Instructions for Use – WHOLE BLOOD

For testing of deceased patients using this device, please refer to the Instructions for Use entitled OraQuick® Ebola Rapid Antigen Test – Instructions for Use – Cadaveric Oral Fluid.

For Use Under Emergency Use Authorization (EUA) Only

For *in-vitro* diagnostic use

NAME AND INTENDED USE

The OraQuick® Ebola Rapid Antigen Test is a single-use immunoassay intended for the qualitative detection of antigens 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 venipuncture whole blood and fingerstick whole blood.

The OraQuick® Ebola Rapid Antigen Test is for the presumptive detection of the 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 OraQuick® Ebola Rapid Antigen 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 virus. The authorized OraQuick® Ebola Rapid Antigen Test is not intended for use for general Ebola infection screening, such as airport screening or contact tracing of individuals without signs and symptoms of Ebola infection. The OraQuick® Ebola Rapid Antigen 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 OraQuick® Ebola Rapid Antigen 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 of Ebola virus.

The level of Ebola virus antigens that would be present in the clinical specimen from individuals with early systemic infection is unknown. The OraQuick® Ebola Rapid Antigen Test was evaluated in a limited clinical study using retrospective clinical specimens from individuals with EVD confirmed by RT-PCR.

SUMMARY AND EXPLANATION OF THE TEST

Ebola hemorrhagic fever is a severe, often-fatal disease in humans and nonhuman primates that has appeared sporadically since its initial recognition in 1976. The Ebola virus is one of three, but one of two human disease causing members of a family of RNA viruses called the Filoviridae. There are four identified subtypes of Ebola virus affecting humans: Bundibugyo virus (BDBV), Sudan virus (SUDV), Taï Forest virus (TAFV), and Ebola virus (EBOV) strains. The presence of Ebola virus antigens indicate that the individual may be currently infected and capable of transmitting the virus.

The OraQuick® Ebola Rapid Antigen Test utilizes a sandwich capture lateral flow immunoassay method to detect Ebola virus antigens. Ebola antigens are captured and visualized by colloidal gold labeled with Ebola antibodies generating a visible line in the test zone for a positive sample.

PRINCIPLES OF THE TEST

The OraQuick® Ebola Rapid Antigen Test is a manually performed, visually read immunoassay for the qualitative detection of Ebola virus in human venipuncture whole blood and fingerstick whole blood. The OraQuick® Ebola Rapid Antigen Test is comprised of both a single-use test device and vial containing a pre-measured amount of a buffered developer solution. The test consists of a sealed pouch with two separate compartments for each component. The OraQuick® Ebola Rapid Antigen Test utilizes a proprietary lateral flow immunoassay procedure.

The assay test strip, which can be viewed through the test device result window, is comprised of a series of components: the blocker pad, the conjugate pad, the nitrocellulose membrane, and finally the absorbent pad. The performance of the assay occurs by hydration and transport of reagents as they interact with the specimen across the strip via chromatographic lateral flow. The conjugate pad contains salts, buffers, and a signal generating reagent consisting of Ebola antibodies conjugated to colloidal gold. Ebola antigens in the patient sample are captured by Ebola antibodies at the Test (T) Zone, which become immobilized on the nitrocellulose membrane and visualized by colloidal gold labeled with Ebola antibodies. The Control (C) Zone immobilized onto the nitrocellulose membrane is visualized by colloidal gold ensuring component elution, reagent activity, and adequate device performance.

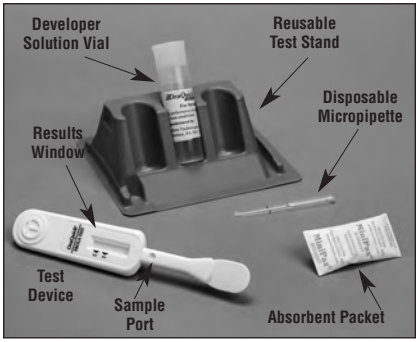
A fingerstick or venipuncture whole blood specimen is collected using a plastic micropipette and transferred to the device, followed by the insertion of the device into the developer vial. The developer solution facilitates the capillary flow of the specimen into the device and onto the assay strip. As the specimen flows through the device, antigens from the specimen are bound by the Ebola antibody labeled gold colorimetric reagent present on the assay strip. If the specimen contains Ebola antigens, the resulting labeled complexes bind to the Test (T) Zone resulting in a purple line. If the specimen does not contain Ebola virus, the labeled complexes do not bind at the Test

