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Canine Monocytotropic Ehrlichiosis (CME) Sampling and Diagnostic Testing Guide. Part 2: The In-House Laboratory Tests

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1. **Smear Examination**

Ehrlichia canis morulae can be identified in peripheral blood smears, central blood smears, buffy coat smears, splenic aspirates, lymph node aspirates, cerebrospinal fluid and bone marrow aspirates from both live animals as well as at post mortem.

A. Blood smears (Peripheral / Central)

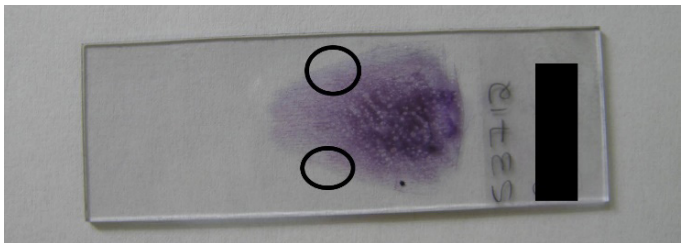


Figure 1. Thin blood smear with a broad multi-layered base and smeared out feathered end where a monolayer of erythrocytes, leukocytes and platelets are available for examination. The circled areas on the sides of the feathered edge are the areas where the examiner is most likely to find Ehrlichia canis organisms.

- Thin blood smears with a feathered end (figure 4) should be prepared from both **peripheral** bloods, collected from a needle prick on the ear pinnae and directly smeared out on a glass slide, as well as **central** blood collected in an EDTA anticoagulated tube from a large vein (cephalic, jugular), from which the thin smears are then prepared and air-dried prior to staining.
- All smears are stained with Diff-Quick® prior to microscopic examination.
- *Ehrlichia canis* organisms are only **visible** under the **100X oil immersion lens**.
- Parasitized monocytes containing *E.canis* morulae are heavier than non-parasitized leukocytes and are pushed out to the periphery of the feathered end of the smear during preparation, coming to lie predominantly along the distal edges of the blood smear feather (figure 4 circles). These smear areas should be thoroughly examined for morulae under the 100X oil immersion lens.

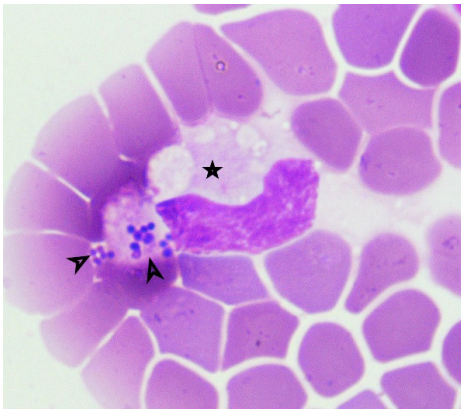


Figure 2. Thin blood smear (100X) demonstrating a blood monocyte with reactive vacuolated cytoplasm (asterisk) and containing multiple Ehrlichia morulae composed of clusters of spherical organisms arranged as a "bunch of grapes" (arrowheads).

- Detecting morulae in monocytes within blood smears (peripheral/central) is diagnostic with morulae appearing as multiple small clustered spherical bodies with a "bunch of grapes" confirmation or as larger single, hyperchromatic spherical bodies (figures 4 and 5). However, blood smear examination lacks sensitivity, as estimated parasitaemia's in early infections are only 1% of circulating leukocytes. Parasitaemias are also extremely low in subclinical disease.

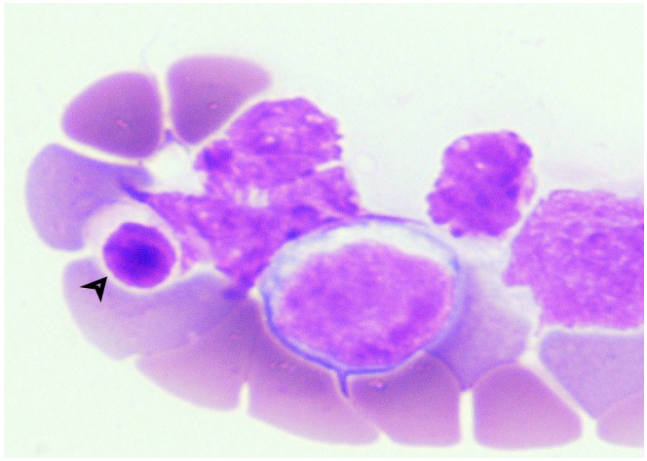


Figure 3. Thin blood smear (100X) with a group of reactive monocytes, one containing a single, large, intracytoplasmic, hyperchromatic round Ehrlichia morula.

- The demonstration of reactive vacuolated monocytes in blood smears collected from suspected CME cases should alert the examiner to hunt carefully for *Ehrlichia* morulae (figure 5 asterisk).
- A negative blood smear evaluation does not exclude *E. canis* infection.

