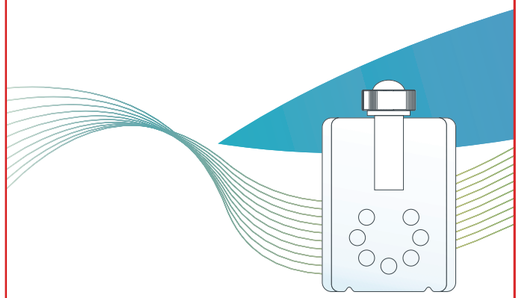
# Leishmania spp.

## Nucleic Acid Test Card

**For veterinary use only**



## Product Name

Product Name: Leishmania spp. Nucleic Acid Test Card

Trade name: Pluslife Leishmania Card

## Intended Use

Leishmaniasis is a vector-borne disease transmitted by sandflies and caused by obligate intracellular protozoa of the genus Leishmania, which are classified in three main forms: cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL). Due to the extremely variable individual incubation times, ranging from from a few months to several years, infested animals can be free of symptoms during that time. The detection of Leishmania DNA can be pointing at an initiating or an existing infection.

The Leishmania spp. Nucleic Acid Test Card is used for in vitro qualitative detection of DNA of Leishmania spp. (L. infantum, L. donovani, L. mexicana) in canine/feline blood.

Positive results are indicative of the presence of Leishmania DNA. The patient infection status needs to be determined in conjunction with test results, medical history, and other diagnostic information. Negative results do not preclude Leishmania infection due to the limitation of the kit and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information.

## Testing Principle

This kit is based on isothermal amplification and enzymatic cleavage probe technology, with conserved regions selected for specific primers and specific probes design. A large number of target sequence's copies were generated in the reaction system during the isothermal amplification. When the probe hybridizes to the complementary sequence, it is cleaved and fluorescence is emitted. The integrated Nucleic Acid Testing Device detects and analyzes fluorescence signal automatically, reporting negative, positive or invalid result. The kit includes an internal control for monitoring of sample collection, processing, and amplification to reduce false negative results.

## Materials Provided

| Article No. and specifications / Component | RM2011100-1<br>1 Test | RM2011100-5<br>5 Tests | RM2011100-10<br>10 Tests | RM2011100-50<br>50 Tests |
|---|---|---|---|---|
| Leishmania Reaction Card (piece) | 1 | 5 | 10 | 50 |
| Nucleic Acid Releasing Agent 02 (1 tube) | 1 | 5 | 10 | 50 |
| Pasteur Pipette (piece) | 1 | 5 | 10 | 50 |
| Waste Bag (piece) | 1 | 5 | 10 | 50 |

**NOTE:** 1. The above components of different batches of kits shall not be used interchangeably.

## Materials Required but Not Provided

Integrated Nucleic Acid Testing Device (Model:PM003), Guangzhou Pluslife Biotech Co., Ltd.

Eight-Channel POC Molecular Analyzer (Model:PM008P), Guangzhou Pluslife Biotech Co., Ltd.

Note: This instructions for use only provides instructions of the operations on PM003. For instructions on PM008P, please refer to the corresponding user manual.

## Storage Conditions and Expiry Date

1.2°C~28°C storage, valid for 13 months.

2.The production date and expiration date are shown on the package label.

## Sample Requirements

Whole blood (EDTA)

## Testing Method

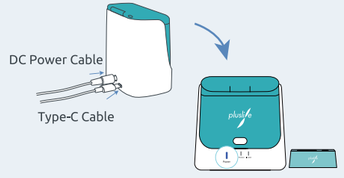
The room temperature should be between 15~28°C. Please read all the instructions carefully before you begin.

### STEP 1: Test Preparation

1. Take out the device, power adapter, and card holder from the Integrated Nucleic Acid Testing Device package.

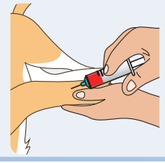
2. Put the Integrated Nucleic Acid Testing Device on a flat surface, connect the power supply, press the button in front of the device to enter the warm-up process (the power light is flashing red). After 2 minutes, the warm-up is completed and in a standby mode (the power light is blue). Connect the Integrated Nucleic Acid Testing Device to a computer with a data line and open the installed Pluslife software.

3.Run a thermostatic dry bath or water bath incubator and heat up to 65°C.




### STEP 2: Sample Collection

1.Perform venipuncture to collect the whole blood.



2.Put the collected whole blood into an EDTA tube (purple cap) and invert the tube 5~6 times.

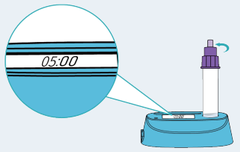
3.The samples should be used immediately after collection. If samples are not tested immediately, they should be refrigerated at 2~8°C for up to 2 days.

