PCRun®

Canine Distemper RNA Molecular Detection Kit

Cat. No.30CDR108

For *in vitro* veterinary diagnostic use only

User Manual

INTENDED USE

PCRun® Canine Distemper RNA Molecular Detection Kit is intended for detection of Canine Distemper Virus RNA isolated from various biological samples collected from canines suspected to be infected with the Distemper Virus (see section on "Sample Collection"). The ability of the kit to discover the Distemper Virus at different stages of the disease depends on the sample used for RNA extraction. The kit contains all the disposable components required for performing an easy and accurate test.

PRINCIPLE

PCRun® Canine Distemper RNA Molecular Detection Kit is a molecular assay based on isothermal amplification of the Nucleocapsid Protein (N) gene. It is intended for the qualitative detection of the Canine Distemper Virus. This kit is designed to be used with a suitable RNA extraction kit and a compatible heat block.

STORAGE AND HANDLING

- Storage 2-25^{0}C (room temperature or refrigerated).
- Avoid exposure to direct sunlight.
- Do not use beyond expiration date stated on the package label.
- Do not freeze!

Precautions:

- The PCRun® assay is not to be used on the specimen directly. An appropriate RNA extraction method must be conducted prior to using this kit.
- In order to avoid the introduction of contaminating RNA into the reaction, clean laboratory examination gloves must be worn while performing the test.
- Open and remove the PCRun® reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRun® reaction tubes to the original aluminum packet together with the desiccator and seal the zip-lock tightly.**
- Do not use kit if the pouch or the components are damaged.
- Each component in this kit is suitable for use only with a specific lot number. Components have been quality control approved as a standard batch unit. Do not mix components from different lot numbers.
- Employ accepted sanitary procedures designated for biological and molecular waste when handling and disposing of the reaction tubes.

BACKGROUND

Canine distemper is a highly contagious disease caused by the RNA virus paramyxovirus. Domestic dogs are considered the reservoir species. Young, unvaccinated puppies and non-immunized older dogs tend to be more susceptible to the disease. The main route of infection is via aerosol droplets from the oral/nasal cavities of infected animals. The Distemper virus initially replicates in the epithelial and lymphoid tissue of the upper respiratory tract. Depending on the level of immunity during the viremic stage, the virus may spread via the bloodstream, replicating in the mononuclear cells, the epithelia of the respiratory and gastrointestinal system as well as the central nervous system (CNS). In cases where CNS invasion occurs, neurons will also become infected. Distemper is often fatal, and dogs that survive usually have permanent, irreparable nervous system damage[1].

DIAGNOSIS

Clinical diagnosis of early canine distemper is difficult due to the broad spectrum of signs that may be confounded with other respiratory and enteric diseases. Diagnosis should be based on history (vaccination and exposure) multiple systemic clinical signs (respiratory, gastrointestinal, neurological and dermatological) and laboratory confirmation.

Initially, infected dogs will develop mild transient fever accompanied by leukopenia, followed by oculo-nasal discharge, fever, coughing, lethargy, reduced appetite, diarrhea and vomiting. In cases of CNS invasion neurological signs will appear such as muscle twitches (Myoclonus), convulsions with jaw chewing movements and partial or complete paralysis. The virus may also cause thickening of the footpads, leading to its nickname "hard pad disease" In milder cases, signs may be similar to other agents of canine infectious respiratory disease complex. Subclinical infection with shedding may occur, depending on the level of host immunity.

Early clinical findings may include lymphopenia and microscopic inclusions in stained WBCs and RBCs. Laboratory findings and CFS analysis are often nonspecific. Multiple methods of detection increase diagnostic suspicion of CDV. Serological tests include in-house IgM titers for detection of recent CDV infection or exposure (Biogal, Immunocomb), IFA and ELISA. Antigen tests include, virus isolation, Immunohistochemistry, Immunochromatography and RNA Polymerase Chain Reaction[2]. Although virus isolation is the gold standard it requires specialized laboratories. Considering the infectious potential and high mortality rates of the disease, the need for carrying out a rapid antemortem diagnosis of distemper is essential. The sensitivity, specificity and rapidity of molecular methods such PCRun, when compared with conventional methods, make molecular techniques preferable for CDV diagnosis[3].

