## Performance Characteristics

The **BioSign® CHW** test was evaluated with clinical samples identified by reference assays including necropsy and a commercially available ELISA method. Relative overall sensitivity was 97.9 %, relative overall specificity was 97.5 % and overall accuracy was 97.7 %. The assay was sensitive enough to detect worm burden as low as 1 adult female worm (sensitivity = 92.3 %, N=13). Both visual reading and DXpress™ reading for each individual test was identical with no discrepant result in this study.

| | | BioSign® Test result | | Total | performance |
|---|---|---|---|---|---|
| | | Positive | Negative | | |
| Necropsy positives[1)] | # of live female worms | | | | |
| | 1 | 12 | 1 | 13 | 92.3% sensitivity |
| | 2 | 11 | 0 | 11 | 100% sensitivity |
| | 3 | 11 | 0 | 11 | 100% sensitivity |
| | ≥ 4 | 12 | 0 | 12 | 100% sensitivity |
| | Total necropsy positives | 46 | 1 | 47 | 97.9% sensitivity |
| Reference assay diagnosed [2)] | Reference negatives | 1 | 39 | 40 | 97.5% specificity |

[1)] Samples were identified with necropsy diagnosis

[2)] Samples were identified with reference assay: commercially available ELISA method

## References


1. Ettinger SJ. Textbook of Veterinary Internal Medicine. 6th ed. St. Louis, USA. Elsevier Saunders 2005, P 1134
2. Brunner CJ, Hendrix CM, Blagburn BL, Hanrahan LA. (1988) Comparison of serologic tests for detection of antigen in canine heartworm infections. J Am Vet Med Assoc. 192:1423-7.
3. Pratt SE, Corwin RM, Selby LA, Rhoades JD. (1981) Prevalence of *Dirofilaria immitis* and *Dipetalonema reconditum* infections in Missouri dogs. J Am Vet Med Assoc. 179:592-3.





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P-5432


# Canine Heartworm Antigen Test Kit

## BioSign® CHW

**For Professional Use**

**Rapid test for detection of adult *Dirofilaria immitis* Antigen In canine serum, plasma and whole blood**

***PBM***

## Intended Use

The **BioSign® CHW** test is an *in vitro* qualitative immunochromatographic assay for the detection of heartworm antigens of heartworm infection in canine serum, plasma or whole blood.

## Summary and Explanation

The canine heartworm, *Dirofilaria immitis*, is a common parasite of dogs worldwide. Transmission occurs through the bite of a mosquito harboring third-stage larvae. Third-stage larvae undergo two molts in the mammalian host to become adults and those continue to migrate to reach the heart and pulmonary arteries. After mating, female parasites produce microfilariae, which circulate in the blood or cutaneous tissues. The mosquito ingests circulating microfilariae while feeding on an infected dog. Within the mosquito, the microfilariae undergo two molts to become the infective third-stage larvae that can be transmitted into another animal.[1]

The heart and lungs are the major organs affected by heartworm in dogs. However, dogs with low worm burdens that receive little cardiopulmonary exercise may never have overt signs of heartworm disease. The physical presence of the heartworm parasite in the pulmonary and right ventricle of canine heart, and the resulting destruction of tissue, causes respiratory and circulatory problems that can be fatal under conditions of stress and vigorous exercise.

One of the common diagnostic procedures for canine heartworm infection is to detect circulating antigens secreted primarily by adult female worms.[2] This is available as an in-clinic test as well as at veterinary reference laboratories. Another common diagnostic method is identification of microfilariae by microscopic examination requiring concentration technique (centrifugation or filtration of blood sample).[3] Approximately 20% of dogs do not test positive for microfilariae even though they have heartworms due to the acquired immunity. Therefore, the antigen test is the preferred diagnostic method for canine heartworm infection. Both the microfilariae and the specific antigens are detectable beginning at 6 months post-infection.

**BioSign® CHW** uses solid-phase immuno-chromatographic technology for the detection of antigens produced by adult female *D. immitis* in canine serum, plasma or whole blood. The test is an immunometric assay using monoclonal antibody to selectively detect the heartworm antigens.

## Reagents

**Materials Provided:** Each BioSign® CHW test kit contains all the necessary reagents to perform the test.

- **BioSign® CHW** test device and disposable sample transfer pipette
- One bottle of developer solution

## Precautions

- For *in vitro* diagnostic use only.
- Do not use a test device or reagent after the expiration date or mix from different kit lots.
- Use a fresh transfer pipette for each specimen. Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling kit reagents or specimens, and thoroughly wash hands afterwards.
- All specimens should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- The **BioSign® CHW** device should remain in its original sealed pouch until ready for use. Do not use the test if the seal is broken or the pouch is damaged.

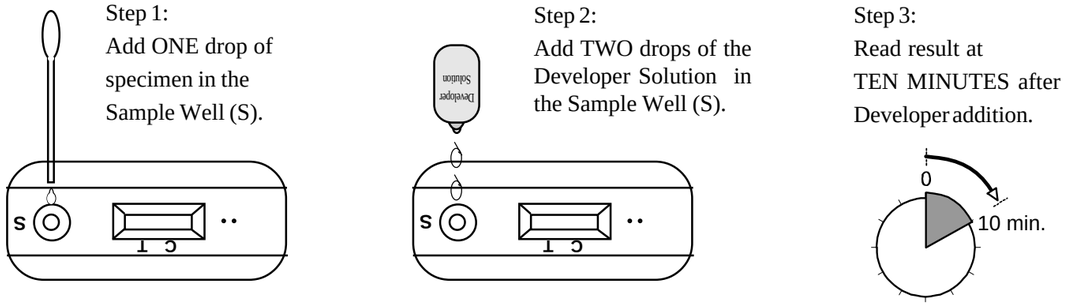
## Storage and Stability

The **BioSign® CHW** test kit should be stored at 2-30°C (35-86°F) in the original sealed pouch. The expiration dating provided was established under these storage conditions.

