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We use this protocol and it's working

Canine Enteric Virus Detection Assays

Come J Thieulent[1], Mariano Carossino[1], Laura Peak[2], Udeni B. R. Balasuriya[1]

[1]Louisiana Animal Disease Diagnostic Laboratory and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA;
[2]Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Come J Thieulent

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 Canine Enteric Virus Detection Assays is intended as an in vitro veterinary reagent set, based on Reverse Transcription quantitative PCR (RT-qPCR), for the detection of canine adenovirus type 1 (CAdV-1), canine distemper virus (CDV), canine enteric coronavirus (CECoV), canine parvovirus (CPV), canine rotavirus A (CRV) and SARS-CoV-2 in rectal swabs and fecal samples.

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GUIDELINES

SHIPPING and STORAGE:

The Canine Enteric Virus 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 Canine Enteric Viruses Detection Assays 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 (LOD95) □ 15 copies/□l.

MATERIALS

Assay 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 canine enteric assay 1 (CEA_1) and CEA_2.

Probe Dye Settings

TaqMan QSYTM Probe set are used as follows:

Assays	Pathogens	Reporter	Quencher
CEA_1	CAdV-1	ABY™	QSY™
	CECoV	FAM™	QSY™
	CDV	JUN™	QSY™
	CPV	VIC™	QSY™
CEA_2	CRV	JUN™	QSY™
	SARS-CoV-2	FAM™	QSY™

Table 2. TaqMan probe set

Other Materials

- Appropriate nucleic acid extraction instrument and kits
- Appropriate real-time PCR instrument calibrated for ABY™, FAM™, JUN™ 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)

Reaction Set Up

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 Canine Enteric Virus Detection Assays do not include an internal control, but positive controls are provided for each of the three assays (CEA_1 and CEA_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™, FAM™, JUN™ 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
CEA_1	CAdV-1/ABY	Ct ≤ 22	Ct > 40
	CECoV/FAM	Ct ≤ 22	
	CDV/JUN	Ct ≤ 22	
	CPV/VIC	Ct ≤ 22	
CEA_2	CRV/JUN	Ct ≤ 26	Ct > 40
	SARS-CoV-2/FAM	Ct ≤ 22	

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
CEA_1	CAdV-1/ABY	38
	CECoV/FAM	40
	CDV/JUN	38
	CPV/VIC	32
CEA_2	CRV/JUN	33
	SARS-CoV-2/FAM	35

Table 4. Ct cut-off values