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Canine Monocytotropic Ehrlichiosis (CME) Sampling and Diagnostic Testing Guide. Part 1: The Diagnostic and Surveillance Tests

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Introduction

Canine monocytotropic ehrlichiosis (CME) is caused by *Ehrlichia canis*, an obligate intracellular, pleomorphic, gram-negative bacterium. Disease transmission is via tick bites, primarily of the brown dog tick (*Rhipicephalus sanguineus*). Bacteria are found within the cytoplasm of monocytes in blood and macrophages in tissue, where they appear as clusters termed morulae. Clinical ehrlichiosis in dogs can be acute, subclinical or chronic.

Clinical disease is characterised by fever, malaise, lethargy, haemorrhagic tendencies (epistaxis, dermal petechiae/ecchymoses, mucous membrane haemorrhages), lymphadenopathy, and splenomegaly. Less common presenting signs include ocular disease (uveitis, chorioretinitis, retinal haemorrhage) and neuromuscular disorders (seizures, stupor, ataxia) associated with meningitis / meningeal haemorrhage. The subclinical disease can last for a few months to years, during which the carrier state exists. The chronic phase of ehrlichiosis is characterised by severe bone marrow suppression (pancytopaenia).

Diagnosis should be based on a combination of consistent clinical signs, antigen detection via polymerase chain reaction (PCR) on EDTA blood and antibody detection on serum. Smear examination, haematology, clotting function tests, and clinical chemistry are used as ancillary diagnostic tests to interpret the PCR and serology analyses better.

In South Africa, it has previously been presumed that co-infection with *Ehrlichia canis* and *Babesia canis rossi* was a common occurrence, with leukopaenia and thrombocytopaenia in cases of canine babesiosis being thought to be indicative of concurrent ehrlichiosis. Rautenbach Y. et al. in their 2018 study into the prevalence of *Babesia* and *Ehrlichia* co-infections, demonstrated that co-infection is, in fact, a rare occurrence. In addition, they demonstrated that leukopaenia and thrombocytopaenia in dogs with babesiosis had an extremely low predictive value for concurrent ehrlichiosis, with both parameters being common features of canine babesiosis as well.



Figure 1. Canine ehrlichiosis with epistaxis, mucous membrane pallor and petechial haemorrhages (arrowhead). Courtesy of Disease Diagnostic Laboratory Services, DPIRD, Perth, Australia.

The thrombocytopaenia of canine ehrlichiosis is frequently associated with bleeding, while low platelet counts associated with canine babesiosis are not.

Laboratory Diagnosis.

Current diagnostic tests for canine ehrlichiosis include the *E. canis* Real-time PCR for antigen detection and ELISA / Immunofluorescent antibody test for antibody detection. For clinical disease investigation, the ideal samples for the detection of *E. canis* are:

- 2-5 mL of EDTA blood for *E.canis* Real-time PCR
- 2-5 mL of clotted blood (preferably a 1-2 mL aliquot of clear serum should be transferred into a plain tube prior to submission) for serology (ELISA / IFA).

Other sample types appropriate for post-mortal tissue PCR testing include dry conjunctival swabs and fresh spleen, lymph node, liver, kidney, lung and bone marrow. However, testing of these sample types may have reduced sensitivity compared to EDTA blood on live animals.

Smear examination, haematology, clotting function tests and clinical chemistry are ancillary diagnostic techniques which should be used in conjunction with the *E.canis* Real-time PCR and serology to provide prognostic clinical data and facilitate the evaluation of response to therapy.

Approach to Diagnostic Testing.

According to the pathophysiology of the disease, PCR detection of *E.canis* on EDTA blood samples is most sensitive in the early stages of infection. In prolonged chronic infections, the sensitivity of PCR decreases as the organism sequesters in peripheral tissues. On the other hand, serological antibody titres in serum may take 7-14 days to develop the following infection.

