ReEBOV™ Antigen Rapid Test
(Ebolavirus VP40 Antigen Detection)

Instructions for Use

For Use Under Emergency Use Authorization (EUA) Only

For *in vitro diagnostic use*

INTENDED USE

ReEBOV™ Antigen Rapid Test is a rapid chromatographic immunoassay intended for the qualitative detection of VP40 antigen from Ebola viruses (*Zaire Ebola virus*, [including the *Zaire Ebola virus* strain detected in the West Africa outbreak 2014], *Sudan Ebola virus*, and *Bundibugyo Ebola virus*) in fingerstick (capillary) whole blood, venous whole blood, or plasma.

The ReEBOV™ Antigen Rapid Test is for the presumptive detection of Ebola Zaire virus (detected in the West Africa outbreak in 2014) in individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors (including geographic locations with high prevalence of Ebola infection). The authorized ReEBOV™ Antigen Rapid Test is intended for circumstances when use of a rapid Ebola test is determined to be more appropriate than use of an authorized Ebola nucleic acid test, which has been demonstrated to be more sensitive in detecting the Ebola Zaire virus. The authorized ReEBOV™ Antigen Rapid Test is not intended for use for general Ebola infection screening, such as airport screening or contact tracing. The ReEBOV™ Antigen Rapid Test is authorized for use in laboratories or facilities adequately equipped, trained, and capable of such testing (including treatment centers and public health clinics).

Testing with the ReEBOV™ Antigen Rapid Test should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. Negative results do not preclude Ebola virus infection and should not be used as the sole basis for patient management decisions. The definitive identification of Ebola Virus disease (EVD) requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of EVD must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification Ebola Virus.

The level of VP40 antigen that would be present in the clinical specimen from individuals with early systemic infection is unknown. The ReEBOV™ Antigen Rapid Test was evaluated in a limited clinical field study using fresh and banked clinical specimens from individuals with EVD confirmed by RT-PCR.

PRINCIPLE OF THE TEST

The Ebola virus (EBOV) encodes seven proteins, including the VP40 matrix protein (i.e. VP40 antigen). Advanced protein chemistry techniques have been used to develop non-infectious, recombinant EBOV VP40 antigen. Affinity purified caprine polyclonal antibody has been developed using the recombinant EBOV VP40 antigen to detect the presence of the native EBOV VP40 antigen in the whole blood or plasma of suspected Ebola Virus Disease (EVD) patients.

The ReEBOV™ Antigen Rapid Test is performed as a dipstick immunoassay. Sample consisting of whole blood from fingerstick or venous collection or plasma is added to the Sample Pad. Inserting the dipstick into a test tube containing the Sample Buffer initiates the flow of sample through the reagent pads and across the nitrocellulose membrane. The EBOV VP40 specific antibody is striped onto nitrocellulose membrane in order to capture EBOV VP40 antigen. The EBOV VP40 specific antibody is also conjugated to gold nanoparticles and deposited in one of the rapid test reagent pads. As the assay develops, the EBOV VP40 antigen present in the sample forms immune-complexes with the anti-EBOV VP40 antibody - gold nanoparticles. As the VP40 antigen bound gold nanoparticles are captured by the Test Line, the deposition of the gold nanoparticles generates a pink to red signal which corresponds to the concentration of EBOV VP40 antigen present in the sample. Excess gold nanoparticles are captured by the Control Line, indicating a valid result. Visual interpretation is made between 15 to 25 minutes signal development time. Refer to the Visual Aid section.

REAGENTS
Store at 2–8°C. Do Not Freeze.

Each ReEBOV™ Antigen Rapid Test kit contains the following reagents

- 2 x 25 ReEBOV™ Antigen Rapid Test Dipsticks in resealable foil pouches with desiccant
- 2 x 7 mL Sample Buffer Dropper Bottles
- 2 x 0.25 mL Lyophilized Negative Control (negative human serum)
- 2 x 0.25 mL Lyophilized Positive Control (recombinant VP40 antigen spiked in negative human serum)
- 2 x 25 Test Tubes with Caps
- 1 Disposable Test Tube Rack
- 1 Visual Aid - ReEBOV™ Antigen Rapid Test Results Card

WARNINGS AND PRECAUTIONS
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- **Ebola Virus is classified as a NIAID Category A Priority Pathogen. Handling of infectious blood and serum requires advanced biocontainment (BSL-4) facilities. Where advanced biocontainment facilities are not available, the use of all possible universal precautions is highly recommended including safety goggles and/or face shields, masks or respiratory equipment, disposable gowning, boots and gloves. It is highly recommended that health care workers are appropriately trained in the donning and doffing of personal protective equipment. All equipment and biohazardous waste should be discarded in appropriately labeled waste bags and incinerated.**
- Specimens should always be treated as infectious and/or biohazardous and sample and assay waste should be discarded according to local safety regulations.
- Follow necessary precautions when handling specimens with this test. Use personal protective equipment (PPE) consistent with current guidelines.

