INTRODUCTION

Pancreatitis-associated protein-1 (PAP-1) is a 16.5 kDa protein that is expressed at high levels during inflammatory bowel disease (IBD) in humans. In studies at Life Diagnostics, we found PAP-1 levels of 9.7±17.7 μg/g (mean ± SD, n=13) and 115±264 μg/g (mean ± SD, n=14) in feces from healthy dogs and dogs with IBD respectively.

The dog PAP-1 VetBio-1 assay uses SPARCL™[1] technology. Two PAP-1 antibody conjugates are used. One to horseradish peroxidase (HRP), the other to acridan; a chemiluminescent substrate. When HRP and acridan conjugates bind to PAP-1 they are brought into proximity. Upon addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of luminescence that is proportional to PAP-1 concentration.

In practice, a blank, a standard and diluted samples (10 μl) are pipetted into test tubes and mixed with 0.5 ml of combined acridan and HRP conjugates. After 45 minutes, the tubes are sequentially inserted into the VetBio-1 luminometer. Luminescence is measured after injection of background reducer, followed by hydrogen peroxide containing trigger-solution. The concentration of PAP-1 is determined from the ratio of the blank-subtracted sample luminescence to the that of the standard.

Equilibrate reagents to room temperature

⇩

Prime the luminometer

⇩

Program the luminometer and save the program

⇩

Dilute samples

⇩

Prepare standard

⇩

Add blank, standard and diluted samples to tubes (10 μl/tube)

⇩

Reconstitute the acridan/HRP conjugate mix (5.5 ml/vial)

⇩

Add conjugate mix to the tubes (0.5 ml/tube)

⇩

Incubate tubes at room temp for 45 min

⇩

Run the Quick Prime program

⇩

Load the program. Test standards and samples

STORAGE

Store the conjugate and PAP-1 standard vials at or below -20°C. The remainder of the kit should be stored at 4°C.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 5 vials **Store at -20°C**
- PAP-1 stock, **Store at or below -20°C**
- Diluent: CSD50-1, 2x50 ml
- Background reducer: BR9-1, 9 ml
- Trigger solution: TS12-1, 12 ml
- 15 ml centrifuge tubes, 2

Materials required but not provided:

- VetBio-1 luminometer
- Precision pipettors and tips
- 12 x 75 mm borosilicate tubes[2]
- 12 x 75 mm test tube racks

[1] SPARCL technology, using acridan- and HRP-conjugated antibodies, was developed by and is licensed from Lumigen Corp.

[2] Only cylindrical 12 x 75 mm borosilicate glass tubes can be used. Do not use polystyrene or polycarbonate tubes.

GENERAL INSTRUCTIONS

1. Please take the time to completely read all instructions before starting your assay**.** Contact us if you need clarification.
2. All reagents used in the assay should be allowed to reach room temperature before use.

LUMINOMETER SETUP

1. Turn the VetBio-1 luminometer on.
2. Place the tubing from injector 1 into the tube containing background reducer. Tube holders are positioned adjacent to the injectors.
3. Place the tube from injector 2 into the tube containing trigger solution.
4. From the Protocol manager on the keypad select “Prime & Wash”, "Prime”, then “Start”.
5. Open the luminometer drawer and insert an empty 12 x 75 mm tube.
6. Close the drawer and click “Start” again.
7. Wait for priming to complete. Open the drawer and discard the tube.
8. From the Protocol manager select “Measure new experiment”” followed by “Dog PAP-1 (Serum)” or “Dog PAP-1 (Feces)”
9. Select “Start”.
10. The experiment setup screen will be displayed. Enter the number of samples you intend to measure, then press “OK”. Please note that the number of samples does not include the two tubes used for the blank and standard.
11. Enter the sample IDs and dilution factor(s), if different than the default dilution. You can also enter experimental comments.
12. After entering information, press save. The experiment will be saved as a “prepared experiment”.

SAMPLE PREPARATION

Serum and Plasma

We recommend testing at a 10-fold dilution.

1. Mix 10 µl of sample with 90 µl of diluent CSD50-1 into a microcentrifuge tube.
2. Use 10.0 ul as described in the procedure section.

Feces

We recommend that feces be extracted and diluted as follows.

1. Accurately weigh approximately 100 mg of feces into a tared 1.5 ml microcentrifuge tube.
2. Add 9 volumes of CSD50-1 or 10 mM Tris, 150 mM NaCl, 1 mM EDTA pH 7.5 (i.e., 0.9 ml to 100 mg of feces).
3. Vortex several times over a 30-minute period to prepare a suspension that is as homogeneous as possible.
4. Centrifuge in a microcentrifuge (5 minutes at 15,000 rpm).
5. Save the supernatant. This represents a 10-fold “dilution” of the fecal sample. Samples may be stored frozen at or below -20°C.
6. For each sample to be tested mix 10 µl of the 10-fold dilution with 90 µl of CSD50-1 diluent to obtain a 100-fold dilution.
7. Test 10.0 ul as described in the procedure section.

STANDARD PREPARATION

1. Tap the PAP-1 standard vial to ensure that the contents are at the bottom.
2. Carefully remove the tear-off seal and stopper (contents are under vacuum).
3. Reconstitute as detailed on the vial label to give the 400 ng/ml standard
4. Use 10 µl as described in the procedure section.