Zone and no line is observed. The intensity of the line color is not directly proportional to the amount of virus present in the specimen. The remaining colloidal gold is transported and bound to the Control (C) Zone. This procedural control serves to demonstrate that the fluid has migrated adequately through the device. A purple line will appear at the C Zone during the performance of all valid tests whether or not the sample is positive or negative for Ebola virus (refer to the *Test Result and Interpretation of Test Result* section in this package insert). Positive results may be interpreted as soon as lines are visible at the Test (T) Zone and Control (C) Zones. Negative results have to be read 30 minutes after inserting the device into the Developer Vial.

MATERIALS PROVIDED

OraQuick® Ebola Rapid Antigen Test Kits are available in the following packaging configurations:

Components of Kit Catalog Number	25 Count Kit 1001-0426	100 Count Kit 1001-0427
Divided Pouch, Each containing: Test Device (1) Absorbent Packet (1) Developer Solution Vial (1) (each vial contains 1.0 mL of a buffered saline solution with an antimicrobial agent)	25	100
Test Stands	25	100
Plastic Micropipettes	30	110
Package Insert	1	1
Quick Reference Guide	1	1



MATERIALS REQUIRED AND AVAILABLE AS AN ACCESSORY TO THE KIT

OraQuick® Ebola Rapid Antigen Test Kit Controls 1001-0425

Ebola Positive Control (1 vial, orange cap, 0.25 mL)
Ebola Negative Control (1 vial, white cap, 0.25 mL)
Package Insert

OraQuick® Ebola Visual Reference Panel 1001-0428

Ebola Limit of Detection (1 device)
Ebola Low Positive (1 device)
Ebola Negative (1 device)
Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

Timer or watch capable of timing 30 minutes
Biohazard waste container
Materials required for venipuncture whole blood specimen collection
Materials required to obtain a fingerstick whole blood specimen

WARNINGS

For *in vitro* Diagnostic Use under Emergency Use Authorization only

- **All testing MUST be conducted under appropriate biosafety conditions in accordance with applicable country, state and local laws and with CDC guidelines.**
- **Specimens should always be treated as infectious and/or biohazardous. The use of all possible universal precautions is highly recommended when handling specimens with this test.**
- **Use personal protective equipment (PPE) consistent with current guidelines including safety goggles and / or face shields, masks or respiratory equipment, disposable gowning, boots, and gloves. Users performing this test should be appropriately trained of the donning and doffing of personal protective equipment.**
- **All personnel conducting testing MUST read and be familiar with Universal Precautions[1], Infection Control for Viral Hemorrhagic Fevers in the African Health Care Setting[2] and in Information for Healthcare Worker in the United States (http://www.cdc.gov/vhf/ebola/healthcare-us/index.html) depending upon their location of testing.**
- **For information on the transport of Ebola virus suspected positive samples, please 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).**
- **All equipment and biohazardous waste should be discarded in accordance with country, state, and local laws and policies.**
- **Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.**
- **This package insert must be read completely before using the product.**
- **Follow the instructions carefully when performing the OraQuick® Ebola Rapid Antigen Test. Failure to do so may cause an inaccurate test result.**

- **This test kit is for use with venipuncture and fingerstick whole blood specimens only. Performance of this assay with other specimen types had not been evaluate and has not been authorized under the EUA.**
- **This test should be performed at temperatures in the range of 15°-40°C (59°-104°F). If stored refrigerated, ensure that the Divided Pouch is brought to operating temperature (15°-40°C, 59°-104°F) before performing testing.**
- **This test is not intended to be used as a screening test on patients without signs & symptoms of Ebola infection or to monitor individuals who are undergoing treatment.**
- **Do not use this test beyond the expiration date printed on the Divided Pouch. Always check expiration date prior to testing.**

Device Handling Precautions

- Use all Pipettes, Test Devices, and Developer Solution Vials only once and dispose of properly (see *Safety Precautions*). **Do not reuse any test components**.
- Inspect the Divided Pouch. If the Divided Pouch has been damaged, discard the Divided Pouch and its contents and select a new Divided Pouch for testing.
- Do not interchange Test Devices and Developer Solution Vials from kits with different lot numbers.
- Avoid microbial contamination and exercise care in handling the kit components.
- Adequate lighting is required to read a test result.