Biosafety information: Refer to CDC Guidance for Collection, Transport, and Submission of Specimens for Ebola Virus Testing (http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/specimens.html)

- The ReEBOV™ Antigen Rapid Test Controls are prepared with pooled negative human serum (Negative Control). The Positive Control is negative human serum to which recombinant EBOV VP40 antigen has been added.
- Human source material used to prepare the Controls included in this kit have been tested and shown to be negative for antibodies to HBsAg, HCV, and HIV 1 & 2 by FDA approved tests. All human blood derivatives, including patient samples, should be handled as potentially infectious material.
- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
- Certain components are labeled with the following: Irritating to eyes (R 36). Avoid contact with skin and eyes (S 24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). If swallowed, seek medical advice immediately and show container or label (S 46).

  Warning  . Biological Risk  .
- Lyophilized Controls should be handled carefully. **Use extreme caution when removing tear off metal cap. Procedure can cause sharp edges to occur which can lead to compromising the integrity of the operator's PPE. Use a hemostat or other appropriate instrument for removing metal cap. Properly discard metal cap seal in appropriate sharps container.**

- For the lyophilized Controls, subsequent removal of the lid for each use should be performed in a manner to minimize the risk of aerosol formation wherever possible.
- Do not use components of this kit that have passed their expiration date.
- Do not mix kit components from different kit lot numbers.
- Results from this test should be interpreted in conjunction with clinical signs, symptoms and travel history of the patient.
- The ReEBOV™ Antigen Rapid Test is for use in laboratories or facilities adequately equipped, trained, and capable of such testing (including treatment centers and public health clinics).

SPECIMEN COLLECTION AND PREPARATION

Whole blood from fingerstick or whole blood collected in EDTA or Citrate Vacutainers can be tested using the ReEBOV™ Antigen Rapid Test dipsticks.

INSTRUCTIONS FOR USE

Materials Provided:
ReEBOV™ Antigen Rapid Test; see "Reagents" for a complete listing.

Materials Required But Not Provided:
- ReEBOV™ Antigen Accessory Kit (Cat. Number 14036)
    - 200 Disposable Lancets
    - 200 Cotton Balls
    - 200 Alcohol Wipes
- Precision pipettors capable of delivering between 10 µL and 250 µL, with appropriate tips
- Deionized water
- In austere testing conditions: disposable gloves, gowning, safety goggles, face shields, respiratory masks; and boots that can be decontaminated

Procedural Notes
- Bring samples and kit reagents to ambient temperature (18-30°C) and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
- Visual interpretation of assay results must be conducted within 15-25 minutes signal development time.
- Incubation temperatures above or below normal room temperature (18-30°C) may contribute to inaccurate results.
- Do not use kit components beyond the expiration date.
- Do not mix kit components from different kit lot numbers.

Assay Procedure – ReEBOV™ Antigen Rapid Test Dipsticks for Fingerstick Whole Blood

1. Remove appropriate amount of dipsticks for testing the required fingerstick whole blood samples.
2. Add 4 drops of Sample Buffer to a test tube provided in the test kit.
3. Perform fingerstick using the disposable lancet provided in the ReEBOV™ Antigen Accessory Kit (Cat. Number 14036), allow a full drop of blood to develop from fingerstick.
4. Touch sample pad of the dipstick to the drop of blood to transfer sample.
5. Insert the ReEBOV™ Antigen Rapid Test dipsticks (arrows down) into test tubes containing the Sample Buffer. Replace the tube caps. **DO NOT** shake, agitate, or invert test tubes during rapid test signal development.
6. Allow the ReEBOV™ Antigen Rapid Test to develop for 15-25 min before performing a visual interpretation using the ReEBOV™ Antigen Rapid Test Results Card and the Results Interpretation visual aid illustrated on page 12.

(See Rapid test instructions for fingerstick whole blood on page 13 for detailed visual instructions.)

Assay Procedure – ReEBOV™ Antigen Rapid Test Dipsticks for Venous Whole Blood and Processed Plasma

1. Remove appropriate amount of dipsticks for testing the required venous whole blood and plasma samples.
2. Add 4 drops of Sample Buffer to a test tube provided in the test kit.
3. Transfer 30 µL of venous whole blood or plasma onto the center of the Sample Pad using a pipettor.
4. Insert the ReEBOV™ Antigen Rapid Test dipsticks (arrows down) into test tubes containing the Sample Buffer. Replace the tube caps. **DO NOT** shake, agitate, or invert test tubes during rapid test signal development.
5. Allow the ReEBOV™ Antigen Rapid Test to develop for 15-25 min before performing a visual interpretation using the ReEBOV™ Antigen Rapid Test Results Card and the Results Interpretation visual aid illustrated on page 12.

(See Rapid test instructions for venous whole blood and processed plasma on page 13 for detailed visual instructions.)