### STEP 3: Sample Processing

1. Transfer 50 μL of whole blood into Nucleic Acid Releasing Agent 02 using Pasteur Pipette, screw on the cap and invert the tube 8 - 10 times.

**NOTE:** Be careful to avoid contact with eyes or skin by the Nucleic Acid Releasing Agent 02. If it happens unfortunately, wipe off the liquid immediately and rinse with plenty of water.

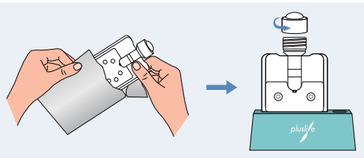
2. Place it in a thermostatic dry bath or water bath system preheated to 65 ℃ and incubate for 5 mins.




### STEP 4: Sample Testing

1.Make sure the Integrated Nucleic Acid Testing Device is in standby (the power light is blue).

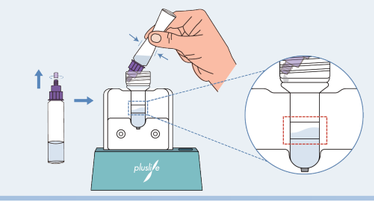

2.Tear open the aluminum foil bag of one Leishmania Reaction Card and take it out, place the Leishmania Reaction Card on the card holder and unscrew the cap of the sample tube on the Leishmania Reaction Card.



**NOTE:** The Leishmania Reaction Card must be proceeded to subsequent operations as soon as possible after the aluminum foil bag has been torn and proceed to the next step immediately when the cap of the tube is unscrewed.

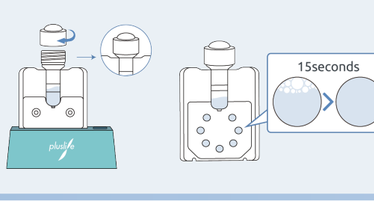
3. Open the top cap of the Nucleic Acid Releasing Agent 02 vial from STEP 3, use one hand to stabilize the card holder, use the other hand to slowly pour the Nucleic Acid Releasing Agent 02 solution on the tube inside wall of the Leishmania Reaction Card between the two liquid injection lines by squeezing the Nucleic Acid Releasing Agent 02 vial wall.

**NOTE:** There are two liquid injection lines marked on the Leishmania Reaction Card sample tube. Add Nucleic Acid Releasing Agent 02 solution into the Leishmania Reaction Card sample tube until the liquid level between the two lines.



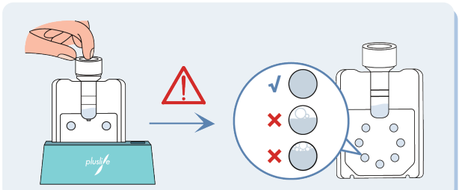
4. Place the Nucleic Acid Releasing Agent 02 vial in waste bag for disposal.

5.Screw the cap of the Leishmania Reaction Card sample tube tightly. Allow the card stand still for 15 seconds.



6. Firmly press the protruding arc-shaped air bag on the sample tube cap of the Leishmania Reaction Card to deform it and recess it into the tube.

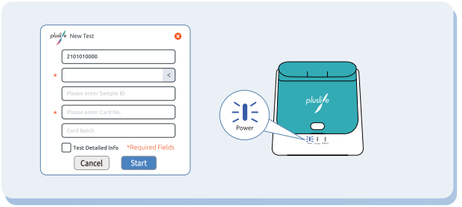
**NOTE:**Discard the card if the bubble volume occupies more than 1/3 of the chamber.



7. Hold the card, shake it up and down for 10 times in about 5 seconds. Then proceed to next step immediately.

8. Open the cabin door of Integrated Nucleic Acid Testing Device, and insert the Reaction Card into the device according to the direction indicated on the Leishmania Reaction Card, and push it to the fixed position of the bottom card slot, close the cabin door.

9. Press start button on the software to start the run. The light is flashing blue during the operation.




10. Wait 15-30 minutes.

11. When the run is completed, result is presented on the software. Record the result in time. The assay is finished.

12. Open the cabin door, take out the Leishmania Reaction Card, and put it into waste bag, seal the waste bag, and dispose of the waste following local regulations.

13. If move on to next test, press the power button to eliminate the last test result (the power indicator is steady on), insert the reaction card to be tested, and then press the power button for the next normal test (back to STEP 1). If not, press the button for over 3 seconds to turn it off.