1. Acute Clinical Disease.

PCR on EDTA blood samples and antibody serology (ELISA / IFA) on serum. The PCR detection of the nucleic acid of *E. canis* in an EDTA blood sample confirms active bacteraemia. Definitive serological diagnosis of acute *E. canis* infections, on the other hand, requires detection of an active *E. canis*-specific antibody response, as indicated by a rising antibody titre of greater than or equal to 4-fold in magnitude when testing paired blood samples by immunofluorescence assay (IFA). Ideally, paired-samples should be collected at least two weeks apart. PCR is the preferred diagnostic assay.

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2. Monitoring Response to Therapy.

The PCR is the preferred test for the monitoring response to treatment, primarily when combined with clinical and other laboratory parameters (platelet counts, leukocyte counts, clotting function tests, clinical chemistry). Persistently elevated antibody titres are well-documented post-exposure, and therefore serology is not recommended for monitoring response to treatment.

3. Disease Surveillance.

The ELISA is used for sero-surveillance of *E. canis* due to its low cost, accessibility, ability to screen large numbers of samples and high sensitivity and specificity. PCR testing of collected tick samples is also an integral part of any surveillance programme.

Results Interpretation Guideline (*Ehrlichia canis*).

ASSAY RESULT INTERPRETATION
Real-time PCR (qPCR) • POSITIVE = Active infection with *E.canis* bacteraemia. • Negative = No acute active infection. • Indeterminate = cannot interpret the result. (Repeat qPCR. If repeat indeterminate again, conventional PCR run).
Conventional PCR • POSITIVE = Active infection with *E.canis* bacteraemia. • Negative = No acute active infection.
Antibody (Ab) IFA/ELISA • Ab POSITIVE + PCR POSITIVE = Active infection with *E.canis* bacteraemia. • Ab POSITIVE + PCR Negative = Previous *E.canis* infection or chronic infection. • Ab Negative + PCR POSITIVE = Acute active *E.canis* infection (previous 10 days)

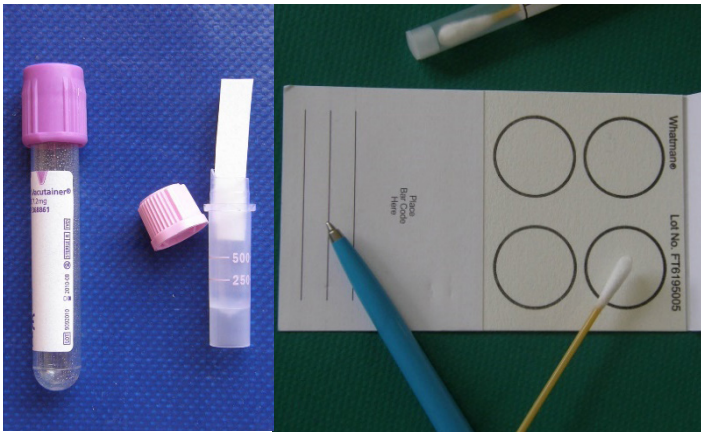


Figure 2. EDTA blood tubes and filter paper (blood-soaked) are the preferred specimen containers for whole blood samples. Dry swabs (without transport media) and FTA cards are the preferred samples for tissue PCR analysis.

Diagnostic Test Characteristics and Applications

A. **Polymerase Chain Reaction - *Ehrlichia canis* Antigen Detection.**

- *Ehrlichia canis* nucleic acid detection (PCR) is a sensitive procedure and the preferred test to detect acutely infected animals. The diagnostic sensitivity of PCR is highly dependent on the stage of the disease. When bacteria are present in circulation, the sensitivity and specificity are believed to be > 90%.
- The PCR can also be used as a confirmatory test on fresh tissues collected at post-mortem examination.
- For whole-blood samples, a minimum of 2ml collected into EDTA or fresh blood-soaked filter paper is required (figure 2). Cerebrospinal fluid collected into an EDTA blood tube is also suitable for PCR testing.
- Dry swabs and FTA cards are used for fresh tissue samples (lymph node, spleen, liver, kidney, lung) and PCR testing (figure 2).
- Acute and convalescent serology should always be used in conjunction with the PCR. Positive serology in the face of negative PCR indicates either previous infection, with clearance of the organism, or chronic infection.
- The *E. canis* PCR is best suited to be used in conjunction with serology to detect acutely and persistently infected animals.
- PCR of splenic aspirates is considered a much more sensitive alternative to blood-PCR. It is useful in distinguishing treated animals with persistent infection from those that retain high antibody titres after successful treatment. However, collecting splenic aspirates from live animals is not without significant risk.
- PCR analysis of joint fluid, CSF or aqueous humour is likely to provide more meaningful diagnostic information for localised infections. A minimum of 0.5 ml of fluid collected into a plane serum tube is required.
- PCR on ticks is useful as a disease surveillance procedure.
- If submission of fresh EDTA samples is not possible, the following samples would still be suitable for testing. EDTA samples stored at 4°C for no longer than two months. EDTA samples frozen at -80°C (regardless of the length of storage). EDTA samples frozen at -20°C for less than one year. EDTA samples stored at room temperature (15-30ºC) for > 4 days are **not** suitable for PCR testing.