Assay Procedure – ReEBOV™ Antigen Rapid Test for Negative or Positive Control

1. Remove appropriate amount of dipsticks for testing one Negative Control, one Positive Control. Positive and Negative Controls should be run at a minimum once every day of testing.
2. Reconstitute one Lyophilized Negative Control and one Lyophilized Positive Control each with 0.250 mL of deionized water for minimum of 5 minutes at ambient temperature. Shake or agitate vial to ensure complete reconstitution of controls. Rehydrated controls are stable for 30 days at 2-8 °C.
3. Add 4 drops of Sample Buffer to a test tube provided in the test kit.
4. For Positive and Negative Controls, add 30 µL to the center of the Sample Pad using a pipettor.
5. Insert the ReEBOV™ Antigen Rapid Test dipsticks (arrows down) into test tubes containing the Sample Buffer. Replace the tube caps. **DO NOT** shake, agitate, or invert test tubes during rapid test signal development.
6. Allow the ReEBOV™ Antigen Rapid Test to develop for 15-25 min before performing a visual interpretation using the ReEBOV™ Antigen Rapid Test Results Card and the Quality Control visual aid illustrated on page 12.
7. If test strip produces an invalid result, user should repeat test with a new test strip.

Results Interpretation – Refer to included Visual Aid on page 12 and/or Visual Aid ReEBOV™ Antigen Rapid Test Results Card.

1. The ReEBOV™ Antigen Rapid Test results should be compared to the Visual Aids included to assist with the interpretation of the results.
2. For a positive patient result on the ReEBOV™ Antigen Rapid Test, a pink to red line should form across the Test Line, and a pink to red line should form across the Control Line.
3. For a negative patient result no line should be detected across the Test Line, and a pink to red line should form across the Control Line.
4. Absence of a pink to red line forming across the Control Line is considered as an "Invalid" result which requires sample retesting.
5. If available, a permanent record should be made by digital photography.

QUALITY CONTROL – Refer to included Visual Aid on page 12 and ReEBOV™ Antigen Rapid Test Results Visual Aid Card.

1. The ReEBOV™ Antigen Rapid Test should form a pink to red line across the Control Line indicating the dipstick is performing properly.
2. Failure of the Control Line to develop constitutes an invalid result and requires retesting.

3. The appearance of pink to red background, streaks or spots in the Test or Control Line area may be due to improper flow of reagents and constitutes an invalid result and requires retesting.
4. The development of partial width or variable intensity Test Line does not constitute an invalid result but retesting may be considered

LIMITATIONS OF THE TEST

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- The ReEBOV™ Antigen Rapid Test detects the presence of EBOV VP40 antigen in suspected patient whole blood. Ongoing studies indicate that circulating EBOV VP40 antigen may be absent or undetectable if the patient has progressed to their humoral immune response and anti-EBOV VP40 antibody titers may have developed.

- Negative results do not preclude Ebola virus infection, particularly within the first 72 hours after appearance of symptoms, and should not be used as the sole basis for patient management decisions. The definitive identification of Ebola Virus disease (EVD) requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The authorized ReEBOV™ Antigen Rapid Test is not intended for use for general Ebola infection screening, such as airport screening or contact tracing and should not be used on asymptomatic individuals.
- Testing patient samples containing excess hemoglobin may result in false negative readings. Testing patient samples containing rheumatoid factor may result in false positive readings.

- Potential ReEBOV™ Antigen Rapid Test cross-reactivity with Ebola vaccines or therapeutics has not been evaluated. Specimens from patients who have received therapeutics or vaccines against Ebola virus may exhibit false positive or other confounding test results.

PERFORMANCE

Field Testing

Corgenix Inc. Study

The clinical performance of the ReEBOV™ Antigen Rapid Test was evaluated using unselected frozen archived plasma samples at the Kenema Government Hospital, Kenema, Sierra Leone. The performance of the ReEBOV™ Antigen Rapid Test was compared to a laboratory validated rRT-PCR assay that utilizes the EBOV Zaire-TM GP primers and probe as outlined in Trombley et al., 2010 [7].

Frozen Plasma Results

	rRT-PCR Positive	rRT-PCR Negative	Total
RDT +	72[1]	2	74
RDT -	44[2]	58	102
Total	116	60	176
Positive Percent Agreement (%)	62.1 (72/116)	95% CI: 53.0% - 70.4%	
Negative Percent Agreement (%)	96.7 (58/60)	95% CI: 88.6% - 99.1%	

[1] "True Positive" (n=72), RT-PCR Ct range 24.69 ± 4.40
[2] "False Negative" (n=44), RT-PCR Ct range 37.01 ± 3.41

World Health Organization (WHO) Study

The ReEBOV™ Antigen Rapid Test was evaluated independently by WHO-supported European Union and African Union field laboratories at Hastings and Prince of Wales (Sierra Leone) using 152 fresh venous whole blood and 140 randomly selected frozen archived plasma specimens. The performance of the ReEBOV™ Antigen Rapid Test was compared to the benchmark assay, RealStar® Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics GmbH):

Fresh Venous Whole Blood Results

	RealStar Positive	RealStar Negative	Total
RDT +	18[1]	12	30
RDT -	5[2]	117	122
Total	23	129	152
Positive Percent Agreement (%)	78.3 (18/23)	95% CI: 58.1% - 90.3%	
Negative Percent Agreement (%)	90.7 (117/129)	95% CI: 84.4% - 94.6%	

