Healvet®

Cortisol Rapid Quantitative Test

INTENDED USE

The Healvet™ Cortisol Rapid Quantitative Test is a fluorescence immunoassay used along with Healvet™ FIA 3000 for quantitative determination of canine or feline Cortisol concentration in canine or feline serum or plasma specimen. Diagnostic and therapeutic effects of adrenal dysfunction

For *in vitro* diagnostic use only.For professional use only.

TEST PRINCIPLE

This test employs a quantitative competitive fluorescence immunoassay technique. A competitive binding assay is based upon the competition of labeled and unlabeled analyte for a limited number of antibody binding sites. Unbound antibodies and immunocomplexes migrate along the nitrocellulose membrane forward to the Test line. The unbound antibodies are then captured by antigens immobilized on the Test line. The more Cortisol in the patient specimen, the more immnocomplexes are formed, thus the less fluorescent-labeled antibodies captured on the test line. The fluorescent signal intensity reflects the amount of Cortisol captured and is processed in Healvet™ FIA 3000.The Cortisol concentration is expressed in nmol/L.

WARNINGS AND PRECAUTIONS

1.This kit is for in vitro diagnostic use only. Do not swallow.
2.Lot Number of all the test components (test device, ID chip and buffer) must match each other. Do not mix components from different kit lots.
3.Inspect the packaging and labels before use. Do not use if the pouch is broken, torn or not well sealed, or the vial looks damaged or leaked.
4.Carefully follow the instructions and procedures described in this insert.
5.Do not use the test device beyond the expiration date. The test device must remain in its original sealed pouch until ready to use. Do not use if the pouch or the device itself is damaged, torn or not fully sealed.
6.A buffer tube should be used for processing one sample only.
7.The operation shall be conducted away from vibration and magnetic field. Healvet™ FIA 3000may generate minute vibration during use, which should be regarded as normal.
8.One pipette tip should be used for one specimen only.
9.Do not touch the test area of the test device
10.All specimens and used test materials are considered as potentially infectious. The used pipette tips, buffer tubes, test devices and specimens must be handled carefully and disposed of in accordance with local regulations and procedures.

MATERIAL

Material Provided

Each box contains:
1.10 individual sealed pouches, each containing:
■ a test Device
■ a desiccant pouch
2.One Test Device ID Chip
3.Instructions for Use
4.10 tubes of Tris-HCl buffer
5. Pipette Tips

Material Required But Not Provided

1.Healvet™ FIA 3000
2.Transfer Pipette
3.Timer
4.Centrifuge

STORAGE AND STABILITY

1.Store the test kit at 4～30℃ up to the expiration date.
2.Once the pouch is opened, the test should be performed within an hour.
3.If removed from refrigerator, allow the testfor 30 minutes to attain room temperature before testing.

SPECIMEN COLLECTION AND PREPARATION

The test can be performed with either serum or plasma.

For Plasma or Serum:

1.Following standard phlebotomy venipuncture procedure, collect whole blood specimen using a blood collection tube.
If plasma sample will be used, use blood collection tube containing an EDTA anticoagulants.If serum sample will be used, use a vessel without anticoagulant.(do not use the serum separator tube)
2.Separate serum or plasma from blood within 2 hours after blood collection. If a specimen appears to be severely hemolyzed, another specimen should be obtained and tested.
3.Optimally, the test should be performed immediately after the specimen collection. If the test cannot be performed within 2 hour after blood collection, store the specimen at 2℃～8℃ for no longer than 48 hours. For long-term storage, specimens shall be kept below -20℃.

Bring all materials to room temperature before use. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly. Only clear, non-hemolyzed specimens can be used.

TEST PROCEDURE

Refer to Healvet™ FIA 3000 Operation Manual for complete instructions for use of the Test device.
1.Set a Test Device on a clean, level horizontal place.
2.Make sure that the Test Device lot No. matches with ID Chip No. Insert ID Chip into the meter. Be aware not to touch the insertion tip of the ID chip.
3.Pipette75μl of prepared sample into the buffer, gentlymix well. Vigorous agitation and foaming should be avoided.
4.Pipette 75μl of mixed sample to add into the sample of the test device. Avoid forming bubbles.
5.Please refer to the Section V Operation in Operation Manual for details.
a) **Quick Test mode:** start the timer right after adding the sample mixture to the sample well. Leave the Test Device at room temperature for 15 minutes. Then insert test device immediately onto the holder of the meter and click Test. The instrument will scan the Test Device automatically and show the test result.
b) **Standard Test mode:** insert the Test Device into the device holder of the Meter right after adding the sample to the sample well, click Test. The meter will start to countdown and read the test result automatically.
6.Results are displayed on the main screen or be printed by click Print.

