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Canine Respiratory Pathogen Detection Assays

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DISCLAIMER

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

ABSTRACT

The Canine Respiratory Pathogen (CRP) 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-2 (CAdV-2), canine distemper (CDV), canine herpesvirus type 1 (CHV-1), canine influenza A virus (CIV), canine parainfluenza (CPiV), canine pneumovirus (CPnV), canine respiratory coronavirus (CRCoV), SARS-CoV-2, *Bordetella bronchiseptica*, *Streptococcus equi* subsp. *zooepidemicus*, *Mycoplasma cynos* and *Mycoplasma canis* in nasal and pharyngeal swab samples.

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

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GUIDELINES

Shipping and Storage

The CRP 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.

Limitation:

1. Strict compliance with the instructions is required for optimal results.
2. Appropriate specimen collection, transport, storage, and processing procedures are required for the optimal performance of this test.
3. The presence of RT-PCR inhibitors may cause false negatives.
4. Results of Canine Influenza A Subtypes Identification Assay need to be interpreted in consideration of all clinical and laboratory findings.

Quality Control:

1. The specificity of each test was validated using a panel of reference and related canine respiratory pathogens.
2. 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}) □ 60 copies/□l.

MATERIALS

Assay description and components

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

Lid color	Component	Number of vials	Volume per vial
Purple	RT-PCR Master mix	3	825 µl
Red	RT mix	1	49.5 µl
Yellow	Primers & probes mix*	3	82.5 µl
Blue	Nuclease free water	1	1.2 ml
Colorless	Positive Controls*	3	20 µl

* 3 tubes of primers & probes and positive controls are provided and correspond to the canine respiratory assays CRA_1, CRA_2, and CRA_3.

Probe dye setting

TaqMan QSY™ Probe set are used as follows:

Assays	Pathogens	Reporter	Quencher
CRA_1	CAdV-2	ABY™	QSY™
	CPiV	FAM™	QSY™
	CDV	JUN™	QSY™
	CIV	VIC™	QSY™
CRA_2	CRCoV	ABY™	QSY™
	SARS-CoV-2	FAM™	QSY™
	CHV	JUN™	QSY™
	CPnV	VIC™	QSY™
CRA_3	*S. equi subsp. zooepidemicus*	ABY™	QSY™
	B. bronchispectica	FAM™	QSY™
	M. canis	JUN™	QSY™
	M. cynos	VIC™	QSY™

Table 2. TaqMan probe set

Material and equipment required but NOT provided.

- 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 Setup

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 CRP Detection Reagents do not include an internal control, but positive controls are provided for each of the three assays (CRA_1, CRA_2 and CRA_3). 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.

Programming the Thermocycler

4 The following fluorescence channels should be selected: ABY™, FAM™, JUN™, and VIC™.

5 ROX™ should be used as a passive reference dye.

6 The standard mode should be selected. Setup cycling condition following table 2:

	A	B	C	D
	Step	Number of cycles	Temp. (°C)	Time (min:sec)
	Reverse transcriptase	1	50	20:00
	Initial activation	1	95	15:00
	Denaturation	40	94	00:45
	Annealing/extension		60	00:75

Table 2. Thermal profile. The data acquisition is performed during the annealing/extension step.

Results interpretation

7 Before results analysis, 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.

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

Assays	Pathogens	Positive Control	Negative Control
CRA_1	CAdV-2/ABY	Ct ≤ 22	Ct > 40
	CPiV/FAM	Ct ≤ 22	
	CDV/JUN	Ct ≤ 22	
	CIV/VIC	Ct ≤ 22	
CRA_2	CRCoV/ABY	Ct ≤ 22	Ct > 40
	SARS-CoV-2/FAM	Ct ≤ 22	
	CHV/JUN	Ct ≤ 22	
	CPnV/VIC	Ct ≤ 22	
CRA_3	*S. equi subsp. zooepidemicus/ABY*	Ct ≤ 24	Ct > 40
	B. bronchispectica/FAM	Ct ≤ 24	
	M. canis/JUN	Ct ≤ 24	
	M. cynos/VIC	Ct ≤ 24	

Table 3. Assays criteria

9 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
CRA_1	CAdV-2/ABY	35
	CPiV/FAM	34
	CDV/JUN	35
	CIV/VIC	34
CRA_2	CRCoV/ABY	34
	SARS-CoV-2/FAM	37
	CHV/JUN	38
	CPnV/VIC	36
CRA_3	*S. equi subsp. zooepidemicus/ABY*	35
	B. bronchispectica/FAM	40
	M. canis/JUN	31
	M. cynos/VIC	35

Table 4. Ct cut-off values