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Feline Respiratory Pathogen Detection Assays

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DISCLAIMER

Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

ABSTRACT

The Feline Respiratory Pathogen (FRP) Detection Assays is intended as an in vitro veterinary reagent set, based on quantitative PCR (qPCR) and Reverse Transcription qPCR (RT-qPCR), for the detection of feline calicivirus (FCV), feline herpesvirus type 1 (FHV-1), influenza A virus (IAV), SARS-CoV-2, *Bordetella bronchiseptica*, *Mycoplasma felis* and *Chlamydia felis* in nasal and pharyngeal swab samples.

Protocol status: Working
We use this protocol and it's working

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GUIDELINES

SHIPPING & STORAGE INFORMATION

The FRP Detection Assays are shipped on dry ice. Reagents should arrive frozen. The Reagents in the purple and red tubes may arrive liquid, this will not result in a reduction in performance.

All reagents should be stored at -20°C upon arrival. All reagents can be stored for a minimum of one year (from the date of shipment) at -20°C without showing a reduction in performance. Positive controls should be stored at -80°C.

LIMITATIONS:

- Strict compliance with the instructions is required for optimal results.
- Appropriate specimen collection, transport, storage, and processing procedures are required for the optimal performance of this test.
- The presence of RT-PCR inhibitors may cause false negatives.
- Results of FRP Detection Reagents need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

- The specificity of each test was validated using a panel of reference and related canine respiratory pathogens.
- The analytical sensitivity of each assay was determined using ten-fold dilution of in vitro transcribed RNA or plasmid copies number. All assays have a limit of detection (LOD_{95}) □ 15 copies/□l.

MATERIALS

ASSAY DESCRPTION & COMPONENTS

The reagents are assembled for 60 reactions (+ 10% extra).

Lid color	Component	Number of vials	Volume per vial
Purple	RT-PCR Master mix	2	825 µl
Red	RT mix	1	33 µl
Yellow	Primers & probes mix*	2	82.5 µl
Blue	Nuclease free water	1	800 µl
Colorless	Positive Controls*	2	20 µl

Table 1. Kit description.

* 2 tubes of primers & probes and positive controls are provided and

correspond to the feline respiratory assay 1 (FRA_1) and FRA_2.

PROBE DYE SETTING

TaqMan Probe settings should be as follows:

Assays	Pathogens	Reporter	Quencher
FRA_1	FCV	ABY™	QSY™
	FHV-1	Cy5™	3IAbRQSp
	SARS-CoV-2	FAM™	QSY™
	IAV	VIC™	QSY™
FRA_2	*B. bronchiseptica*	FAM™	QSY™
	C. felis	ABY™	QSY™
	M. felis	VIC™	QSY™

Table 2. TaqMan probe set

OTHER MATERIALS:

- Appropriate nucleic acid extraction instrument and kits
- Appropriate real-time PCR instrument calibrated for ABY™, Cy5, FAM™ and VIC™ dyes (e.g., Applied Biosystems 7500 Fast Real-time PCR machine)
- Vortex and benchtop centrifuge
- Appropriate 96-well reaction plate or reaction tubes with corresponding closing tape or caps
- Pipettes & tips
- Personal Protective Equipment (PPE)

1 Thaw all reagents on ice.

2 Centrifuge all reagents on a benchtop centrifuge to ensure no liquid is in cap and keep on ice

Note

The FRP Detection Reagents do not include an internal control, but positive controls are provided for each of the two assays (FRA_1 and FRA_2). A positive and a negative control should be run simultaneously with each sample setup.

3 Setup the Master Mix according to the following table 1:

Reagents	Volume per reaction (µl)
RT-PCR Master Mix	12.5
RT mix	0.25
Primers & probes mix	1.25
Nuclease free water	6
Total volume per Master Mix	**20**
DNA/RNA template	5
Total Volume per reaction	**25**

Table 1. Reaction mix preparation

PROGRAMMING THE THERMOCYCLER

4 Select the following fluorescence channels: ABY™, Cy5, FAM™, and VIC™.

Note

ROX™ should be used as a passive reference dye.

5 The standard mode should be selected, following the table below:

Step	Number of cycles	Temp. (°C)	Time (min:sec)
Reverse transcription	1	50	20:00
PCR initial heat activation	1	95	15:00
Denaturation	40	94	00:45
Annealing/ extension		60#	00:75

Data acquisition

Table 2. Thermo profile

RESULTS INTERPRETATION

6 Before analysis of results, the threshold value of each fluorescent dye must be manually set in the region of exponential amplification, typically 0.1 × ΔRn value at the plateau phase.

7 Each assay is considered valid if the following criteria are met:

Assays	Pathogens	Positive Control	Negative Control
FRA_1	FCV/ABY	Ct ≤ 22	Ct > 40
	FHV-1/Cy5	Ct ≤ 22	
	SARS-CoV-2/FAM	Ct ≤ 22	
	IAV/VIC	Ct ≤ 22	
FRA_2	*B. bronchiseptica*/FAM	Ct ≤ 24	Ct > 40
	C. felis/ABY	Ct ≤ 24	
	M. felis/VIC	Ct ≤ 24	

Table 3. Positive control validation criteria

8 The results are qualitative (Positive or Negative). A specimen is considered positive if the Ct value obtained is below the following Ct cut-off values:

Assays	Pathogens	Ct Cut-off
FRA_1	FCV/ABY	37
	FHV-1/Cy5	35
	SARS-CoV-2/FAM	33
	IAV/VIC	34
FRA_2	*B. bronchiseptica/FAM*	38
	C. felis/ABY	37
	M. felis/VIC	37

Table 4. Ct cut-off values