Ebola Antigen Rapid Test

(Whole Blood/Serum/Plasma)

A rapid test for the qualitative detection of Ebola virus antigens in human whole blood, serum or plasma specimens.
Please read the package insert carefully before using.
For professional in vitro diagnostic use only.

【SPECIFICATION】

25 Tests/Kit, 40 Tests/Kit

【INTENDED USE】

The iCARE Ebola Antigen Rapid Test is a rapid, serological, lateral flow chromatographic immunoassay for the qualitative detection of antigens from Ebola viruses in human whole blood, serum or plasma specimens as an aid in the diagnosis of Ebola virus infection.

【SUMMARY】

The Ebola virus causes an acute, serious illness which is often fatal if untreated. Ebola virus disease (EVD) first appeared in 1976 in 2 simultaneous outbreaks, one in Nzara, Sudan, and the other in Yambuku, Democratic Republic of Congo. The latter occurred in a village near the Ebola River, from which the disease takes its name.

Electron micrographs of members of genus Ebolavirus show them to have the characteristic thread-like structure of a filovirus. EBOV VP30 is around 288 amino acids long. The virions are tubular in general form but variable in overall shape and may appear as the classic shepherd's crook or eyebolt, as a U or a 6, or coiled, circular, or branched; laboratory techniques, such as centrifugation, may be the origin of some of these formations. Virions are generally 80 nm in diameter with a lipid bilayer anchoring the glycoprotein which projects 7 to 10 nm long spikes from its surface. They are of variable length, typically around 800 nm, but may be up to 1000 nm long. In the center of the virion is a structure called nucleocapsid, which is formed by the helically wound viral genomic RNA complexed with the proteins NP, VP35, VP30, and L. It has a diameter of 80 nm and contains a central channel of 20–30 nm in diameter. Virally encoded glycoprotein (GP) spikes 10 nm long and 10 nm apart are present on the outer viral envelope of the virion, which is derived from the host cell membrane. Between envelope and nucleocapsid, in the so-called matrix space, the viral proteins VP40 and VP24 are located.

The virus family Filoviridae includes 3 genera: Cuevavirus, Marburgvirus, and Ebolavirus. There are 5 species that have been identified: Zaire, Bundibugyo, Sudan, Reston and Taï Forest. The first 3, Bundibugyo Ebolavirus, Zaire Ebolavirus, and Sudan Ebolavirus have been associated with large outbreaks in Africa. The virus causing the 2014 west African outbreak belongs to the Zaire species.

Ebola then spreads through human-to-human transmission via direct contact (through broken skin or mucous membranes) with the blood, secretions, organs or other bodily fluids of infected people, and with surfaces and materials (e.g., bedding, clothing) contaminated with these fluids.

The incubation period, that is, the time interval from infection with the virus to onset of symptoms is 2 to 21 days. Humans are not infectious until they develop symptoms. First symptoms are the sudden onset of fever fatigue, muscle pain, headache and sore throat. This is followed by vomiting, diarrhoea, rash, symptoms of impaired kidney and liver function, and in some cases, both internal and external bleeding (e.g., oozing from the gums, blood in the stools). Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes.

It can be difficult to distinguish EVD from other infectious diseases such as malaria, typhoid fever and meningitis. Confirmation that symptoms are caused by Ebola virus infection are made using the following investigations:

- antibody-capture enzyme-linked immunosorbent assay (ELISA)
- antigen-capture detection tests
- serum neutralization test
- reverse transcriptase polymerase chain reaction (RT-PCR) assay
- electron microscopy
- virus isolation by cell culture.

Samples from patients are an extreme biohazard risk; laboratory testing on non-inactivated samples should be conducted under maximum biological containment conditions.

The Ebola Antigen Rapid Test is a simple, visual qualitative test that detects Ebola virus antigen in human whole blood, serum or plasma specimens. This assay is based on immunochromatography and can give a result at 15-20 minutes.

【TEST PRINCIPLE】

The Ebola Antigen Rapid Test is a qualitative membrane-based immunoassay for the detection of antigens from Ebola viruses in human whole blood, serum or plasma specimens. After specimen is added to the specimen well (S) on the sample pad, it moves through the conjugate pad and mobilizes gold anti-Ebola conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with anti-Ebola antibody that is coated on the test line region (T). If the specimen contains Ebola virus antigen, a colored line will appear in the test line region (T), indicating a positive result. If the specimen does not contain Ebola virus antigen, a colored line will not appear in this region, indicating a negative result. An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control line, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test device.