CONJUGATE PREPARATION

1. The acridan and HRP conjugate mix should be prepared just before use.
2. Tap the vial to ensure that the contents are at the bottom of the vial before carefully removing the stopper.
3. Add 5.5 ml of diluent CSD50-1 to the vial. Insert the stopper and mix gently by inversion several times.
4. Each vial of reconstituted conjugate mix provides enough reagent to measure 0 and 400 ng/ml standards and up to eight samples.[3]

PROCEDURE

STEP 1

1. Determine the number of 12 x 75 mm borosilicate glass tubes required (one each for the blank, standard, and the samples). Ensure that all the tubes you intend to use fit easily in the VetBio-1 sample holder before beginning the test.
2. Pipet 10 µl of diluent into tube 1. This serves as the zero standard or blank.
3. Pipet 10 µl of the 400 ng/ml PAP-1 standard into tube 2.
4. Pipet 10 µl of the diluted samples into tubes 3, 4, 5…
5. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available. Avoid creating bubbles.
6. Incubate the mixtures at room temperature for 45 minutes.

[3] If using multiple vials of conjugates to measure more than eight samples, combine the reconstituted contents of all vials and mix briefly before dispensing 0.5 ml aliquots into the reaction tubes.

STEP 2

1. Two to three minutes before the end of the incubation step, insert an empty tube into the sample holder. Select “QuickPrime” from the Protocol manager, close the drawer and press start. Discard the tube after completion. This step ensures that the injectors are fully loaded.
2. Recall your saved experiment and press start.
3. After the end of the incubation, insert tube 1 (Blank) into the sample holder of the luminometer and close the drawer. When the luminescence value is recorded on the screen open the drawer and remove the tube.
4. Similarly measure luminescence of the standard (tube 2), followed by the samples (tubes 3, 4, 5…).
5. CRP concentrations of the samples are automatically calculated.
6. After measurement of the last sample, select “End”.
7. Results will be saved and can be recalled. They may be exported as Excel or pdf files via a USB stick.

RESULTS

If RLU/s values for diluted samples are between the blank and standard, assume that the calculated values are correct. If RLU/s values significantly exceed that of the standard, PAP-1 concentrations may be underestimated. If an accurate measurement is required, such samples should be retested at a higher dilution.

LUMINOMETER MAINTENANCE

The luminometer injectors must be cleaned with distilled or deionized water at the end of each day of use to avoid clogging of the injector ports.

1. From the Protocol manager screen select “Prime & Wash”.
2. Select “Backprime” to return unused reagents to the respective tubes.
3. If future use of the reagents is intended store the sealed tubes in a refrigerator.
4. Place the tubing from injectors 1 and 2 into separate 15 ml centrifuge tubes containing distilled or deionized water.
5. Select “Wash” from the “Prime & Wash” screen. Press “Start”, insert an empty tube into the luminometer, close the drawer and press “Start”.
6. Discard the tube.
7. Leave the injector tubing immersed in water.
8. Switch the luminometer off. It should not be left on when not in use.

REPLICATES

Replicate measurements of the blank/standard and samples can be measured. This requires minor editing of the program.

1. In Protocol manager, select “Measure new experiment” and the program you would like to edit.
2. Press “Edit”.
3. You can change the sample dilution factor, the number of replicates for the blank and standard, and the number of replicates for samples (leave the standard concentration as 400 ng/ml).
4. Press Start, enter the number of samples, and save the experiment.

Please note that these settings will be saved for future runs unless re-edited.

ASSAY PERFORMANCE

Typical data: The table below shows results from an assay in which the zero standard (blank), 40 ng/ml standard, and eight fecal extracts were tested as singlets.

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Dog PAP-1 (Feces) Exp#001 10/08/2021 15:35:06

Single Assay	Start by :	DOOR	Blank :	ON
Measurement	Delay [s] :	2.600	Time [s] :	0.800
Injector 1	Delay [s] :	1.000	Volume [µL] :	50
Injector 2	Delay [s] :	2.000	Volume [µL] :	100

Sample	Dilution	Rep	RLU/s	Conc [µg/mL]
Blank			1,182	0
Standard			172,952	0.4
2	100		370,942	86.106
3	100		56,299	12.835
4	100		1,972	0.18397
5	100		112,229	25.859
6	100		43,909	9.9496
7	100		417,475	96.942
8	100		97,998	22.545
9	100		42,409	9.6003

Reproducibility: Four fecal extracts were tested in quadruplicate in three separate assays. Intra-assay and inter-assay variability are listed below.

	Intra-assay			Inter-assay		
Sample	µg/ml	SD	CV	µg/ml	SD	CV
1	29.82	0.45	1.5	28.84	1.18	4.1
5	23.25	1.04	4.5	22.14	1.15	5.2
8	22.94	0.41	1.8	22.07	1.13	5.1
10	9.43	0.55	5.9	9.33	0.40	4.3

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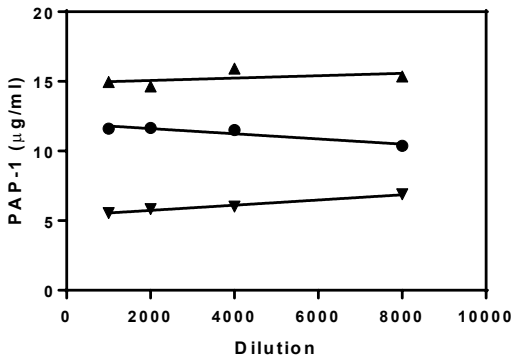
Linearity: To assess the linearity of the assay, fecal extracts containing PAP-1 at concentrations of 6.1, 11.3 and 15.2 μg/ml was serially diluted with diluent to produce values within the dynamic range of the assay.



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