B. Buffy Coat Smears

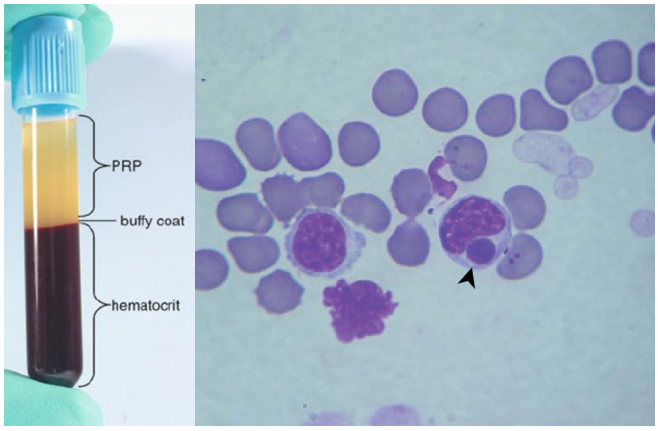


Figure 4. The buffy coat is created by centrifuging the whole blood sample in EDTA and is largely composed of leukocytes and platelets. Monocytes are, therefore, condensed into leukocyte-rich smears facilitating the demonstration of Ehrlichia morulae

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- The sensitivity of detecting Ehrlichia morulae in the blood is markedly increased (up to ± 40%) by examining buffy-coat smears (figure 7).
- Buffy coat smears are prepared from fresh whole blood collected into EDTA anticoagulant and should be produced within 2 hours of blood collection.
- After preparing the thin blood smears from the EDTA sample, the sample should be centrifuged to separate the sample into plasma (PRP), buffy coat (white blood cells and platelets) and erythrocytes (haematocrit) (figure 7).
- After centrifugation, remove and discard the plasma leaving a small amount just above the buffy coat.
- Then aspirate the remaining plasma and buffy coat and mix this aliquot thoroughly.
- Prepare thin and thick smears from the buffy coat aliquot, air dry and stain with Diff-Quick® for examination under 100X oil immersion.

C. Splenic Aspirate Smears.

- Splenic aspirate smears are more likely to contain *Ehrlichia* morulae than blood smears or buffy coat smears. The spleen is suspected to be the last organ to host the bacteria before elimination. Splenic aspirates produce thick smears.
- There is a significant risk of intra-abdominal haemorrhage when aspirating splenic tissue through the abdominal wall of a live animal. Therefore, this procedure should be **carefully considered** in the **live animal** and rather reserved for use as part of a post-mortem examination.



Figure 5. Splenic aspirate smears produce thick smears. They should be performed with extreme caution in the live animal.

- Detection of morulae in splenic aspirate smears stained with Diff-Quick® is more likely than from buffy coat smears, particularly in sub-clinically infected animals.

*Lymph node Aspirate Smears have low sensitivity in detecting the presence of *Ehrlichia* morulae.

2. **Haematology.**



Figure 6: Lavender top EDTA anticoagulant blood tube for haematology.

- For haematology, blood is collected into an EDTA anticoagulant.
- Thrombocytopaenia is the most frequent haematological abnormality (in >80% of cases) of all forms of ehrlichiosis. Also, a common haematological change in canine babesiosis.
- Thrombocytopaenia is frequently accompanied by leukopenia and non-regenerative anaemia in acute ehrlichiosis.
- Thrombocytopaenia alone is usually the only haematological abnormality in subclinical disease.
- Pancytopenia is a feature of chronic disease.

3. **Clotting Function Tests.**

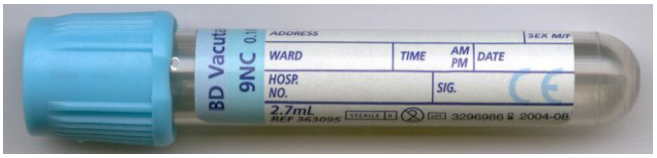


Figure 7. Citrate tubes are the only tube recommended for coagulation assays.

- The Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) are measured to evaluate clotting times.
- Sampling is very important: The preferred anticoagulant is sodium citrate (figure 10), with a ratio of anticoagulant-to-blood of 1:9. Under-filling citrate tubes with blood causes a relative excess of citrate that falsely prolongs the clotting times. The animal should be bled straight into the citrate tube.
- A citrated blood sample from a normal animal of the same species should be submitted to provide a helpful control.
- APTT and PT assays are still valid if performed on plasma kept refrigerated at 4°C for up to 48 hours after collection.

