OCT 23, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn2o9pg5d/v1

Protocol Citation: Come J Thieulent, Mariano Carossino, Laura Peak, Udeni B. R. Balasuriya 2023. Canine Influenza A Subtype Identification Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn2o9pg5d/v1

MANUSCRIPT CITATION: Development and validation of a panel of one-step 4-plex qPCR/RT-qPCR assays for simultaneous detection SARS-CoV-2 and other pathogens associated with canine infectious respiratory disease complex. Thieulent C. J., Carossino M., Peak L., Strother K., Wolfson W., Balasuriya U. B. R. Applied Microbiology and Biotechnology.

Canine Influenza A Subtype Identification Assay

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

[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 Influenza A Subtype Identification Assay is intended as an in vitro veterinary reagent set, based on Reverse Transcription quantitative PCR (RT-qPCR), for the detection of canine influenza A virus (CIV) and identification of CIV H3N2, CIV H3N8 and CIV H1N1 in nasal and pharyngeal swab samples.

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

Created: Mar 29, 2023

Last Modified: Oct 23, 2023

PROTOCOL integer ID: 79703

Funders Acknowledgement:
Vet-LIRN
Grant ID: 1 U18 FD007514

GUIDELINES

Storage and Shipping

The Canine Influenza A Subtype Identification Assay is 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 Subtype 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. All assays have a limit of detection (LOD_{95}) □ 12 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	1	825 µl
Red	RT mix	1	16.5 µl
Yellow	Primers & probes mix	1	82.5 µl
Blue	Nuclease free water	1	400 µl
Colorless	Positive Controls	1	20 µl

Probe dye setting

TaqMan QSY™ Probe sets are used as follows:

Assays	Pathogens	Reporter	Quencher
CRA_4	CIV – H1N1	ABY™	QSY™
	CIV – H3N2	FAM™	QSY™
	CIV – H3N8	JUN™	QSY™
	CIV – Matrix	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

Thaw all reagents on ice.

1

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

> **Note**
>
> The Canine Influenza A Subtypes Identification Assay does not include an internal control, but positive controls are provided. 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:

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. Thermal profile

Results interpretation

7 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.

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

Assays	Pathogens	Positive Control	Negative Control
CRA_4	CIV – H1N1/ABY	Ct ≤ 20	Ct > 40
	CIV – H3N2/FAM	Ct ≤ 20	
	CIV – H3N8/JUN	Ct ≤ 20	
	CIV – Matrix/VIC	Ct ≤ 20	

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_4	CIV – H1N1/ABY	34
	CIV – H3N2/FAM	32
	CIV – H3N8/JUN	35
	CIV – Matrix/VIC	38

Table 4. Ct cut-off values