[1] "True Positive" (n=18), RealStar® Ct range 20.17 ± 2.96
[2] "False Negative" (n=5), RealStar® Ct range 26.09 ± 3.14

Frozen Plasma Results

	RealStar Positive	RealStar Negative	Total
RDT +	71[1]	18	89
RDT -	3[2]	48	51
Total	74	66	140
Positive Percent Agreement (%)	95.9 (71/74)	95% CI: 88.7% - 98.6%	
Negative Percent Agreement (%)	72.7 (48/66)	95% CI: 61.0% - 82.0%	

[1] "True Positive" (n=71), RealStar® Ct range 20.19 ± 4.79
[2] "False Negative" (n=3), RealStar® Ct range 29.67 ± 0.62

Analytical Testing
Analytical testing of the ReEBOV™ Antigen Rapid Test was conducted in BSL-4 (The University of Texas at Galveston, Galveston TX), BSL-3 (Tulane University, New Orleans LA; CDC, Ft. Collins CO) and BSL-2 (Corgenix Inc., Broomfield CO) laboratories in 2014.

Analytical Sensitivity

A Limit of Detection (LOD) range finding study identified 1.00E+06 pfu/mL as the tentative LOD for Zaire Ebola virus spiked into pooled venous whole blood. This dilution level was confirmed as the LOD by all 20 replicates testing positive. A virus dilution of 1.00E+06 pfu/mL is equivalent to 3.00E+04 pfu/test.

The LOD in venous whole blood sample matrix was also confirmed in an additional LOD confirmation study using rVP40 by testing 36 and 33 replicates at 3.12E+02 ng/mL (9.40E+00 ng/test) and 6.25E+02 ng/mL (1.88E+01 ng/test), respectively. The final confirmed LOD in this additional study was determined to be the lowest concentration resulting in positive detection at which >95% of all replicates were positive.

Table 1. LOD Summary Data

	LOD –Virus in Venous Whole Blood	LOD – Antigen in Venous Whole Blood
Limits of Detection (LOD)	1.00E+06 PFU/mL or 3.00E+04 PFU/Test Ebola Guinea Virus	6.25E+02 ng/mL or 1.88E+01 ng/Test Ebola rVP40 Antigen

Analytical Specificity:

Reactivity: Reactivity of the ReEBOV™ Antigen Rapid Test was evaluated for additional isolates of Ebola virus and Marburg virus in an analytical study. This study testing 3 to 5 replicates was performed using negative serum (negative scores).

Table 2. Reactivity - Nearest Neighbor Testing

Virus	Type-Strain (Isolate)	Concentration Tested (pfu/mL)	Negative Scores	Reactivity
Ebola Virus	Zaire (Mayinga)	3.30E+06	+++	Yes
Ebola Virus	Zaire (Mayinga)	1.30E+05	+++++	Yes
Ebola Virus	Sudan-Gulu (200011676)	3.75E+05	+++	Yes
Ebola Virus	Sudan-Gulu (200011676)	3.75E+04	+++	Yes
Ebola Virus	Bundibugyo (200706291)	1.38E+04	+++	Yes
Ebola Virus	Bundibugyo (200706291)	1.38E+03	+++	Yes
Ebola Virus	Reston (AZ-1435)	6.75E+03	---	None
Marburg Virus	Musoke	2.20E+05	---	None

Reactivity: ReEBOV™ Antigen Rapid Test exhibits reactivity to the three strains of Ebola virus (Zaire, Sudan, and Bundibugyo) known to cause EVD in humans. Ebola Reston is only known to cause hemorrhagic fever in non-human primates. Although Marburg virus is from the same family, it possesses very low amino acid sequence identity (34%) to Zaire Ebola virus VP40.

Cross Reactivity: Cross-reactivity of the ReEBOV™ Antigen Rapid Test was evaluated by testing additional viral, bacterial, or parasitic pathogens. This study testing 3 replicates was performed using rVP40 protein spiked into serum at 6.0 ng/test (positive scores) and negative serum (negative scores). The cross-reactivity study results are presented in Table 3 below.