Figure 3. Plain serum and clot activator serum tubes are the preferred samples

B. ***E. canis* Antibody Detection – Serology**

i. Indirect Immuno-Fluorescent Antibody (IFA) test.

- Serology is the most used screening method. A minimum of 0.5 ml of clotted blood is required. Following collection of blood into serum tubes with/without clot activator (figure 3), allow samples to stand for 20-30 minutes at room

temperature to form a clot. They should be centrifuged, and the serum poured off.

- The preferred samples are an acute serum sample collected as early as possible during the disease syndrome, and a convalescent sample collected two to four weeks later. However, note that seroconversion in dogs can take up to 4-5 weeks post-infection.
- The Indirect Immuno-Fluorescent Antibody (IFA) test is the gold standard serological test for CME. The IFA test is considered to have excellent sensitivity. Cross-reactivity with antibodies against closely related *Ehrlichia* species and low-level cross-reactivity to *Anaplasma phagocytophilum* has been documented.
- Positive serology is commonly accepted at titres greater than 1:64 – 1:80, but it varies between laboratories. Antibody titres are lower in sub-clinically infected dogs.
- A negative result in a case with high clinical suspicion should have a PCR run on EDTA whole blood or spleen aspirates and the serology repeated in 4 – 6 weeks.
- Antibodies can be detected in cerebrospinal fluid, but any positive result must be confirmed with PCR.
- Positive IFA titres in asymptomatic dogs merely indicate previous exposure.
- The role of circulating *E. canis* specific antibodies in eliminating intracellular infection is minimal. Therefore, high antibody titres do not provide protection should the animal be challenged.
- Treated dogs should be retested by IFA at two months post-treatment. If the IFA titre does not drop significantly (>16-fold), additional treatments will be required. PCR is the preferred test for monitoring response to therapy.
- In these assays, antibodies to E. canis can cross-react with other Ehrlichia species, but this is probably of limited importance in South Africa, with only *E. canis* and *E. ruminantium* known to occur.

ii. Enzyme-linked Immunosorbent Assay (ELISA).

- Most ELISA methods are only semi-quantitative, so IFA is preferred wherever possible.
- ELISA assays accurately identified positive cases (by IFA) when IFA titres were > 1:320 but were unreliable when titres were between 1:80 – 1:320.
- The application of the ELISA is ideally suited for disease surveillance rather than individual animal diagnostics.
- The ELISA assay has no application in monitoring response to therapy; an *E.canis* PCR should be employed for this purpose.

REFERENCES

1. Greene CE. 2012. *Infectious Diseases of the Dog and Cat*. 4th edn. Elsevier, St Louis, Missouri.
2. Kaneko J, Harvey J, Bruss M. 2008. *Clinical Biochemistry of Domestic Animals*. 6th edn. Academic Press, Ames, Iowa.
3. Last, RD, Hill JM, Vorster JH *et al*. 2010. *Vetdiagnostix Laboratory Manual*. 2nd edn. Serrati Publishers, Pretoria.
4. Rautenbach Y, Schoeman J, Goddard A. 2018. Evidence of canine babesiosis and Ehrlichia co-infection in the predictive value of hematology. *Onderstepoort Journal of Veterinary Research* 85(1), a1626. https://doi.org/ 10.4102/ojvr.v85i1.1626.
5. Weiss DJ, Wardrop KJ. 2010. *Schalm's Veterinary Hematology*. 6th edn, Wiley-Blackwell, Ames, Iowa.
6. Commonwealth Scientific and Industrial Research Organisation (CSIRO). 2021. *Diagnostic testing for Ehrlichia canis at the CSIRO Australian Centre for Disease Preparedness*, Version 3.