【MATERIALS PROVIDED】

- Test device individually foil pouched with a desiccant
- Sample diluent
- Dropper
- Package insert

【MATERIALS REQUIRED BUT NOT PROVIDED】

Timer, Specimen collection containers

【WARNINGS AND PRECAUTIONS】

1. For *in vitro* diagnostic use only. Do not reuse the test.
2. Do not freeze the test kit or its components.
3. These instructions must be carefully read and strictly followed by a trained healthcare professional to achieve accurate results. All users have to read the instructions before performing test.
4. The test is only for the detection of Ebola virus antigen, not for any other viruses or pathogens.
5. Inadequate or inappropriate specimen collection, storage, and transportation are likely to result in false negative test results.
6. Do not use hemolyzed blood specimens for testing.
7. Do not eat, drink or smoke in the area where handling specimens or performing the test.
8. Do not use the test kit beyond its expiration date.
9. Do not mix components from different kit lots.
10. Leave test device sealed in its foil pouch until just before use. Do not use the test device if the pouch is damaged or the seal is broken.
11. To avoid contamination or inaccurate test result, do not touch the reaction area of test device when performing the test.
12. Wear appropriate personal protection equipment and gloves when performing the test, collecting and handling patient specimens.
13. Dispose of all used test devices and potentially contaminated materials in a biohazard container as if they were infectious waste and dispose according to applicable local laws and regulations.

【STORAGE AND STABILITY】

1. The test kit should be stored either at room temperature or refrigerated (2-40°C), away from direct sunlight. Do not freeze the kit or expose the kit to temperatures over 40°C.
2. The shelf life of the kit is as indicated on the outer package (24 months from date of manufacture).
3. This test kit is stable until the expiration date marked on the outer package and foil pouch. Ensure all test components are at room temperature (15-40°C) before use.
4. Perform the test immediately after taking out the test device from the foil pouch.

【SAMPLE COLLECTION AND PREPARATION】

Consider any materials of human origin as infectious and handle them using standard biosafety procedures. The test can be performed using whole blood (from venipuncture or fingerstick), serum or plasma specimens. Follow standard laboratory procedures to collect specimens.

Plasma/Serum

1. Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
2. To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
3. To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C if not tested immediately. Specimens can be stored at 2-8°C for up to 3 days, and should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles (no more than 3 times). Prior to testing, equilibrate frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity so as to avoid interference on result interpretation.

Whole Blood

Collect whole blood by either fingertip puncture or by venipuncture into collection tube containing EDTA, citrate or heparin for plasma. Do not use any hemolyzed blood for testing.

Do not freeze a whole blood specimen, otherwise the red blood cell will break, which may cause hemolysis. Whole blood specimens should be stored in refrigeration (2-8°C) if not tested immediately. The specimens must be tested within 24 hours after collection.

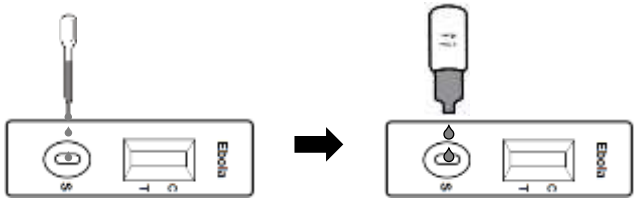
【TEST PREPARATION】

Before testing, open the package and equilibrate the test device, sample diluent, specimens and/or controls to room temperature, and shake the sample diluent gently before use. The most suitable temperature condition to perform the test is room temperature (15-40°C). If the test kit is stored at room temperature, it can be opened and used immediately.

【TEST PROCEDURES】

1. Take out the test device from sealed foil pouch and place on a dry, clean and level surface.
2. Be sure to label the device with specimen's ID number.
3. Fill the pipette dropper with the specimen. Hold the dropper vertically and transfer 3 drops of whole blood/serum/plasma specimen (approximately 30 µL) into the specimen well (S) making sure that there are no air bubbles. Then add 2 drops of sample diluent (approximately 80-100 µL) to the diluent well (S) immediately. See illustration below.
4. Start the timer.

5. Wait for the colored line(s) to appear. Read test results at 15–20 minutes. Do not interpret the result after 20 minutes.



【INTERPRETATION OF TEST RESULTS】

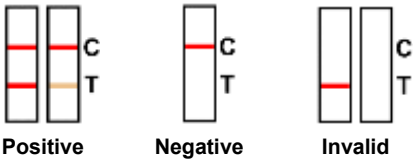
(Please refer to the illustrations below)

POSITIVE: Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

NOTE: The intensity of the color in the test line region (T) will vary depending on the concentration of Ebola virus antigen present in the specimen. Therefore, the presence of any test line (T), no matter how faint, within the designated observation time, indicates a positive result.

