



PCRRun®

Parvovirus Molecular Detection Kit (For Blood Only)

Cat. No.30CFP104

For *in vitro* veterinarian diagnostic use only

User Manual

INTENDED USE

PCRRun® Parvovirus Molecular Detection Kit is intended for detection of canine and feline parvovirus (canine CPV-2a, 2b and 2c) in DNA isolated from whole blood. The kit contains all the disposable components required for performing an easy and accurate test.

PRINCIPLE

PCRRun® is a molecular assay based on the isothermal amplification of part of the VP2 capsid protein gene. It is intended for the qualitative detection of canine and feline parvovirus and can be most effectively used during the acute phase of disease. The kit is designed to be used with DNA extracted from whole blood samples using PCRRun® Sample Prep and a compatible heat block.

STORAGE AND HANDLING

- Storage 2-25°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 PCRRun® assay is not to be used on the specimen directly. An appropriate nucleic acid extraction method must be conducted prior to using this kit.
- In order to avoid the introduction of contaminating DNA into the reaction, clean laboratory examination gloves must be worn while performing the test.
- Open and remove the PCRRun® reaction tubes from the sealed pouches only immediately prior to their use.
- **Return unused PCRRun® 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 parvovirus (CPV) and feline panleukopenia virus (FPLV) are autonomous parvoviruses which are the causative agent of parvoviral infections in dogs and panleukopenia in cats (otherwise known as feline infectious enteritis, feline parvoviral enteritis and feline distemper). Although thought to be species-specific, new variants of CPV have developed the potential to cause disease also in cats¹. Infections with CPV and FPLV result in highly contagious diseases with worldwide distribution in wild and domestic animals. Virus particles can be transmitted to a susceptible host by oronasal route. Settings such as animal shelters, pet shops, kennels and research colonies are ideal for spread and transmission of the viruses. Canines and felines of all ages can be infected but severe disease is most common in unvaccinated or partially vaccinated puppies and kittens between 6 weeks and 4 months old. The period of greatest susceptibility to infection is when maternal antibodies are waning and vaccine-induced immunity is not fully developed. Both CPV and FPLV are

associated with high morbidity and mortality, but infections can range from subclinical to severe depending on the immune status and the age of the animal. The virus initially infects and replicates in oropharyngeal lymphoid tissue. They have an affinity for rapidly dividing cells and for this reason, cells lining the intestinal tract and those making up the bone marrow are most affected. During the acute phase of the infection, FPLV and CPV particles may be shed in saliva, vomit, feces or urine.² Parvovirus are ubiquitous, very stable and resistant to most disinfectants making them a source of infections for susceptible animals.

DIAGNOSIS

Dogs and cats affected by acute infections with CPV or FPLV, display similar enteric diseases symptoms. The first clinical sign, pyrexia (3 - 5 days after virus infection), is followed by viral shedding in high titers in the feces. The virus primarily attacks the lining of the gastrointestinal tract leading to ulceration, shedding of the intestinal epithelium, diarrhea (less frequent in cats), vomiting, dehydration, malnutrition, anemia, and often death. Complete blood counts show decreased white blood cells. A tentative diagnosis of CPV or FPLV viral infection is based on the general history (exposure to contamination), vaccination history, clinical signs and supportive blood tests^{3,4}. Serological tests can be used to determine disease status, but must be performed on paired sera to determine whether there is a rise in titer between the acute and convalescent sample. A definitive diagnosis can be made based on positive fecal ELISA or immunochromatography antigen tests. These tests are considered to be moderately sensitive, but false negatives often occur. Several molecular methods directed at the detection of CPV and FPLV in blood and fecal samples have reported greater sensitivity than conventional immunochromatography and are progressively replacing the antigen procedure as the standard procedure for virus surveillance and diagnosis.

KIT CONTENTS

Components	Contents	Amount
Aluminum pouch Cat No. 03CPV100	PCRRun® strip of 4 lyophilized Parvovirus single reaction tubes	1
Detection device Cat No. 03100010	Aluminium pouch with disposable nucleic acid detection device.	4
Capillary tubes Cat No. 03200020	Disposable plastic capillary tubes 20 µl*	10

*Accurate laboratory pipettes with aerosol barrier tips can be used in place of the capillary tubes.

EQUIPMENT TO BE SUPPLIED BY USER:

- Recommended PCRRun® Sample Prep kit cat # 30PRE104
- Heat block which maintains 60°C – compatible with 0.2 PCR tubes
Heat block can be supplied by Biogal
- Timer
- Small scissors
- Fine tipped permanent marker
- Protective laboratory gloves

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for detecting nucleic acid extracted from 50 µl of whole blood using PCRRun® Sample Prep Kit (Cat No. 30PRE108). Blood samples can be collected in EDTA, heparin or sodium citrate. Anticoagulants have a substantial consequence on PCR reactions therefore it is imperative that the optimal volume of blood is collected as indicated on the tube. For best results, recently acquired samples and freshly prepared DNA extracts are recommended for use with the PCRRun® kit.