The SAVA Stress Management Hotline is there to assist members who are experiencing personal problems by offering access to professional counselling/advice.

The hotline can assist with referrals or simply offer much needed emotional support when anxiety, depression, anger, grief, loneliness and fear are at their highest.



The following SAVA members are available on the SAVA stress management hotline. If required, they will refer you to professionals.

Ken Pettey	082 882 7356	ken.pettey@gmail.com
Tod Collins	083 350 1662	tcollins@isat.co.za
Aileen Pypers	072 599 8737	aileen.vet@gmail.com
Willem Schultheiss	082 323 7019	willem.schultheiss@ceva.com
Mike Lowry	084 581 2624	mikelowry@sai.co.za

QUESTION 1

In which of the following blood cells are the morulae of Ehrlichia canis bacteria encountered?

a. Lymphocytes
b. Monocytes
c. Erythrocytes
d. Platelets
e. Neutrophils

QUESTION 2

What is the most common clinical presenting sign of chronic ehrlichiosis?

a. Fever
b. Haemorrhagic tendencies
c. lymphadenopathy
d. Splenomegaly
e. Pancytopenia

QUESTION 3

Which of the following clinical parameters are common features of both canine ehrlichiosis and babesiosis?

a. Epistaxis
b. lymphadenopathy
c. uveitis
d. Thrombocytopenia
e. Retinal haemorrhage

QUESTION 4

Which is the most definitive test for the diagnosis of acute clinical ehrlichiosis in dogs?

a. Polymerase chain reaction (PCR)
b. Smear examination
c. Haematology
d. Clotting function test
e. Clinical chemistry

QUESTION 5

Which of the following is the correct interpretation of an E. canis antibody positive PCR negative result?

a. Active infection with bacteraemia
b. No current or previous infection
c. Previous infection or chronic infection
d. Acute infection in the previous 10 days
e. Indeterminate result

QUESTION 6

The diagnostic sensitivity of the E.canis PCR is most dependent on which of the following factors?

a. Low platelet count
b. Pancytopenia
c. Active bacteraemia
d. Abnormal clotting function
e. Hypergammaglobinaemia

QUESTION 7

In suspected localised E. canis associated meningitis which would be the preferred diagnostic assay?

a. PCR on EDTA blood
b. IFA on serum
c. Cytological examination of CSF for morulae
d. PCR on CSF
e. IFA on CSF

QUESTION 8

Which of the following EDTA samples would not be considered suitable for E.canis PCR testing?

a. Fresh EDTA blood sample transported on ice to the laboratory
b. EDTA blood samples stored at 4°C for less than 2 months
c. EDTA blood samples frozen at -20°C for less than one year
d. EDTA blood samples frozen at -80°C
e. EDTA blood samples stored at room temperature for > 5 days

QUESTION 9

Which of the following statements about Ehrlichia canis IFA serology is not correct?

a. Allow samples to stand for 20 to 30 minutes at room temperature post collection
b. Acute and convalescent serum samples collected 4 weeks apart are the preferred samples
c. A negative result even in patients with heart clinical suspicion would exclude Ehrlichia canis infection
d. A positive IFA titer in an asymptomatic dog merely indicates previous exposure
e. Antibodies to Ehrlichia canis can cross-react with other Ehrlichia species in the test

QUESTION 10

Which of the following statements about E.canis ELISA serology is correct?

a. ELISA is the preferred serological method for disease diagnostics
b. ELISA assay is ideally suited for disease surveillance
c. Ehrlichia ELISA accurately identifies positive cases with IFA titers of 1:80 – 1:320
d. Ehrlichia ELISA is a fully quantitative assay
e. Ehrlichia ELISA is the preferred essay for monitoring response to therapy

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