Test protocol

Before you begin

- Allow reagents to come to 18° C–26° C (64º F–79º F).
- Swirl gently to mix.
- Use timer for all incubation steps (such as a smart phone).
- The bottles containing Reagent 1, 2, 3, and 4 require a dropper cap for running the assay. Before use, replace the bottle closures that accompany each of these solutions during shipment with a dropper cap.
- Obtain materials from accessory pack: precision pipette, plate cover, and a wash bottle.
- Make sure there is an adequate supply of distilled or deionized water available for the two wash steps.
- IDEXX recommends no more than 30 samples per testing run (four strips, including controls).

Dispense controls and samples into ELISA plate

Using precision pipette:
- Dispense 100 μL (one full pipette tip) of Negative Control into one well.
- Change tip and dispense 100 μL (one full pipette tip) of Positive Control into one well.
- Change tip and dispense 100 μL (one full pipette tip) of sample into appropriate wells. Use a fresh tip for each sample.

Note: Use a fresh tip for each sample.

Dispense Reagent 1 (Detector Solution)

- Dispense 3 drops of Reagent 1 into each well.
- Cover the wells with the plate cover, gently tap the plate 10 times and incubate for 7 minutes at 18ºC–26ºC (64ºF–79ºF).

Dispense Reagent 2 (Conjugate Solution)

- Dispense 3 drops of Reagent 2 into each well.
- Cover the wells with the plate cover, gently tap the plate 10 times and incubate for 7 minutes at 18ºC–26ºC (64ºF–79ºF).

Dispense Reagent 3 (TMB Substrate)

- Dispense 3 drops of Reagent 3 into each well.
- Cover the wells with the plate cover, gently tap the plate 10 times and incubate for 7 minutes at 18ºC–26ºC (64ºF–79ºF).

Dispense Reagent 4 (Stop Solution)

- Dispense 3 drops Reagent 4 into each well.
- Gently tap the plate 10 times.

Read plate and interpret results

Read plate and assess validity

For valid results, the positive control well must appear blue. If the positive control does not turn blue, all results for the assay are invalid. Repeat the test following a thorough review of the kit insert.

Interpret results

The pregnancy status of each animal is determined by comparing the color development of the sample well to the negative control.

Pregnant

If blue color is visible in the sample well and is greater than that of the negative control well, then PAGs are present and the animal is considered pregnant.

Not pregnant

If the color development of the sample well is less than or the same as the negative control well, the animal is considered not pregnant (open).

Doubtful/Suspect

Sample wells that are difficult to interpret visually should be considered doubtful/suspect; pregnancy recheck is recommended.

Pregnant Negative control

Pregnant Open

Pregnant Pregnant

Negative control Open

This protocol is a summary of the steps required to perform the IDEXX Rapid Visual Pregnancy Test. For complete instructions, refer to the package insert. For use with bovine whole blood (EDTA), plasma (EDTA), or serum.

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