Table 3. Cross-reactant Testing

Virus/Microbe/ Parasite	Type-Strain (Isolate)	Concentration Tested	Positive Scores	Negative Scores	Reactivity
Lassa Virus	Josiah (CDC#57562)	8.80E+04 PFU/mL	ND	---	None
Lassa Virus	Nigeria-CladeII (Saurwald)	1.50E+04 PFU/mL	ND	---	None
Junin Virus	Espindola (P3790)	2.25E+04 PFU/mL	ND	---	None
CCHF Virus	IBAR 10200	5.00E+04 PFU/mL	+++	---	None
Yellow Fever Virus	CDC Vaccine Strain	Not Available	+++	---	None
Rift Valley Fever Virus	CDC Vaccine Strain	Not Available	+++	---	None
Chikungunya Virus	CDC Strain	Not Available	+++	---	None
Flaviviridae	DENV-1	>1.00E+05 PFU/mL	+++	---	None
Flaviviridae	DENV-2	>1.00E+05 PFU/mL	+-+[1]	---	None
Flaviviridae	DENV-3	>1.00E+05 PFU/mL	+++	---	None
Flaviviridae	DENV-4	>1.00E+05 PFU/mL	+++	---	None
HIV-1	JC/pMT4R5	1.00E+03 PFU/mL	+++	---	None
HIV-1	Ba-1/MT4R5	1.00E+03 PFU/mL	+++	---	None
HIV-1	JFM/pMT4R5	1.00E+03 PFU/mL	+++	---	None
Herpesviridae	CMV-Townes	8.47E+04 PFU/mL	+++	---	None
SIV	17E/MH161	1.00E+03 PFU/mL	+++	---	None
EBV	B95-8	1.00E+03 PFU/mL	+++	---	None
HSV1	NA	5.50E+04 PFU/mL	+++	---	None
Influenza	A/P8/8/34 H1N1	9.00E+09 PFU/mL	+++	---	None
Paramyxoviridae	Measles - Edmonston	4.67E+03 PFU/mL	+++	-+-[1]	None
Rhabodvirus	VSV	Unknown	+++	---	None
Arenaviridae (NW)	Pichindae	2.81E+04 PFU/mL	+++	---	None
Pseudomonas	*P. aeruginosa*	1.00E+05 cell/mL	+++	---	None
Shigella	Serogroup A	Unknown	+++	---	None
Streptococcus	*S. pneumoniae*	1.00E+08 cell/mL	+++	---	None
Salmonella	*S. typhi*	1.00E+06 cell/mL	+++	---	None
Yersinia	*Y.pseudotuberculosis*	1.00E+06 cell/mL	+++	---	None
Trypanosoma	*T. cruzi*	1.00E+08 parasite/mL	+++	---	None
Plasmodium	*P. falciparum*	1.00E+07 parasite/mL	+++	---	None
Toxoplasma	*T. gondii*	Unknown	+++	---	None

Note: ND = Not Done

[1] At least 2/3 replicates generated correct results. Acceptance criteria for this study were met.

Cross Reactivity: ReEBOV™ Antigen Rapid Test did not exhibit cross-reactivity with any of the viral, bacterial, or parasitic pathogens tested, except for 1 out of 3 replicates of Measles-Edmonston. The panel represents a range of human pathogens known to produce signs and symptoms associated with hemorrhagic fevers, malaria, viral infection, and non-specific signs of febrile illness.

Interfering Substances:

The impact of potentially interfering substances on the ReEBOV™ Antigen Rapid Test was evaluated. The evaluation was conducted to demonstrate that the potential interferents do not generate false positive results in known negative specimens, and do not lead to false negative results in known positive specimens. This study testing 5 replicates was performed using rVP40 protein spiked into serum at 6.0 ng/test (positive scores) and negative serum (negative scores). The interfering substances study results are presented in Table 4 below.

Table 4. Interfering Substance Testing

Interfering Substance	Concentration Tested	Solvent	Results[1]
Hemoglobin	100 mg/mL	npH_2O	Fail
Hemoglobin	20 g/dL	npH_2O	Fail
Serum Protein	50 mg/mL	npH_2O	Pass
Serum Protein	35 mg/mL	npH_2O	Pass
HAMA	800 ng/mL	Serum	Pass
HAMA	182.1 units/mL	Serum	Pass
HAMA	2.65 units/mL	Serum	Pass
Rheumatoid Factor (RF)	60 units/mL	npH_2O	Pass
Rheumatoid Factor (RF)	15 units/mL	npH_2O	Pass
Rheumatoid Factor (RF)	2000 IU/mL	Serum	Fail
Rheumatoid Factor (RF) Dilution	1050 IU/mL	NA	Fail
Rheumatoid Factor (RF) Dilution	350 IU/mL	NA	Fail
Rheumatoid Factor (RF) Dilution	117 IU/mL	NA	Fail
Rheumatoid Factor (RF) Dilution	39 IU/mL	NA	Pass
Bilirubin	25 mg/mL (High)	0.1N NaOH	Pass
Bilirubin	15 mg/dL (High)	0.1N NaOH	Pass
Bilirubin	1 mg/dL (Low)	0.1N NaOH	Pass
Conjugated Bilirubin	5 mg/dL (High)	npH_2O	Pass
Conjugated Bilirubin	0.2 mg/dL (Low)	npH_2O	Pass
EDTA (Short Draw)	25 mM	npH_2O	Pass
Ribavirin (Loading Dose 75kg Adult)	4.5 g/dL (30 mg/Kg/5L)	npH_2O	Pass
Arthemeter (Loading Dose 75kg Adult)	0.48 g/dL (3.2 mg/Kg/5L)	npH_2O	Pass
Artesunate (Loading Dose 152.4 mg Adult)	0.314 g/dL	DMSO	Pass
Quinidine Gluconate	15 µmol/L	npH_2O	Pass
Quinidine Gluconate	6.2 µmol/L	npH_2O	Pass
Quinine	148 µmol/L	npH_2O	Pass
Doxycycline	67.5 µmol/L	npH_2O	Pass
Ampicillin	152 µmol/L	npH_2O	Pass
Ciprofloxacin	30.2 µmol/L	0.1N HCL	Pass
Acetaminophen (Paracetamol)	199 µmol/L	npH_2O	Pass
Acetaminophen (Paracetamol)	33 µmol/L	npH_2O	Pass
Ibuprofen	2425 µmol/L	0.1N NaOH	Pass
Aspirin (ASA)	21.6 mg/mL	Ethanol	Pass
Promethazine	4.22 µmol/L	npH_2O	Pass
ALT	2000 units/L	npH_2O	Pass
AST	2000 units/L	npH_2O	Pass