INTERPRETATION OF RESULTS

1.The Healvet™ FIA 3000calculates Cortisol test results automatically and displays the concentration of Cortisol on the screen right after correctly adding the sample to the sample well for 15 minutes. For further information, refer to the Operation Manual for the Healvet™ FIA 3000.
2.Healvet™ FIA 3000 will prompt " Abnormal C-line " when insufficient sample volume or liquid is not fully crawled across the test line, then recommends adding sample again.

QUALITY CONTROL

Each Healvet™ Cortisol Rapid Quantitative Test contains an internal control for routine quality control requirements. This internal quality control is performed each time a patient sample is tested, If an invalid result from the internal control occurs, the meterwill display an error message, indicating that another test should be conducted.

LIMITATIONS OF PROCEDURE

1.This test is developed for testing canine or feline serum or plasma specimen.
2.The results of Healvet™ Cortisol Rapid Quantitative Test should be evaluated with all clinical and laboratory data available. If test results do not agree with the clinical evaluation, additional tests should be performed accordingly.
3.The false positive results include cross-reactions with some components of blood from individual to antibodies; and non-specific adhesion of some components in canine or feline blood that have similar epitopes to capture and detector antibodies. In the case of false negative results, the most common factors are: non-responsiveness of antigen to the antibodies by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; instability of Cortisol antigen, resulting in degradation with time and, or temperature, such that they become no longer recognizable by antibodies; and degraded other test components. The performance of the test is highly sensitive to the storage and handling conditions of kits and sample specimens at optimal conditions.
4.There is the possibility that factors such as technical or procedural errors, as well as additional substances in blood specimens that are not listed in the Cross-Reactivity section, may interfere the test and cause erroneous results.

INTERPRETATION

Reference range of Cortisol in canine or feline blood: （1 ug/dL=27.64 nmol/L）
1.Detection range: 10~1000 nmol/L
2.ACTH stimulation test: intramuscular injection of ACTH 2.2 IU/kg, the maximum does not exceed 40 IU, cortisol after 2 hours. The diagnosis is as follows:

Pre-stimulus Cortisol value	Cortisol value after stimulation	Diagnostic value
<55nmol/L	<55nmol/L	It needs to be evaluated in combination with cortisol test value after stimulation, Addison's disease.
	55-166nmol/L	Uncertain
55-166nmol/L	55-166nmol/L	Uncertain
	166-500nmol/L	Basically normal
	500-663nmol/L	Suspected Cushing's syndrome
	>663nmol/L	Cushing's syndrome requires high-dose dexamethasone suppression test to distinguish between ATH or PDH.

3.LDDST
Intravenous injection of hexadecadrol 0.01mg/kg ， and cortisol value was measured 4 hours and 8 hours later. The diagnosis was as follows:

type	Cortisol base value	Cortisol value after 4 hours	Cortisol value after 8 hours	Diagnostic value
LDDST	normal	＜39nmol/L (1.4ug/dL)	＜39nmol/L (1.4ug/dL)	normal
	normal or increased	＞39nmol/L and ＞50% base value	＞39nmol/L and ＞50% base value	Cushing's syndrome
	normal or increased	＜39nmol/L or ＜50% base value	＞39nmol/L and ＞50% base value	PDH
	normal or increased	＞39nmol/L or ＞50% base value	＞39nmol/L and ＜50% base value	PDH
	normal or increased	＜39nmol/L or ＜50% base value	＞39nmol/L and ＜50% base value	PDH

4. HDDST

Intravenous injection of hexadecadrol 0.1mg/kg ，and cortisol value was measured 4hours and 8 hours later. The diagnosis was as follows:

type	Cortisol base value	Cortisol value after 4 hours	Cortisol value after 8 hours	Diagnostic value
HDDST	normal or increased	＜39nmol/L or ＜50% base value	＞39nmol/L and ＞50% base value	PDH
	normal or increased	＞39nmol/L and ＞50% base value	＜39nmol/L or ＜50% base value	PDH
	normal or increased	＜39nmol/L or ＜50% base value	＜39nmol/L or ＜50% base value	PDH
	normal or increased	＞39nmol/L and ＞50% base value	＞39nmol/L and ＞50% base value	More tests are needed to distinguish PDH and primary adrenal hyperactivity

Each Laboratory should establish a reference range that is representative of the population to be evaluated. The diagnosis must be made in conjunction with other clinical symptoms and tests.

MANUFACTURED BY

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