NEGATIVE: One colored line appears in the control line region (C). No line appears in the test line region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, stop using the test kit immediately and contact your local distributor.



【QUALITY CONTROL】

1. **Internal Control:** An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control line, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test device.
2. **External Control:** Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】

1. The test is only used for the qualitative detection of Ebola virus antigen in human whole blood, serum or plasma specimens by healthcare professionals. The intensity of the test line does not have a linear correlation with the antigen level in the specimen.
2. The test does not indicate the number of Ebola virus antigen in the specimens, and should not be used as the sole criteria for the diagnosis of Ebola virus infection.
3. A negative test result may occur if the level of Ebola virus antigen in a specimen is below the detection limits of the test.
4. A negative result indicates the Ebola virus antigen is not present in the specimen. However, A negative result at any time does not preclude the possibility of Ebola virus infection.
5. Ebola virus infection can develop rapidly. If symptoms are suspicious or persist while test result from the Ebola Antigen Rapid Test is negative or non-reactive, additional testing using alternative clinical methods is recommended, such as RT-PCR.
6. Test results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

【PERFORMANCE CHARACTERISTIC】

1. Clinical Performance

The Ebola Antigen Rapid Test has been evaluated with a reference commercial RT-PCR Assay (the Comparator) using clinical specimens. Test results are presented in the table below.

Ebola Antigen Rapid Test	**RT-PCR**		
	Positive	**Negative**	**Total**
Positive	102	1	103
Negative	18	299	317
Total	120	300	420

Sensitivity (Positive Percent Agreement): 85.00% = 102/120 (95% CI: 77.53%~90.30%)

Specificity (Negative Percent Agreement): 99.66% = 299/300 (95% CI: 98.14%~99.94%)

Accuracy (Overall Percent Agreement): 95.47% = (102+299)/420(95% CI: 93.04%~97.09%)

2. Precision

Within-run and between-run precisions have been determined by testing 10 replicates on the same four specimens: a negative, a

weak positive, a medium positive and a strong positive. The negative, weak positive, medium positive and strong positive specimens were correctly identified in all the tests performed during each run.

3. Analytical Sensitivity (Limit of detection, LoD)

The Ebola Antigen Rapid Test was used to evaluate the Limit of Detection (LoD) for Ebola Inactivated Virus in venous whole blood specimen, and the tentative LoD was identified at $1.7x10^6$ $TCID_{50}$/mL. This tentative LoD was confirmed as the LoD by 19 out of 20 replicates testing positive with the same Ebola Inactivated Virus at this concentration.

4. **Hook Effect**

No hook effect was found with Ebola virus antigen when tested at the concentration up to 10,000 times of the established LoD.

5. Cross-Reactivity

No cross-reactivity was observed by testing the following positive specimens respectively: Yellow Fever, Rift Valley Fever, Chikungunya virus, Influenza A, Influenza B, Rotavirus, Adenovirus, RSV, Enterovirus, Salmonella, Salmonella typhi, Plasmodium falciparum (malaria), Plasmodium vivax (malaria) and Dengue.

6. Interference

The following potentially interfering substances were added to Ebola virus negative and positive specimens. Test results demonstrate that performance of the Ebola Antigen Rapid Test was not affected by the listed potentially interfering substances at the concentrations tested.

Bilirubin	25 mg/dL
Hemoglobin	20 g/dL
HAMA	2460 ng/mL
Protein	5 g/dL

【REFERENCES】

1. Taylor DJ, Leach RW, Bruenn J. Filoviruses are ancient and integrated into mammalian genomes.BMC Evol Biol, 2010, 10(1): 193.
2. Suzuki Y, Gojobori T. The origin and evolution of Ebola and Marburg viruses. Mol Biol Evol, 1997,14(8): 800−806.
3. Feldmann H, Geisbert TW. Ebola haemorrhagic fever. The Lancet, 2011,377(9768): 849−862.
4. Formenty P, Boesch C, Wyers M, et al. Ebola virus outbreak among wild chimpanzees living in a rain forest of C.te d'Ivoire. J Infect Dis, 1999,179(Suppl 1): S120−S126.
5. Barrette RW, Metwally SA, Rowland JM, et al.Discovery of swine as a host for the Reston Ebolavirus. Science, 2009, 325(5937): 204−206.
6. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. Trends Microbiol, 2007, 15(9):408−416.

【INDEX OF SYMBOLS】

	Consult instruction for use		For *in vitro* diagnostic use only		Do not use if package is damaged		Temperature limit
	Lot number		Use by		Do not reuse		Contains sufficient for <X> tests
	Keep dry		Manufacturer		Date of manufacture		Keep away from sunlight