### STEP 5: Interpretation of Test Results

1.The results of the device are determined as follows:

| Phenomenon | Description | Result determination | Suggestions |
|---|---|---|---|
| Power Positive Negative | Positive indicator light on | The sample was determined to be positive for Leishmania. | In case of a positive result: a) Export the data on the computer for analysis of the detection results of Leishmania. |
| Power Positive Negative | Negative indicator light on | The sample was determined to be negative for Leishmania. | In case of a negative result: a) If symptoms of leishmaniasis appear, do a new test. |
| Power Positive Negative | All lights on at the same time | Invalid result. Internal control failed to be detected | In case of invalid result: a) No conclusion can be made with this result. b) Perform a new test. c) If the problem persists, please contact the local distributor for assistance. |

2.The results of a leishmaniasis testes are viewed using the analysis software installed in the computer, as shown in the following table:

| Test Results | Results Determination |
|---|---|
| POSITIVE | The sample was determined to be Leishmania POSITIVE. |
| NEGATIVE | The sample was determined to be Leishmania NEGATIVE. |
| INVALID | Invalid result. ① The reaction was prematurely terminated. ② Internal control failed to be detected*. |

***Note:**

Possible reasons: 1) The sample quantity is insufficient. 2)The reaction is inhibited. 3) The operation error.

Suggestions: 1) Repeat test using a new Leishmania Reaction Card. 2) If test still fails, collect a new sample for retesting, or please contact the local distributor for assistance.

## Limitations of Detection Methods

1. The test results from this kit are only for clinical reference and should be used in conjunction with signs/symptoms, medical history, other laboratory test results for the cat for a comprehensive analysis and interpretation. They should not be used as the sole basis for clinical diagnosis and treatment.

2. False negative results may occur if the sample contains an insufficient amount of virus.

3. False positive results may occur if cross-contamination of the sample or contamination from the laboratory environment occurs during sample handling.

## Product Performance Index

1. Sensitivity (Limit of Detection): ＞10000 copies/mL.

2. Specificity: This kit does not cross-react with other pathogens with similar symptoms, e.g. Anaplasma, Babesia, Ehrlichia and others protozoon, such as Endotrypanum monterogeii, Blechomonas wendygibsoni, Trypanosoma avium or Trypanosoma cruzi.

3. Repeatability: The intra-assay repeatability detection rate is 100% and the inter-assay repeatability detection rate is 100%.

## Precautions

1.This kit is for in vitro diagnostic use only, please read this instruction carefully before use, and operate strictly in accordance with the instruction.

2.The correct collection of samples and accurate operation according to the inspection method are critical to the accuracy of the test results.

3.Avoid excessively high environment temperature. If the kit is stored at a lower temperature, it must be returned to room temperature before opening to avoid moisture condensation.

4.When opening the cover of the Leishmania Reaction Card, do not touch the reaction tube and the inside of the tube cover.

5.Make sure there are no damage of the Leishmania Reaction Card bag, and no liquid leakage of the Nucleic Acid Releasing Agent 02. Do not use them if any leakage occurs.

6.If there are clinical symptoms, even if the test result is negative, the test result should be reassessed by the doctor.

7.All parts of this kit are for external use and should not be swallowed.

8.Avoid contact with eyes or skin by the Nucleic Acid Releasing Agent 02 solution.

9.The validity period must be checked before the test. The test kit shall not be used after the expiry date indicated on the outer packaging.

10.Exposure of the contents of the Leishmania Reaction Card is likely to cause contamination, and the Leishmania Reaction Card seal and its components shall not be damaged any time.

11.Disposal: all parts used have a potential risk of infection. Please use the provided waste bag for disposal.

12.The freeze-dried reaction microspheres are very easy to deliquesce. The sealed package of Leishmania Reaction Card should not be opened too early. If it is not used for testing as soon as possible after opening the package, the Leishmania Reaction Card cannot be used.

13.If the collection and sample processing are not well controlled, resulting in cross-contamination, false positives may occur.

14.A variety of factors during the storage, transportation and use of reagents may cause performance changes, such as sample collection, sample processing, and non-standard operation in the testing process. Please strictly follow the instructions. Due to the characteristics of the sample collection process and the virus infection process itself, there may be false-negative results caused by insufficient sample size. It should be combined with other clinical diagnosis and treatment information to make a comprehensive judgment, and retest if necessary.

15.Do not disassemble the Reaction Card, whether it is used or not.

## Manufacturer

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## Explanation of Symbols

| | | | |
|---|---|---|---|
| | Consult instruction for use | | Keep dry |
| | Use-by date | LOT | Batch number |
| | Temperature Limit | REF | Catalogue number |
| | Manufacturer | | Date of manufacture |
| | Do not re-use | | Do not use if package is damaged and consult instructions for use |
| | Biological risks | | Keep away from sunlight |
| n | Contains sufficient for <n> tests | | Fragile,handle with care |
| | This way up | | Do not roll |
| n | Stacking limit by number | | |

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