KIT CONTENTS

Contents	Amount
PCRun® strip of 8 lyophilized Distemper single reaction tubes	1
PCRun® buffer to re-dissolve lyophilized reaction pellets	1 Vial, 200 µl
Aluminium pouch with disposable nucleic acid detection device.	8

EQUIPMENT TO BE SUPPLIED BY USER:

- RNA extraction kit suitable for use with PCR reactions (Recommended Kit 30PRE308).
- Heat block which maintains 60^{0}C – compatible with 0.2 PCR tubes. Heat block can be supplied by Biogal
- Accurate laboratory pipettes with aerosol barrier tips for volumes 5 and 15 ul.
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves

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SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting Distemper Virus associated ribonucleic acid extracted from various sample types when employing RNA extraction kits suitable for use with Reverse Transcription (RT) PCR. Relevant samples for the molecular detection of CDV RNA include whole blood, CSF and urine, as well as saliva, nasal, rectal and conjunctival swabs. Suggested samples according to the stages of disease are (i) whole blood during febrile periods; (ii) nasal and conjunctival swabs for acute infection in dogs displaying conjunctivitis and/or respiratory signs; (iii) rectal swabs or feces for dogs with gastrointestinal signs; (iv) urine for dogs with neurological signs. It must be noted that urine contains PCR inhibitors, therefore an appropriate sample prep must be used. The use of two different body fluids (e.g. urine and CSF) may increase the RT-PCR sensitivity for antemortem diagnosis of distemper[2]. When using PCR for diagnostic purposes it is imperative to be aware of the vaccination history of the patient. Certain PCR protocols will detect virus from the vaccine for a few weeks post-vaccination[3].

Extracted RNA can potentially degrade when not handled or maintained under optimal conditions, therefore the highest biological standards should be employed. Disposable gloves and RNase free pipette tips are essential for sampling RNA. For best results only recently acquired samples and freshly prepared RNA extracts are suitable. Unless otherwise recommended, samples and RNA extracts must be maintained at -20 to -80^{0}C for short periods of time. Extracted RNA must be maintained on ice while performing this test.

PROTOCOL - PCRUN® REACTION

1. Prepare a clean working area for the assay. Work area should be be decontaminated with commercial household bleach (3.5%) diluted 1:10 with water.

2. Prepare all parts of the assay:

- √ **Extracted RNA sample**
- √ Pouch with reaction tubes
- √ PCRun® buffer
- √ Laboratory pipettes and barrier tips for 5 and 15 ul volume
- √ Fine tipped permanent marker

3. Switch on the heat block and adjust to 60^{0}C. Once the block has reached the target temperature, continue with the reaction.

4. Remove the PCRun® strip from its protective pouch. Take care to return the unused tubes to the aluminium envelope and seal completely with zip-lock to maintain a dry environment. Eight individual reaction tubes are connected by a thin plastic spacer. Employing a small clean scissors, disconnect the required number of tubes without disturbing the lids. Tap the tubes lightly on a surface and observe that the small white pellet is located on the bottom of the tube.

5. Label the lid of the tubes clearly for sample identification.

6. Carefully open the lid of the reaction tubes, one at a time. Dispense 15 µl of PCRun® Buffer to each reaction tube. Close the lid and incubate the mixture at room temperature for 1 minute to allow the pellet to completely dissolve.

7. Add 5 µl of RNA sample into the PCRun® reaction tube and mix thoroughly. Close the lid of the tube firmly and tap the tube on a surface to bring all the fluid to the bottom of the tube.

8. Place the reaction tube into the appropriate hole in the pre heated block (60°C) and incubate for exactly 1 hour. Do not open the tube cover during or at the end of the incubation period.

9. At the end of the incubation period (1 hr) remove the tube from the heat block and analyze immediately with the disposable nucleic acid detection device.

