

1. What is the best samples for cytology and molecular testing

The best samples for cytology/ histopathology :

Cytology can be performed on the following samples: Skin lesions, bone marrow, and fine needle aspirations from spleen, lymph nodes and liver

Cutaneous Tissue Biopsies: Leishmania parasites can be found in the skin, including intact tissue, especially in the facial region or in the ears. Skin biopsies should be collected from the internal surface of the ear using 5.0-mm sterile punches. The tissue should be expressed into a clean vial containing a small volume of sterile physiological fluid such as saline and maintained at 4 °C for up to one day or at -20 °C for longer periods of time

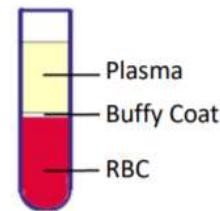
The best samples for molecular testing:

In general, the best target tissue, in decreasing order of sensitivity, is bone marrow/lymph nodes > skin > conjunctive > buffy coat > peripheral blood.

1. **Bone marrow aspirates** should be collected in the presence of heparin in order to prevent clotting. The sample should be expressed into a sterile vial and can be maintained a 4 °C for 24 hrs or at -20 °C for long term storage.
2. **Lymph node aspirates.** Lymph node aspirate the popliteal glands, located at the posterior surface of the knee joint in the “Knee Pit”) are the chosen lymph node for sample for sampling.
3. The aspirate should be expressed into a sterile vial containing 0.5 ml of physiological fluid. Make sure that the tissue is immersed in the fluid. The sample can be maintained a 4 °C for 24 hrs or at -20 °C for long term storage.
4. **Skin swabs** should be obtained from cutaneous lesions. Rub vigorously the fresher part of the lesion with a swab. Open a clean sterile tube, insert about one-third of the swab stick, then snap the handle so that it breaks and the swab tip falls into the tube. Close the tube tightly and maintain at 4C for up to one day or -20C for longer storage.
5. **Conjunctival Swabs** should be obtained separately from both lower conjunctivas using appropriate sterile swabs moistened with physiological saline. The best type of swab to be used for sampling are Flocked Swabs. If this type of swab is not available swabs used for collection of bacterial material are suitable. Collection is performed by pulling the lower eyelid outwards to expose the conjunctival sac. Insert the tip of the swab into the sac and firmly rub the inside of the eyelid to remove the epithelial cells. (Oral and nasal swabs taken in the same manor can also be employed.) Conjunctival samples are often full of mucus and contain few or no cells, therefore, care must be taken to collect suitable samples. Open a clean sterile tube, insert about one-third of the swab stick, then snap the handle so that it breaks and the swab tip falls into the tube. Close the tube tightly and maintain at 4 °C for up to one day or -20 °C for longer storage.
6. **Blood:** The Leishmania pathogen has a high tropism for macrophage therefore in some cases whole blood collected in the anticoagulant EDTA can be a medium for sampling. A sample enriched with white blood cells (**buffy coat**) is preferred. Samples should be maintained at 4 °C until processing.

Buffy coat collection:

- Centrifuge EDTA tube in a standard clinical centrifuge at ~2500 RPM for 10 minutes at room temperature.
- Carefully remove as much plasma as possible and discard.
- Remove the buffy coat cells and place into an appropriate tube. Some blood cells and plasma may be removed together with the buffy coat.


2. Which is the best test to use in my clinic for make the diagnosis?

Two types of tests are available to be perform in the clinic:

- Lateral flow serology which is a quick "yes or no" test and serves as a first screening for Leishmania infantum infection.
- The in-clinic PCR test for the specific detection of Leishmania infantum DNA in the sample

The combination of both tests provides an optimal tool for the diagnosis of Leishmaniasis

3. Allopurinol, Domperidone or Milteferon - which of medications are used the recommended best treatment for the chronic phase of the disease

Several drugs and protocols are used for the treatment of Leishmaniasis. Sometimes, a combination of 2 different drugs is required. The protocols differ according to the disease stage, drugs availability in a specific country, costs and client compliance. A recommended detailed protocol for each stage is available online at Leishvet.org : [Clinical staging and treatment](http://Leishvet.org)

4. It's very impressive to be able to make a diagnosis with PCR technique. Can you give us other disease examples for which we can make a diagnosis with PCR?

PCRUn in clinic molecular detection kits are available for the following pathogens:

1. Canine *Ehrlichia canis*
2. Canine *Anaplasma platys*
3. Canine *Babesia canis/vogeli*
4. Canine *Babesia gibsoni*
5. Canine Pathogenic Leptospira
6. Canine *Leishmania infantum*
7. Canine Parvovirus
8. Canine Distemper
9. Feline Panleukopenia
10. Feline *Mycoplasma haemofelis*
11. Feline FeLV Provirus
12. Feline FeLV virus

Dogs suffering from Leishmaniasis are prone to coinfections. In this case Clinical signs and routine laboratory findings can make the diagnosis even more complex. Therefore it is recommended to test for other vector borne diseases when testing for *Leishmania infantum* infection.

5. If I do not have a quantitative serology test available what is the next preferred test for staging diagnosis:

When quantitative serology is not available, the next preferred step for staging is a lateral flow qualitative serological test and PCR.

Note that for a full comprehensive staging all the following parameters are required:
Physical examination, CBC, biochemistry, urinalysis, UPC, serology and PCR