Unless otherwise recommended, samples and DNA extracts can be maintained at 2-8°C for up to 24 hours or -20°C for an extended period of time.

PROTOCOL - PCRun® REACTION

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

- ✓ Extracted DNA sample
- ✓ Pouch with reaction tubes
- ✓ Capillary tubes for dispensing 20 µl volume
- ✓ Fine tipped permanent marker

3. Switch on the heat block and adjust to 60°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. Four individual reaction tubes are connected by a thin plastic spacer. Employing 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. Employing the 20 µl disposable capillary tube, dispense 20 µl of DNA extracted with PCRun® Sample Prep kit into the reaction tube. Make sure that the entire content of the capillary tube has been emptied into the PCRun® reaction tube. Tap the tube on a surface to bring all the fluid to the bottom of the tube. Incubate the mixture at room temperature for 1 minute to allow the pellet to completely dissolve.

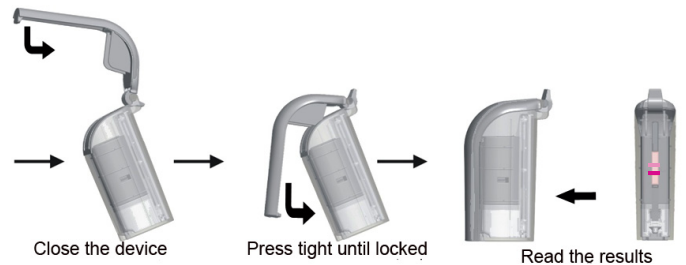
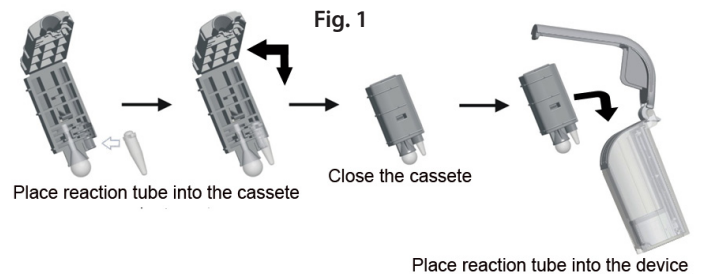
7. Place the reaction tube into the appropriate hole in the pre heated block (60°C) and incubate for **exactly 25 minutes**. The timing is very important for this reaction therefore it is recommended that a timer be set to announce the end of the incubation period. Do not open the tube cover during or at the end of the incubation period.

8. At the end of the incubation period (25 minutes) remove the tube from the heat block and analyze **immediately** with the disposable nucleic acid detection device. **It is important to perform the analysis promptly following termination of the 25-minute incubation in the heat block.**

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).

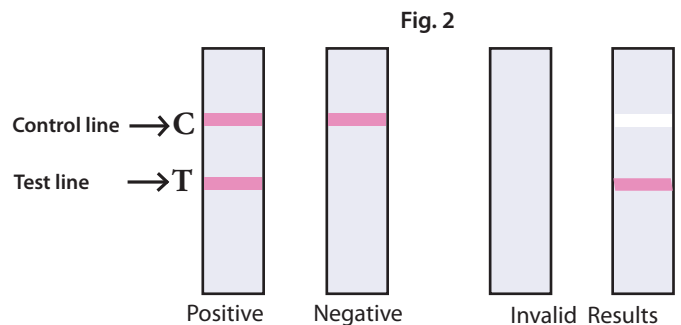
1. Verify the presence of fluid in the bulb.
2. Mark each chamber with the sample ID.
3. Align the lid section of the PCRun® reaction tube with the wide partition located beside the buffer bulb. Apply light pressure to attach the reaction tube to the Amplicon Cartridge (Figure 1).
4. Fold the Amplicon 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.



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 canine or feline Parvovirus.
2. **Negative Result** - a single control line appears. The appearance of a control line only, indicates the absence of the canine or feline Parvovirus DNA or that the copy number is below the detection limit.



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 blood PCR alone is not sufficient for diagnosis of parvoviral infection as recently vaccinated animals may have positive PCR results. Diagnosis should be based on a combination of history, clinical signs, laboratory parameters and the PCR test results.

ANALYTICAL SENSITIVITY

The PCRun® reaction can detect 10³ copies of the target gene in pure DNA.

REFERENCES

1. Characterisation of canine parvovirus strains isolated from cats with feline panleukopenia (2010). Decaro O. et al. Research in Veterinary Science, Vol 89, Issue 2, pp 275-278
2. Infectious Diseases of the Dog and Cat. Fourth edition. Saunders – Elsevier
3. Feline parvovirus infection and associated diseases - Review. (2014) Stuetzer B, Hartmann K. Vet. J. Aug;201(2):150-5
4. An updated TaqMan real-time PCR for canine and feline parvoviruses. (2013). Andre Felipe Streke et al. Journal of Virological Methods. Vol 193. Issue 1 October, pp 6-8



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