	107.6	
	25	79.4	77.1	102.9	
	50	95.7	102.1	93.7	
F3	-	44.0			99.9
	10	55.0	54.0	101.8	
	25	68.7	69.0	99.5	
	50	92.4	94.0	98.3	
F4	-	24.0			97.9
	10	35.7	34.0	104.9	
	25	49.5	49.0	100.9	
	50	65.1	74.0	88.0	

O/E — observed/expected

Table 3.

Precision of the radioimmunoassay for serum α_1 -proteinase inhibitor concentrations in the common marmoset (intra-assay validation)

Serum Sample	Marmoset α_1 -PI Concentration ($\mu\text{g/g}$)					%CV
	I	II	III	IV	Mean	
S1	36.8	37.2	39.7	40.4	38.5	4.6
S2	40.0	37.4	39.7	38.1	38.8	2.8
S3	32.0	30.2	31.8	34.3	32.1	4.6
S4	32.6	33.2	34.8	34.6	33.8	2.8
S5	36.1	33.8	39.0	38.5	36.8	5.6
S6	32.3	37.9	42.6	41.8	38.6	10.6
S7	34.1	33.8	32.7	32.9	33.4	1.7
S8	30.4	31.7	32.8	30.0	31.2	3.5

CV: coefficient of variation

Table 3b.

Precision of the radioimmunoassay for fecal α_1 -proteinase inhibitor concentration in the common marmoset (intra-assay validation)

Fecal Sample	Marmoset α_1 -PI Concentration ($\mu\text{g/g}$)					%CV
	I	II	III	IV	Mean	
F1	25.4	26.5	26.9	25.7	26.2	2.3
F2	58.1	59.6	57.3	55.3	57.6	2.7
F3	40.7	41.9	43.5	44.0	42.5	3.1
F4	25.3	25.2	26.2	28.0	26.2	4.3
F5	51.6	50.5	48.9	55.0	51.5	4.3
F6	31.3	30.1	29.9	29.6	30.2	2.2
F7	12.1	11.4	12.9	12.9	12.3	5.1
F8	17.0	16.2	18.2	17.0	17.1	4.0

CV: coefficient of variation

Table 4a.

Reproducibility of the radioimmunoassay for serum α_1 -proteinase inhibitor concentrations in the common marmoset (inter-assay validation)

Serum Samples	Marmoset α_1 -PI Concentration ($\mu\text{g/g}$)				% CV
	I	II	III	Mean	
S1	40.1	38.5	42.9	40.5	5.6
S2	39.8	40.3	47.3	42.5	9.9
S3	41.5	41.2	46.0	42.9	6.3
S4	42.8	40.0	44.3	42.3	5.2
S5	40.6	41.3	40.3	40.7	1.3
S6	37.2	34.0	39.9	37.0	8.0
S7	36.7	35.9	41.7	38.1	8.2
S8	37.7	33.5	39.6	36.9	8.4

CV: coefficient of variation

Table 4b.

Reproducibility of the radioimmunoassay for fecal α_1 -proteinase inhibitor concentrations in the common marmoset (inter-assay validation)

Fecal Samples	Marmoset α_1 -PI Concentration ($\mu\text{g/g}$)				% CV
	I	II	III	Mean	
F1	60.93	60.14	61.29	60.79	0.97
F2	59.02	59.73	62.06	60.27	2.64
F3	73.85	77.42	78.57	76.61	3.21
F4	67.14	76.26	74.50	72.63	6.66
F5	39.98	42.83	42.59	41.80	3.79
F6	93.12	102.35	96.80	97.42	4.77
F7	84.19	89.94	87.20	87.11	3.30
F8	74.63	73.16	73.37	73.72	1.08

CV: coefficient of variation

Table 5.

Concentrations of serum and fecal alpha₁-proteinase inhibitor (α₁-PI) in healthy marmosets (n=30)

	Concentration of marmoset α ₁ - PI		
	Serum concentration (mg/L)	Three day mean fecal concentration (μg/g)	Three day maximum fecal concentration (μg/g)
Reference interval	1046 – 1484	32 – 124	39 – 159
Median ± SD	1225 ± 130	64 ± 27	78 ± 36
Range	983 – 1516	31–134	38–190