Note: NA = Not Applicable

[1]Testing condition is considered a "Pass" if the median reading scores of 5 replicates does not differ from that of the appropriate solvent control

Interfering Substances: No interference was observed for Bilirubin, HAMA, Qunidine Gluconate, Acetaminophen, or Intralipids at all levels tested. Hemoglobin did cause false-negatives at the 20 g/dL (= 200 mg/mL) or higher while Rheumatoid Factor did cause a false-positive signal from 117 to 2000 IU/mL but previous results indicate that it is a non-interferent at 60 IU/mL and below. Combined results of Interfering Substances testing demonstrate that only higher levels of Hemoglobin and Rheumatoid Factor (>60IU/mL) cause false-negative or false-positive results, respectively. Excessive levels of Hemoglobin

and Bilirubin in clinical samples is listed as a limitation for the assay due to interference with signal development or results interpretation due to elevated background.

ReEBOV Antigen Rapid Test Signal Kinetics

Rapid test signal development time was monitored by reflectance measurements (mV) taken by the ESEQuant Lateral FlowReader (LFR; Qiagen GmbH) during the development of a moderate strength signal using clinical sample (G6527).

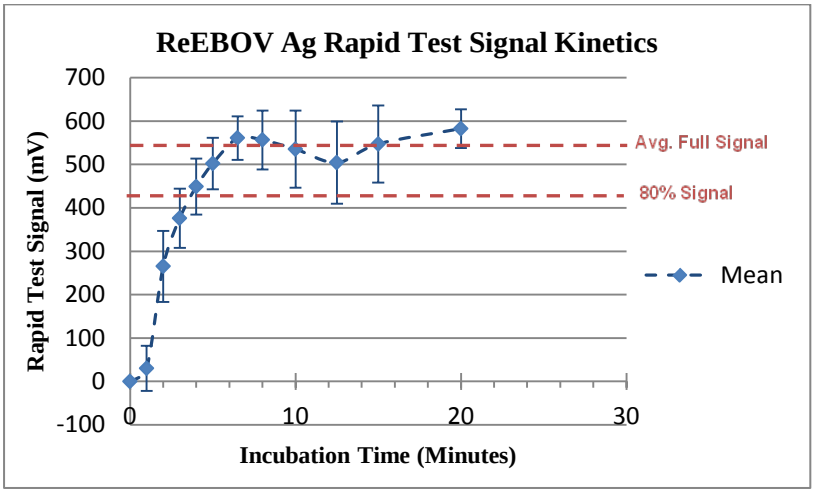


Figure 1. Full signal (>433mV) developed within 5 minutes and was at average full strength (541mV) measurement by 8 minutes. Signal is maintained up to 25 minutes.

Rapid Test Signal Development: In order to ensure proper signal development, it is recommended that results be read between 15 to 25 minutes.

High-dose Hook Effect:

A high-dose hook effect refers to the false negative result which can be seen when very high levels of target are present in a tested sample. A study was conducted to evaluate if a hook effect occurs for the ReEBOV™ Antigen Rapid Test by testing increasing rVP40 antigen concentrations in serum – test line intensity was measured using the ESEQuant LFR. Results of this study are presented below:

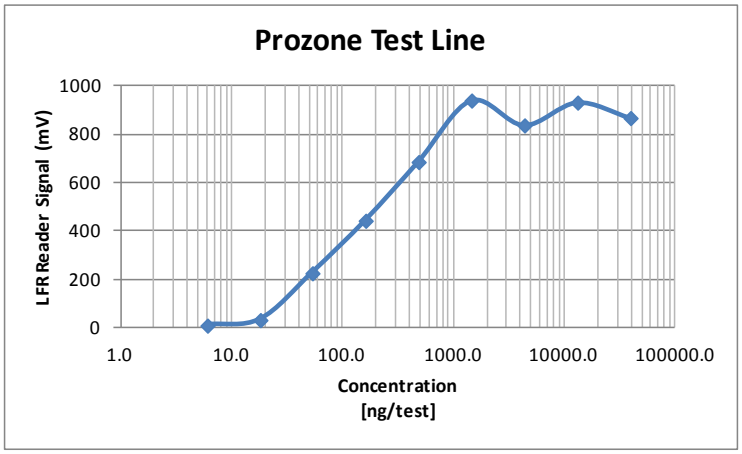


Figure 2 . LFI Reader signals are maintained above 800mV from 1,440 to 39,000 ng/Test.