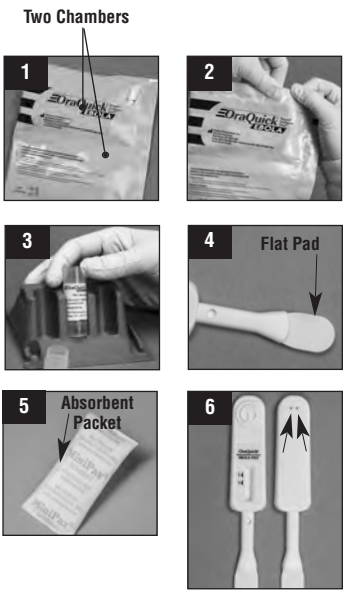
STORAGE INSTRUCTIONS

Store unused OraQuick® Ebola Rapid Antigen Tests unopened at 2°- 30°C (36°-86°F). Do not open the Divided Pouch until you are ready to perform a test. If stored refrigerated, ensure that the Divided Pouch is brought to operating temperature (15°- 40°C, 59°- 104°F) before opening.

DIRECTIONS FOR USE

GENERAL TEST PREPARATION

1. Follow *Safety Precautions* section in this package insert.
2. Gather the materials you will need.
3. Allow the OraQuick® Ebola Rapid Antigen Tests to come to operating temperature (15°- 40°C, 59°- 104°F) before use. Refer to the External Quality Control section in this package insert to determine when the Kit Controls should be run.
4. Set an OraQuick® Test Stand at your workspace, using only the stand provided.
5. Open the two chambers of the OraQuick® Divided Pouch ("Pouch") by tearing at the notches on the top of each side of the Pouch (*see pictures 1 and 2*).
6. Remove the Developer Solution Vial ("Vial") from the Pouch. Hold the Vial firmly in your hand. Carefully remove the cap from the Vial by gently rocking the cap back and forth while pulling it off. Set the cap on your workspace cover.
7. Slide the Vial into the top of one of the slots in the Stand. **DO NOT** force the vial into the Stand from the front of the slot as splashing may occur. Make sure the Vial is pushed all the way to the bottom of the slot in the Stand (*see picture 3*).
8. Remove the Device from the Pouch. **DO NOT** touch the Flat Pad (*see picture 4*). Place the device on a flat clean surface. **(Note: to keep the collection pad clean, avoid contact with any laboratory or other surfaces including hands of the operator)** Check to make sure that an Absorbent Packet is included with the Device (*see picture 5*). If no Absorbent Packet is present, discard the Device and obtain a new Pouch for testing.
   NOTE: DO NOT cover the two holes in the back of the Device with labels or other materials. Doing so may cause an Invalid result (*see picture 6*).



SPECIMEN COLLECTION AND TESTING PROCEDURE

The OraQuick® Ebola Rapid Antigen Test can be used for testing venipuncture whole blood and fingerstick whole blood specimens. Refer to the specific testing procedure below.

FINGERSTICK WHOLE BLOOD AND VENIPUNCTURE PROCEDURE

STEP 1: COLLECT

STEP 1A: FINGERSTICK WHOLE BLOOD

1. Using an antiseptic wipe, clean the finger of the person being tested. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger pad. Hold the finger downward. Apply gentle pressure beside the point of the puncture. Avoid squeezing the finger to make it bleed (*see picture 7*). Wipe away this first drop of blood with a sterile gauze pad. Allow a new drop of blood to form.
2. Pick up an unused plastic micropipette by the handle (*see picture 8*). Hold the micropipette horizontally and touch the tip to the drop of blood, and slowly draw the blood up (*see picture 9*). Filling of the micropipette is automatic, do not squeeze while sampling. Make sure that the micropipette is filled up to the indicator line with blood and there are no bubbles present (*see picture 10*). If a bubble is present, discard collected sample and obtain a new sample using a new micropipette.

   NOTE: If the micropipette is dropped or comes in contact with any other surface, discard it in a biohazard waste container. Get a new micropipette for the collection of the blood sample.

