ONE STEP Rabies Antigen Test

For veterinary diagnostic use only

Anigen Rapid Rabies Ag Test Kit

■ Principles

Anigen Rapid Rabies Ag Test Kit is a chromatographic immunoassay for the qualitative detection of rabies virus antigen in fresh brain tissue of canine, bovine or raccoon dog.

Anigen Rapid Rabies Ag Test Kit has two letters which are test(T) line and control(C) line on the surface of device. Test line and control line in the result window are not visible before applying any samples. The control line is a reference line which indicates the test is performing properly. The control line has to appear every time when the test has performed. If the rabies virus antigens are present in the sample, a purple test line would appear in the result window.

The highly selective antibodies to rabies virus are used as a capture and detector in the assay. These antibodies are capable of detecting rabies virus antigen with a high accuracy.

■ Materials provided (10 Tests/Kit)

1) Ten(10) Anigen Rapid Rabies Ag Test devices
2) Ten(10) Assay diluents tubes
3) Ten(10) Disposable swabs
4) Ten(10) Disposable droppers
5) One(1) Instructions for use

■ Materials required, but not provided

1) Timer

■ Precautions

1) The test kit is for canine, bovine or raccoon dog use only. Do not use for other animals.
2) The test device is sensitive to humidity as well as heat. Perform the test immediately after removing the test device from the foil pouch.
3) Do not reuse the test components.
4) Apply the sample using a disposable dropper vertically.
5) Do not touch the membrane in the result window of test device.
6) Do not use the test kit beyond the stated expiration date marked on the package label.
7) Do not use the test kit if the pouch is damaged or the seal is broken.
8) Do not mix components from different lot numbers because the components in this kit have been quality control tested as standard batch unit.
9) All samples should be handled as being potentially infectious. Wear protective gloves while handling samples. Wash hands thoroughly afterwards.
10) Decontaminate and dispose of all samples, used kits and potentially contaminated materials safely in accordance with national and local regulations.

■ Storage and Stability

1) Store the test kit at 2~30°C. **DO NOT FREEZE.**
2) Do not store the test kit in the direct sunlight.
3) The test kit is stable within the expiration date marked on the package label.

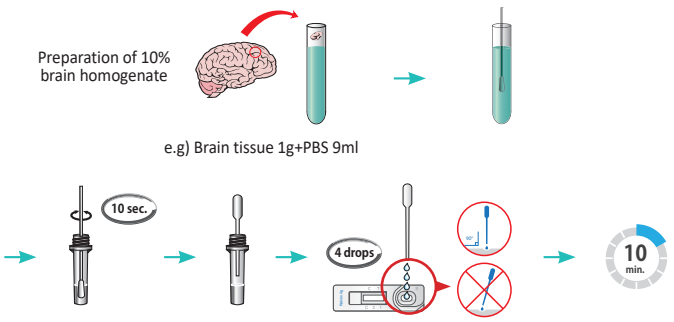
■ Collection and Preparation of Sample

1) Brain tissues from canine, bovine, or raccoon dog should be used for this test. Sample collection method is detailed in the next page.
2) Preparation of 10% brain homogenate
   ① Collect 1g of brain tissue (It is recommended that a pool of brain tissue that including the brain stem should be collected and tested) in a clean tube.
   ② Decompose 1g of brain tissue with any appropriate tools.
   ③ Mix 9㎖ of PBS(Phosphate buffer saline) with well decomposed brain tissue (1g) in the tube.

■ Procedure of the Test

1) All reagents and samples must be at room temperature (15~30°C) before use.
2) Collect the samples from brain homogenates using the disposable swab.
3) Insert the swab into the assay diluents tube.
4) Mix the swab until the sample has been dissolved into the assay diluents.
5) Remove the test device from the foil pouch, and place it on a flat and dry surface.
6) Using a disposable dropper, take the supernatant sample in the tube.
7) Add four (4) drops of mixed sample into the sample hole, drop by drop vertically.
8) Start the timer. The sample will flow across the result window. If it does not appear after 1 minute, add one more drop of mixed sample to the sample hole.
9) Interpret test results at 5~10 minutes. Do not read after 20 minutes.

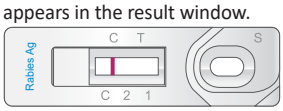
[Figure for test procedure]



■ Interpretation of the Result

1) Negative result

Only control("C") line appears in the result window.

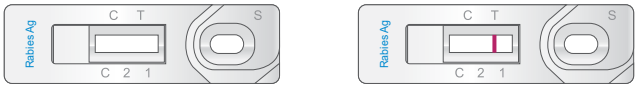


2) Positive result

Test("T") line and control("C") line in the result window indicate the presence of rabies antigen.

3) Invalid Result

If the control (“C”) line does not appear, the result might be considered invalid. The sample should be retested.



■ Limitations of the Test

1) Although the Anigen Rapid Rabies Ag Test kit is very accurate in detecting Rabies virus antigen, a low incidence of false results can occur. Other clinically or laboratory tests might be required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should be diagnosed by the veterinarian after all clinical and laboratory findings have been evaluated. The detection limit of this kit is about $3.3\times10^{3.0}TCID_{50}$/ml in mice and 10^{5}~10^{6} $TCID_{50}$/ml of CVS-Ⅱ and EBL-Ⅰ.
2) The reading window may show a light pink background coloration; this will not affect the accuracy of the results.
3) BioNote and its distributors cannot be held responsible for the consequences of misuse or misinterpretation of the results given by the test.