Haematology Predictors

- **Significantly depressed leukocyte counts, and platelet counts, low haematocrit, hypokalaemia and prolonged activated partial thromboplastin time (APTT), have high predictive value for mortality.**
- **Total leukocyte counts > 5.8 x 10^3/µL; platelet counts >89.5 x 10^3/µL; haematocrit >33.5%, APTT <14.5 seconds and potassium >4.75mmol/L are strong positive predictors of survival.**
- **The greater the magnitude of the thrombocytopaenia, the greater the possibility for a positive PCR result.**

4. **Clinical Chemistry**

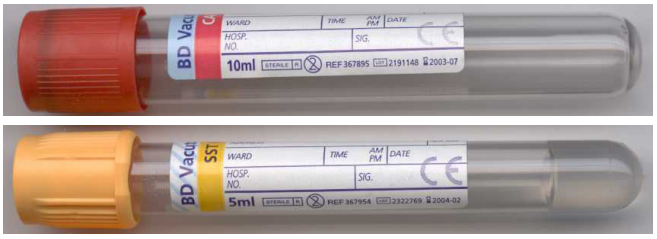


Figure 8. Plane serum (red-stopper) and clot-activator serum (yellow stopper) tubes are the preferred blood tubes for clinical chemistry analysis and serum electrophoresis.

- Clinical chemistries are performed on separated serum collected into plain serum or clot-activator serum tubes (figure 11).
- The most consistent clinical chemistry abnormalities observed in cases of CME include hyperproteinaemia, hypergammaglobinaemia, hypoalbuminaemia and elevated alanine aminotransferase (ALT) and alkaline phosphatase (ALP).
- Serum electrophoresis usually demonstrates a polyclonal hypergammaglobinaemia with monoclonal gammopathies reported in individual cases. Therefore, a monoclonal gammopathy **is not exclusive** to CME.

5. **Post-Mortem Samples.**

- The preferred fresh post-mortem samples for PCR (fresh tissue, dry swab, FTA card) include spleen, lymph node, liver, kidney, lung and bone marrow. Spleen would be considered the best sample and most likely to contain *E.canis* nucleic acid at detectable levels.
- Samples collected into 10% buffered formalin for histopathology should include spleen, lymph node, liver, kidney, lung, brain and bone marrow.

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MULTIPLE-CHOICE QUESTIONS

QUESTION 1

At which location in a thin peripheral blood smear are *Ehrlichia canis* bacterial organisms most likely to be found?

a. Tip of the feathered end of the smear
b. Lateral distal edges of the feathered end of the smear
c. Centrally at the base of the feathered end of the smear
d. Thick portion within the body of the smear
e. Thick base of the smear

QUESTION 2

Which of these cytological features within peripheral or central blood smears should alert the examiner to the possible presence of *Ehrlichia canis* bacterial morulae?

a. Presence of reactive vacuolated monocytes
b. Presence of giant platelets
c. Increased numbers of nucleated erythrocytes
d. Increased numbers of lymphocytes
e. Prominent erythrocyte anisocytosis

QUESTION 3

Of these types of smears, which provides the highest sensitivity in detecting *Ehrlichia canis* bacterial morulae?

a. Thin peripheral blood smear
b. Thick peripheral blood smear
c. Central blood smear
d. Buffy coat smear
e. Lymph node aspirate smear

QUESTION 4

From which type of blood sample are the buffy coat smears prepared?

a. Plain Serum tube
b. EDTA tube
c. Heparin tube
d. Sodium tube
e. Clot activator serum tube

QUESTION 5

At post-mortem, where are *Ehrlichia canis* bacterial morulae most likely to be found?

a. Lymph node
b. Bone marrow
c. Liver
d. Kidney
e. Spleen

QUESTION 6

What is the most frequently encountered haematological abnormality in dogs with acute clinical ehrlichiosis?

a. Leukopenia
b. Monocytosis
c. Thrombocytopenia
d. Lymphocytosis
e. Neutrophilia

QUESTION 7

Which of the following haematological factors <u>does not</u> have a high predictive value for mortality?

a. Marked leukopenia
b. Marked thrombocytopenia
c. Hypokalaemia
d. Low haematocrit
e. Rapid activated partial thromboplastin time (APTT)

QUESTION 8

Which of the following clinical chemistry abnormalities is not a consistent finding in clinical ehrlichiosis of dogs?

a. Hypoproteinemia
b. Hypergammaglobinaemia
c. Hypoalbuminemia
d. Elevated alanine aminotransferase (ALT)
e. Elevated alkaline phosphatase (ALP)

QUESTION 9

Which of the following abnormal laboratory parameters is most frequently encountered in chronic ehrlichiosis?

a. Monoclonal hypergammaglobinaemia
b. Polyclonal hypergammaglobinaemia
c. Hyperproteinemia
d. Pancytopenia
e. Leukopenia

QUESTION 10

At post-mortem, which tissue should always be collected for *Ehrlichia canis* PCR analysis?

a. Bone marrow
b. Lymph node
c. Spleen
d. Kidney
e. Lung

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