ONE STEP Rabies Antigen Test

For veterinary 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. The two letters on the surface of the test device stands for test (T) line and control (C) line. Test and control line in the result window are not visible before applying any sample. The control line is a reference line which indicates the test is working properly and therefore must appear each time the test is performed. If rabies virus antigens are present in the sample, a purple test line will appear in the result window.
Highly selective antibodies to rabies virus are used as a capture and detector in the assay, which are capable of detecting rabies virus antigen with a high accuracy.

Materials provided (10 Tests/Kit)

1) Ten (10) Anigen Rapid Rabies Ag Test device
2) Ten (10) Sample tube
3) Ten (10) Assay diluent tube
4) Ten (10) Disposable swab
5) Ten (10) Disposable dropper
6) One (1) Instructions for use

Materials required, but not provided

1) Timer

Precautions

1) The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.
2) Do not reuse the test components.
3) Apply the sample using a disposable dropper vertically.
4) Do not touch the membrane in the result window of test device.
5) Do not use the test kit beyond the stated expiration date marked on the package label.
6) Do not use the test kit if the pouch is damaged or the seal is broken.
7) Do not mix components from different lot numbers because the components in this kit have been quality control tested as standard batch unit.
8) All samples should be handled as being potentially infectious. Wear protective gloves while handling samples. Wash hands thoroughly afterwards.
9) 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.

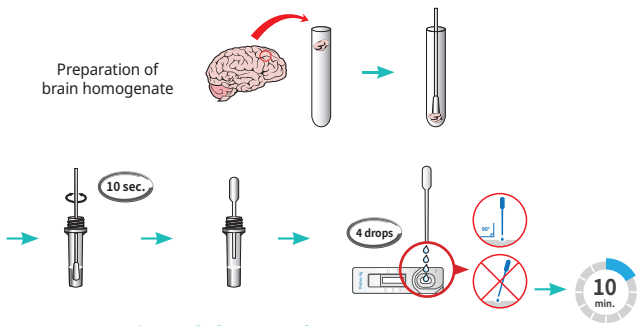
Collection and Preparation of Sample

1) Brain tissues should be used for this test. Sample collection is similar to that of other diagnostic tests for rabies, e.g. DFA, RT-PCR or dRIT and is detailed in the next page.
2) Preparation of 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 sample tube.
   ② With the swab provided in the kit, rub the brain tissue against the inside of the tube with the swab until the brain consistency is a smooth paste and the swab is coated with brain.

Procedure of the Test

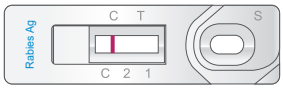
1) All reagents and samples must be at room temperature (15~30°C) before use.
2) Collect the samples from brain homogenates using the disposable swab.
3) Insert the swab into the assay diluent tube.
4) Mix the swab until the sample has been dissolved into the assay diluent.
5) Remove the test device from the foil pouch, and place it on a flat and dry surface.
6) Using a disposable dropper, take the supernatant sample in the assay diluent 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]

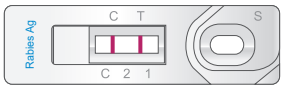


Interpretation of the Result

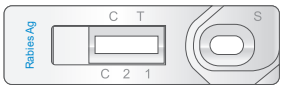
1) Negative result
Only control ("C") line appears in the result window.

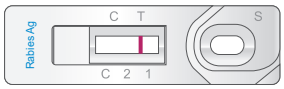


2) Positive result
Test ("T") line and control ("C") line in the result window indicate the presence of rabies antigen.



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. While a positive reaction on the test line is proof of rabies, a negative result does not rule out an infection. Therefore, any LFD negative sample may be subject to further testing. Additionally, if you suspect a faulty result, we recommend you perform other clinical tests or confirmatory test at a lab to confirm the result.
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.

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Detailed Sample Collection Procedures

1. Precautions

Precautions should be taken when handling central nervous system tissues from suspected rabies cases. Protective equipment (such as gloves, face shield, mask) should always be worn and precautions must be taken to prevent aerosols. Cutting tools, scissors and scalpels, should be used with care to prevent injury and contamination.

2. Brain tissue preparation

Ideal sample for the test

1) A fresh, unfixed brain sample is suitable for the test. Preferably sample should be taken within 48 hours 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.

3. Collection of brain samples

Ideally, the brain is collected following the opening of the skull in a necropsy room, and the appropriate samples are collected, preferably brain stem, Ammon's horn, thalamus, cerebral cortex, cerebellum and medulla oblongata (*Option A*). Alternatively, methods of collecting some brain samples without opening the skull can also be applied, particularly under field conditions (*Option B*).

Option 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 (Figure 1). Additionally, if hippocampus tissue is needed, this area 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.

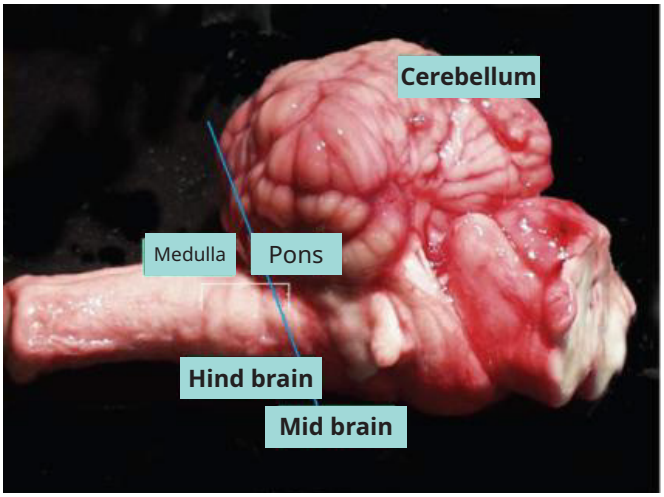

Figure 1. Lateral view of brain with cerebrum removed to show the extension of brain stem beneath the cerebellum (Source: CDC)

Option B

Bend the head to expose the occipital region, cut the skin and neck muscles over the joint between the occipital bone and the atlas vertebra using a disposable scalpel (cut from ear to ear following the line of the skull), open the atlanto-occipital joint by cutting the dorsal membrane and the meninges to access the foramen magnum (cut between the last vertebrae and skull to expose the base of the skull and the spinal cord).

Figure 2: Demonstration of accessing the foramen magnum from the dorsal site (Source: WHO)

A sample containing portions of medulla oblongata, base of the cerebellum, Ammon's horn region and cerebral cortex can be obtained by introduction of an approximately 5 mm in diameter sturdy plastic cylinder (e.g. 1–2 ml truncated syringe, artificial insemination sheath, 2 ml disposable plastic pipette with tip removed, or similar) into the occipital foramen in the direction of the eye.

Alternatively, scalpel and thumb forceps can be used to harvest a complete cross-section of brain stem accessed through the foramen magnum, followed by retrieval of portions of cerebellum using a plastic pipette.

5. Reference

1) WOAH (2023): Chapter - Rabies (Infection with Rabies virus and other Lyssaviruses). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.
2) Chapter 7: Laboratory techniques in rabies, 5th edition; Rupprecht, C.E., Fooks, A.R., Abela-Ridder, B., Eds.; World Health Organization: Geneva, 2018; pp 67–72.
3) CDC (2016): Protocol for Postmortem Diagnosis of Rabies in Animals by Direct.
Fluorescent Antibody Testing,
www.cdc.gov/rabies/pdf/rabiesdfaspv2.pdf

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