Gentaur

Doc. No.: I1801-8E
Issued date: Oct. 04, 2016

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Figure of Detailed Sample Collection Procedures

1. Brain tissue preparation

Ideal sample for the test

1) A fresh, unfixed brain sample is suitable for the test. Sample is preferable to taken within 48hours after death and repeated freeze–thaw cycles should be avoided. Refrigeration will preserve a sample for 48 hours.
2) Chemical fixation can alter tissue to make a sample unsuitable for testing.
3) Sample should be treated with extra caution to avoid contamination.

2. Sampling Spot

Option A: Brain stem + Cerebellum (preferred)

It is easier to take sample, because a cross section of the midbrain area would include all tissues necessary for rabies diagnosis.

Option B: Brain stem + Hippocampus

It needs extra sampling for hippocampus.

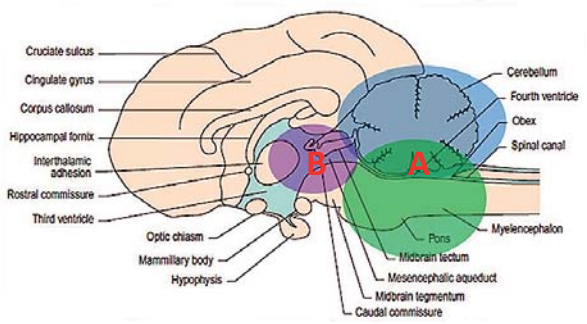

Figure 1 - Sampling spot; Option A; blue + green, Option B; purple + green, hippocampus are not visible in this sagittal plane.
<Image from: Veterinary Neuroanatomy>

3. Preparation of 10% brain homogenate

1) Collect brain tissue in a clean tube and decomposing it with any appropriate tools.
2) After decompensing, mix the sample with PBS
3) The ratio of brain tissue to PBS should be 1:9. For example, you should mix 9ml of PBS with well 1g of decompensated brain tissue.

4. Details for brain sampling

Option A

Lateral view of brain with cerebrum removed to show the extension of brain stem beneath the cerebellum (Figure A). A rabies diagnosis should include an observation of the cut surface of a cross section of the brain stem (through the medulla, pons, or midbrain area) and the cerebellum (through each hemisphere and the vermis). For example, a cross section of the midbrain area (blue line) would include all tissues necessary for rabies diagnosis; Surface of cut section through midbrain area showing convoluted gray matter and white foliar regions of the cerebellum, and the cerebellar connection to pons and medulla (Figure B).
<Image from: Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing >

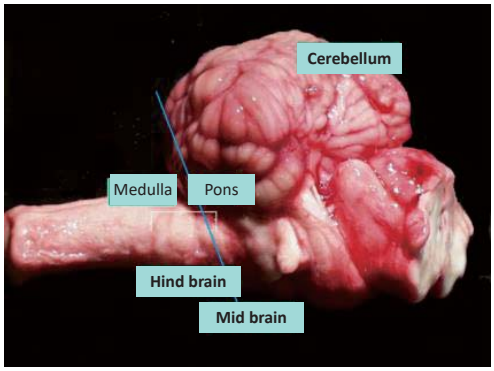

Figure A

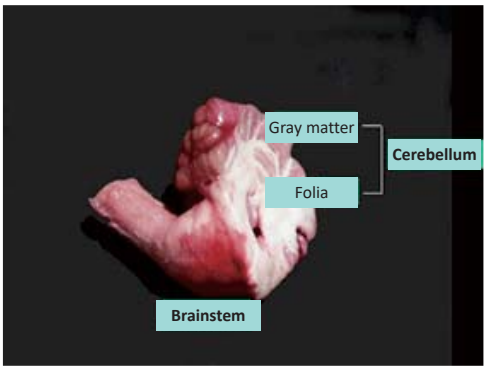

Figure B

Hippocampus for Option B

Dorsal view of cut through the cerebrum exposing the hippocampus (Figure C), Dorsal view of the two horn shaped protrusions of the hippocampus (Figure D). If the cerebellum is unavailable, Option B test can be made by taking sample from horns of the hippocampus.
<Image from: Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing>

Hippocampus tissue is needed for option B, which is buried deep in the temporal lobe near the center of the brain and is only visible when the brain is dissected. The lateral horn-shaped protrusions of the hippocampus are the reason for its alternative name, Ammon`s horn.

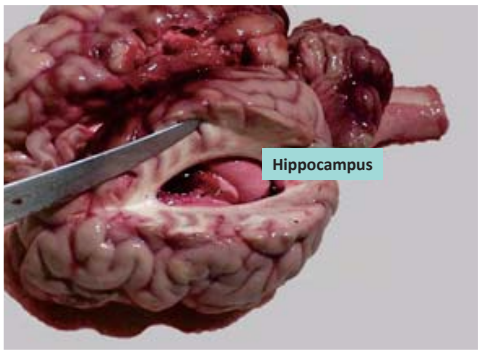

Figure C

Figure D

5. Reference

www.cdc.gov/rabies/pdf/rabiesdfaspv2.pdf

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