High-dose Hook Effect: ReEBOV™ Antigen Rapid Test does not generate High-dose Hook or Prozone effect within the range of the assay.

Fingerstick Whole Blood Specificity Study:

To support a fingerstick whole blood claim, a specificity study was carried out testing duplicate fingerstick whole blood specimens collected from 20 volunteers (collected from two different fingers per donor). The ReEBOV™ Antigen Rapid Test was run and read by one operator. Thirty nine (39) of the 40 fingerstick whole blood specimens tested in this study were negative; one specimen generated a trace signal, resulting in a percent negative agreement against the expected negative results of 97.5% (95% CI 86.8% – 99.9%).

Reading Reproducibility Study:

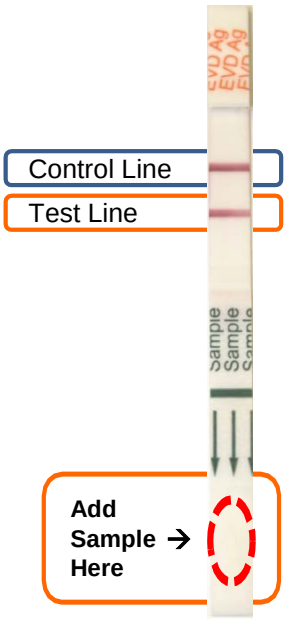
To assess reproducibility of reading the ReEBOV™ Antigen Rapid Test, venous whole blood samples spiked with various levels of rVP40 antigen were tested using the ReEBOV™ Antigen Rapid Test and read by three individual readers. The results of this study are presented in Table 5 below:

Table 5. Reading Reproducibility Results

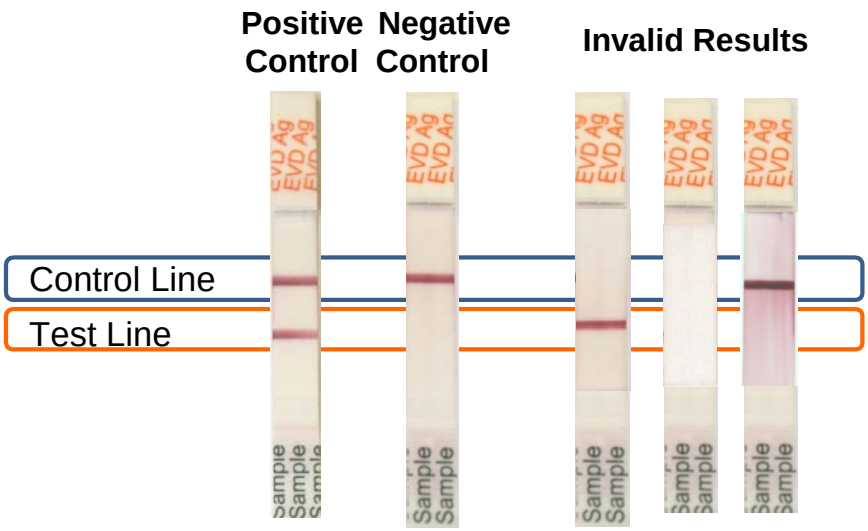
Spiked or Un-spiked	**Spike Level (rVP40 in ng/test)**	**# of Specimens**	**Observations by 3 Individual Readers**			
			# of Specimens and % of Total Specimens at Each Spike Level			
			3/3 positive	2/3 positive	1/3 positive	0/3 Positive
Un-spiked	0	75	0% (0/75)	0% (0/75)	8% (6/75)	92% (69/75)
Spiked	9.4	36	47.2% (17/36)	11.1% (4/36)	22.2% (8/36)	19.4% (7/36)
Spiked	18.8	30	96.7% (29/30)	0% (0/30)	3.3% (1/30)	0% (0/30)
Spiked	37.5	5	100% (5/5)	0% (0/5)	0% (0/5)	0% (0/5)
Spiked	75.0	7	85.7% (6/7)	0% (0/7)	0% (0/7)	14.3% (1/7)
Spiked	150	6	100% (6/6)	0% (0/6)	0% (0/6)	0% (0/6)
Spiked	300	4	75% (3/4)	0% (0/4)	0% (0/4)	25% (1/4)
Spiked	600	6	100% (7/7)	0% (0/7)	0% (0/7)	0% (0/7)
Total		94				