ANALYSIS OF PCRun® REACTION WITH THE DISPOSABLE NUCLEIC ACID DETECTION DEVICE

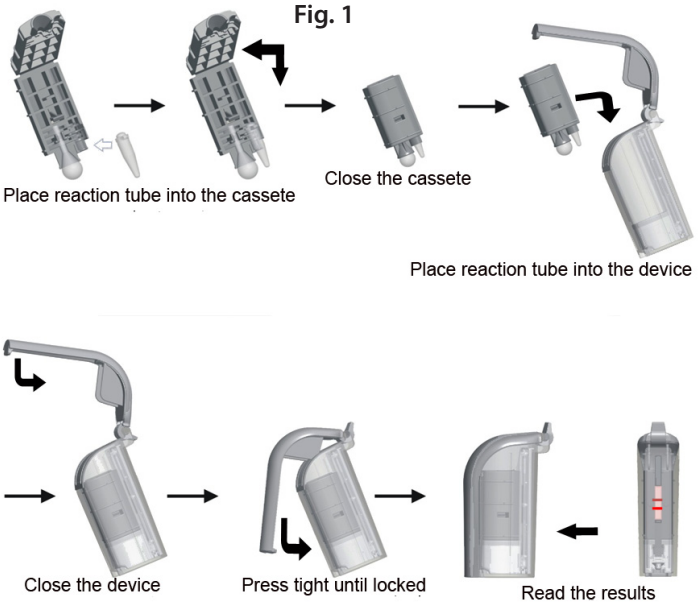
One disposable nucleic acid detection device is needed for each test. Open and remove the components of the detection device. The device consists of two plastic parts, the Amplicon Cartridge containing a plastic buffer bulb and the Detection Chamber containing the lateral flow strip (Figure 1).

1. Verify the presence of fluid in the bulb.
2. Mark each chamber with the sample ID.
3. Align the lid section of the PCRun® reaction tube with the wide partition located beside the buffer bulb.

Apply light pressure to attach the reaction tube to the Amplicon Cartridge.

4. Fold the Amplicon Cartridge in two and snap closed. Place the cartridge into the Detection Chamber with the bulb facing downwards and away from the chamber lever.
5. Push the lever downwards to lock the device.
6. Wait for 15-30 minutes to read the results. Results read after 30 minutes are invalid.

Fig. 1

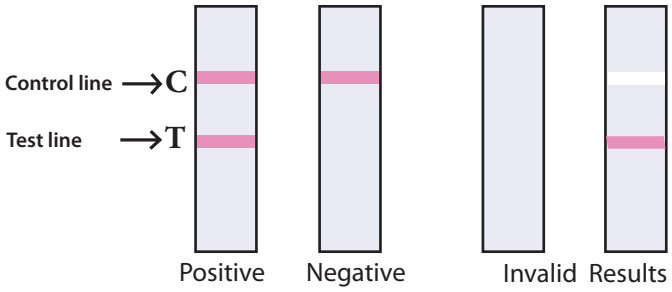


READING AND INTERPRETING THE RESULTS

A valid test must present a red control band. The control line must appear regardless of a positive or negative result (Figure 2).

1. **Positive Result** - two bands appear, the upper control line and the lower test line. The appearance of both control line and test line indicates the presence of the Canine Distemper Virus.
2. **Negative Result** – a single control line appears. The appearance of a control line only, indicates the absence of the Canine Distemper Virus or that the copy number is below the detection limit.

Fig. 2



LIMITATIONS

As with any diagnostic kit, the results obtained must be used as an adjunct to other clinical and laboratory findings. The accuracy of the test results depends on the quality of the sample and adherence to protocol. A negative result may be obtained if the specimen is inadequate or target pathogen concentration is below the sensitivity of the test.
A positive PCRun® result is not sufficient for a diagnosis of Canine Distemper, as recently vaccinated animals may have positive PCR results.
Diagnosis should be based on a combination of history, clinical signs, laboratory parameters and the PCRun® test results.

REFERENCES

1. Canine Distemper Virus. WASAVA Clinical Brief Nov 2014. S. Techangamsuwan and M.A. Kennedy.

2. Detection of canine distemper virus in dogs by real-time RT-PCR. J. virol Methods. 2006 Sept;136(1-2):171-176. Elia, Buonavoglia et al.

3. Real-time reverse transcription polymerase chain reaction method for detection of Canine distemper virus modified live vaccine shedding for differentiation from infection with wild-type strains. Journal of Veterinary Diagnostic Investigation 2014, Vol. 26(1) 27–34 2014. Wilkes, Kennedy et al.