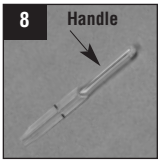
STEP 1B: VENIPUNCTURE WHOLE BLOOD

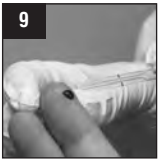
1. Using standard venous phlebotomy procedures collect a whole blood sample using a tube containing EDTA (lavender top) anticoagulant. If the specimens are not tested at the time of collection, the specimen may be stored at 2°-30°C (36°-86°F) for up to 24 hours.
2. Prior to testing, mix the blood tube gently by inversion several times to ensure a homogeneous sample.
3. Pick up an unused micropipette by the handle (*see picture 11*). Hold the micropipette, place it in the blood tube, and slowly draw the blood up. Filling of the micropipette is automatic, do not squeeze while sampling (*see picture 12*). Make sure that the micropipette is filled up to the indicator line with blood and there are no bubbles present (*see picture 13*). If a bubble is present, discard collected sample and obtain a new sample using a new micropipette.

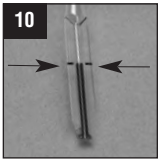
   NOTE: If the micropipette is dropped or comes in contact with any other surface, discard it in a biohazard waste container. Get a new micropipette for the collection of the blood sample.

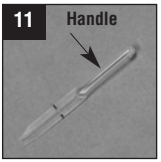
STEP 2: TEST

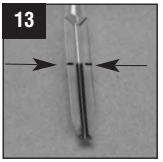
1. Deposit the blood sample through the sample port on the device by compressing the micropipette directly above the sample port (*see picture 14).*
2. Insert the Flat Pad of the Device all the way into the Developer Vial (*see picture 15*). Make sure that the Flat Pad touches the bottom of the Vial. The Result Window on the Device should be facing towards you (*see picture 16*).
3. Start timing the test (*see picture 17*). **DO NOT** remove the Device from the Vial while the test is running. Red fluid will appear and travel up the Result Window. The red fluid will gradually disappear as the test develops (*see picture 18*). Read the results in a fully lighted area. Postive results may be interpreted as soon as lines are visible at the Test (T) Zone and Control (C) Zone and have been observed as early as 4 minutes. Negative results have to be read 30 minutes after inserting the device into the Developer Vial.
4. Refer to the T*est Result and Interpretation of Test Result* section in this package insert.











GENERAL TEST CLEAN-UP

1. Dispose of the used test materials in a biohazard waste container. All equipment and biohazardous waste should be discarded in accordance with country, state, and local laws and policies.
2. Change your gloves between each test to prevent contamination. Throw away the used gloves in a biohazard waste container.
3. Use a freshly prepared 10% solution of bleach to clean up any spills.

QUALITY CONTROL

Built-in Control Features

The OraQuick® Ebola Rapid Antigen Test has a built-in procedural control that demonstrates assay validity. A purple line in the Control ("C") area of the Result Window indicates that the fluid migrated appropriately through the Test Device. The Control line will appear on all valid tests, whether or not the sample is positive or negative for Ebola Antigens. (Refer to *Test Result and Interpretation of Test Result* section of this package insert).

External Quality Control

OraQuick® Ebola Rapid Antigen Test Kit Controls must be used with the OraQuick® Ebola Rapid Antigen Test. The Kit Controls are specifically formulated and manufactured to ensure performance of the Test, and are used to verify your ability to properly perform the test and interpret the results. The Ebola Positive Control will produce a positive test result and has been manufactured to produce a faint Test ("T") line. The Ebola Negative Control will produce a negative test result (Refer to *Test Result and Interpretation of Test Result* section of this package insert). Use of kit control reagents manufactured by any other source may not produce the required results, and therefore, will not meet the requirements for an adequate quality assurance program for the OraQuick® Ebola Rapid Antigen Test. If external controls do not produce expected results, patient testing should not be performed. Contact OraSure Technologies' Customer Care if the Kit Control reagents do not produce the expected results.

Run the External Controls under the following circumstances:

- Each new operator prior to performing testing on patient specimens,
- When opening a new test kit lot,
- Whenever a new shipment of test kits is received,
- If the temperature of the test kit storage area falls outside of 2°-30°C (36°-86°F),
- If the temperature of the testing area falls outside of 15°-40°C (59°-104°F), and
- At periodic intervals as dictated by local, state and country laws and by the user facility.

Test Procedure for External Controls:

Refer to the OraQuick® Ebola Rapid Antigen Test Kit Control package insert for full instruction on the use of these reagents. It is the responsibility of each laboratory using the OraQuick® Ebola Rapid Antigen Test to establish an adequate quality assurance program to ensure the performance of the device under its specific locations and conditions of use.