Visual Aid

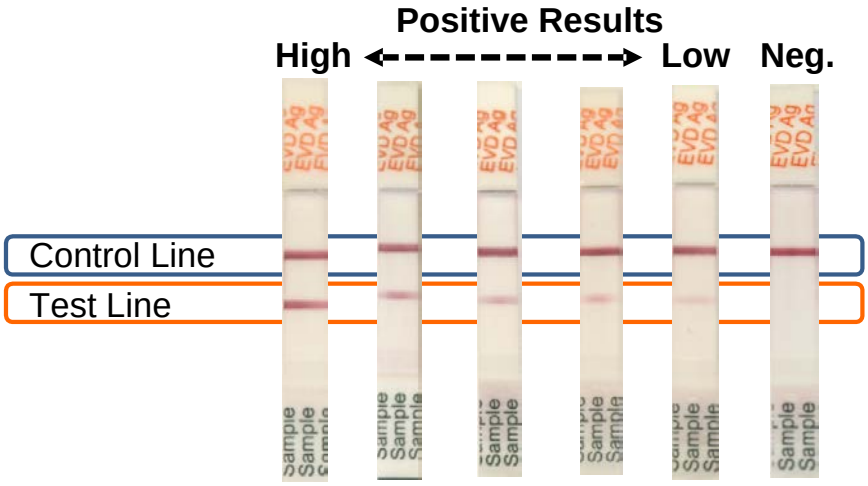
Rapid Test Dipstick



Quality Control

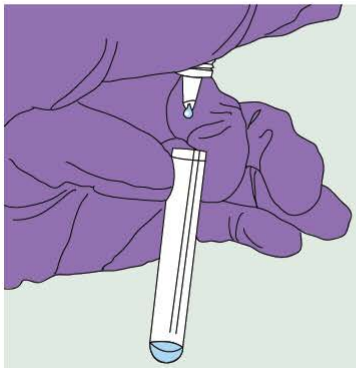


Results Interpretation



ReEBOV™ Antigen Rapid Test Instructions

For Detection of Ebolavirus VP40 Antigen

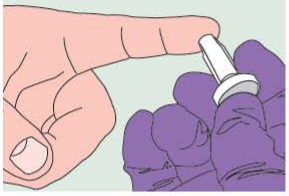


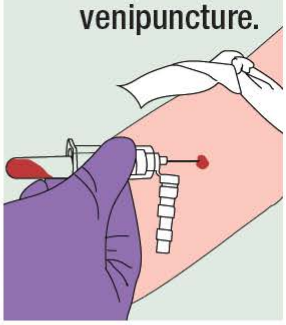
1. Add 4 drops Sample Buffer to plastic tube.

2A. Use safety lancet to perform finger stick.

or

2B. Use a venipuncture.

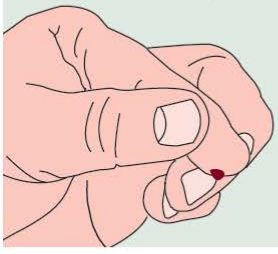




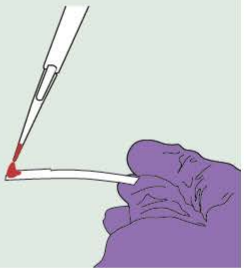
3A. Allow large drop of blood to develop.

or

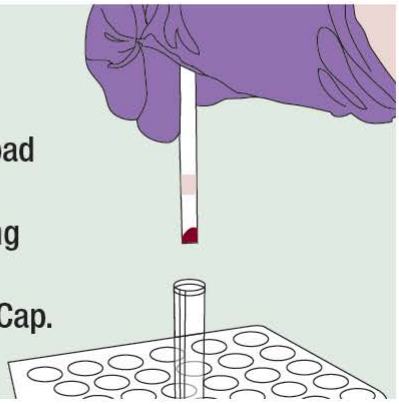
3B. Remove blood with pipette



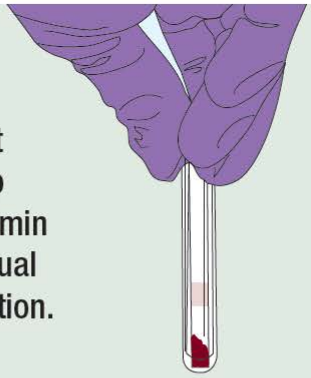
4. Transfer drop of blood on to ReEBOV Rapid Test sample pad.



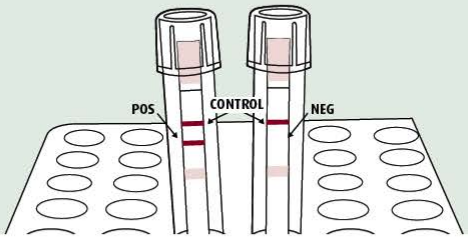
5. Place ReEBOV sample pad into tube containing sample. Replace Cap.



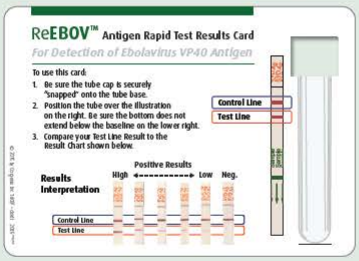
6. Allow ReEBOV Rapid Test to develop for 15-25 min before visual interpretation.



7. Visual Interpretation
   - Top line is control stripe
   - Bottom line is a positive test line



8. Use the Visual Aid card provided to assist in result interpretation.



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Symbol Legend

									
Batch Code	Use by/ Expiry Date	Temperature Limitations	Warning	Catalog Number	Manufacturer	Consult Instructions for Use (Package Insert)	Biological Risk	Caution	For *In Vitro* Diagnostic Use

Warranty
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