## Specimen Collection and Preparation

- Whole blood collected over heparin, citrate or EDTA can be used. Mix whole blood by inversion and test as outlined in the Test Procedure. Collected whole blood can be stored between 2 - 8 °C for 24 hours.
- Turbid serum samples should be centrifuged for 15 minutes at approximately 1,000 relative centrifugal force.

## Test Procedure




- Test should be performed as soon as possible after sample collection. For periods of less than 24 hours, specimens can be refrigerated between 2–8 °C. For storage longer than 24 hours, plasma or serum should be stored at temperatures below -20 °C. Do not freeze whole blood samples.
- Allow specimens to warm to room temperature (18–30°C) prior to testing. The frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing.
- If specimens are to be shipped, they should be packed in compliance with regional regulations covering the transportation of etiologic agents.

## Procedure

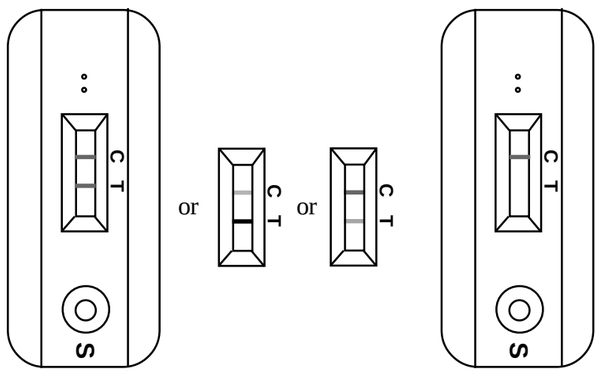
### Procedural Notes

The instructions below must be followed to achieve optimal test reactivity with the specimens. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens or **BioSign® CHW** devices have been stored in a refrigerator, allow them to warm to room temperature before opening the foil pouch for testing.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid cross-contamination, use a new disposable pipette for each specimen.
- Label the device with the specimen name or control number.
- After testing, dispose of the **BioSign® CHW** device and the specimen dispenser following good laboratory practices. Consider each material that comes into contact with the specimen to be potentially infectious.

### Test Procedure (see diagram above)

1. Add one drop of the sample to the Sample well (S) using a disposable pipette, holding it vertically about 1 inch above the Sample well (S), without touching the well.
2. Add two drops of the Developer solution into the Sample well (S), immediately following complete absorption of sample into the sample well. To ensure that the correct solution volume is added, hold the bottle vertically while dispensing the solution.
3. Read the test results at 10 minutes.




2 Lines = **Positive** (+) 1 Line = **Negative** (–)

## Interpretation of Results

**Positive:** Two colored lines, one line at the Control position (C) and the other line at the Test position (T), indicate that antigens from *D. immitis have* been detected.

***Note**: The test line may appear before the control line forms in most strong positive cases. The test line may appear after the control line in weak positive cases, and the control line may be darker than the test line. The three possible positive cases, therefore, are:*

a. Two strong colored lines, one at the Test position (T) and one at the Control position (C).
b. One strong colored line at the Test position (T) and one light colored line at the Control position (C).
c. One light colored line at the Test position (T) and one strong colored line at the Control position (C).

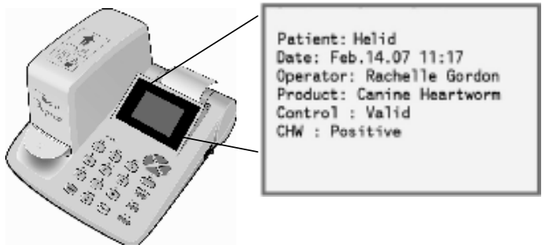
**Negative:** Only one colored line at the Control position (C), with no distinct colored line at the Test position (T) indicates that antigens from *D. immitis* have not been detected.

**Invalid**: A distinctive colored line should always appear at the Control position (C). If no line forms at the Control position (C) after 10 minutes, the test is invalid and should be repeated with a new **BioSign® CHW** test.

**Alternative Reading Method**: Testing sample using the DXpress™ Reader:

1. Scan device barcode and patient ID using the barcode reader, or type them in using key pad on the DXpress™ Reader.
2. Place the test device on a flat surface.
3. Add one drop of the sample to the Sample well (S) using a disposable pipette, holding it vertically.
4. Add two drops of the Developer solution into the Sample well (S), immediately following complete absorption of sample into the sample well. To ensure that the correct solution volume is added, hold the bottle vertically while dispensing the solution
5. Place the test device in the DXpress™ Reader tray. Close the tray and begin operation of the reader.

6. After 10 minutes of incubation the DXpress™ Reader will automatically display the results on the screen. The result can also be printed out. See the DXpress™ Reader user manual for more detail.




## Limitations

- The assay must be performed strictly in accordance with these instructions to obtain accurate, reproducible results.
- The **BioSign® CHW** test is for *in vitro* diagnostic use only.
- This test will indicate only the presence or absence of *D. immitis* antigens in the specimen, and should not be used as the only basis for the diagnosis of heartworm infection. For diagnosis of canine heartworm infection, the result must be considered with other clinical information available to the veterinarian.
- **BioSign® CHW** has not been shown to detect infections consisting of only male heartworms.

## User Quality Control

A colored line at the Control position (C) can be considered an internal procedural control. If the test has been performed correctly and the device is working properly, a distinct colored line will always appear. If a test result is not clear, a new test should be performed. If the problem persists, contact PBM's Technical Services for assistance.