Qualification for New Operators

The OraQuick® Ebola Visual Reference Panel is available separately for use with the OraQuick® Ebola Rapid Antigen Test. The OraQuick® Ebola Visual Reference Panel includes potential test results including negative, low positive, and the limit of detection of the device. New operators must be able to correctly interpret all devices in the OraQuick® Ebola Visual Reference Panel prior to using the OraQuick® Ebola Rapid Antigen Test device with patient samples. Failure to read at low intensities can result in the inability to detect specimens near the limit of detection of the OraQuick® Ebola Rapid Antigen Test and may result in false negative results.

TEST RESULT AND INTERPRETATION OF TEST RESULT

Positive results may be interpreted as soon as lines are visible in the Test (T) Zone and the Control (C) Zone and have been observed as early as 4 minutes. Negative results have to be read 30 minutes after inserting the device into the Developer Vial.

NEGATIVE

A test is **Negative** if:

A purple line appears in the C Zone and NO line appears next to the T Zone (*see picture 19*).

A negative test result is interpreted as Ebola antigen not detected in the specimen. The patient is presumed negative for Ebola antigen.

A negative result does not preclude Ebola virus infection.

POSITIVE

A test is **Positive** if:

A purple line appears in the C Zone and a purple line appears in the T Zone. Lines may vary in intensity. The test is positive regardless of how faint these lines appear (*see pictures 20, 21, and 22*).

A Positive test result is interpreted as Ebola antigen detected in the specimen. The patient is presumed positive for Ebola antigen.

Individuals with a positive result in the OraQuick® Ebola Rapid Antigen Test should undergo appropriate clinical follow-up, which may include supplemental PCR testing.

INVALID

A test is **Invalid** if any of the following occurs:

- **NO** purple line appears in the C Zone (*see picture 23*), or
- any partial line on one side of the C or T Zones (*see pictures 25 and 26*), or
- a purple background in the Result Window makes it difficult to read the result after 30 minutes (*see picture 24*)

An **Invalid** test result means that there was a problem running the test, either related to the specimen or to the Test Device. **An Invalid result cannot be interpreted. An invalid test result needs to be repeated with a fresh sample and a new device. Please contact OraSure Technologies' Customer Care if you are unable to obtain a valid test result upon repeat testing.**

LIMITATIONS OF THE TEST

1. Weak positive samples may take longer to develop and can take the entire 30 minutes for a test line to be present. Therefore, all negative test results must be read 30 minutes after inserting the device in the Developer Vial. Negative test result must not be reported prior to reading the device at 30 minutes.
2. Reading any test result after 30 minutes may yield inaccurate test results.
3. Clinical performance of this device was evaluated with a limited number of retrospective samples.
4. Testing with the OraQuick® Ebola Rapid Antigen Test should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens.
5. Assay results are for the presumptive identification of Ebola virus. The definitive identification of Ebola virus requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of Ebola virus must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification Ebola virus.
6. 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.
7. Potential cross reactivity of the OraQuick® Ebola Rapid Antigen Test 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.
8. Testing samples with concentrations of Rheumatoid Factor above 1050 IU/mL may result in false positive results.
9. Cross-reactivity with organisms other than those tested in the Cross-reactivity Study have not been assessed and may lead to erroneous results.

PERFORMANCE CHARACTERISTICS

WHOLE BLOOD CLINICAL PERFORMANCE

A total of 75 retrospective, remnant whole blood samples collected from patients in West Africa (Sierra Leone) during the 2014-2015 Ebola outbreak were tested with the OraQuick® Ebola Rapid Antigen Test. These samples were tested in West Africa using an U.S. FDA Emergency Use authorized (EUA) Ebola Virus Real-time RT-PCR Assay. The whole blood samples were stored frozen thereafter until testing with the OraQuick® Ebola Rapid Antigen Test. Testing was performed in a randomized, blinded manner. OraQuick® Ebola Rapid Antigen Test performance in comparison to the results generated by the EUA Ebola Virus Real-time RT-PCR Assay (the Comparator) were calculated based on OraQuick® Ebola Rapid Antigen Test results that were read at 30 minutes.

Positive and Negative Percent Agreement against the Comparator

	Percent Agreement	95% CI*
Positive Percent Agreement	84.0% (21/25)§	63.92% - 95.46%
Negative Percent Agreement	98.0% (49/50)	89.35% - 99.95%

*Calculated using Clopper-Pearson exact method

§Includes specimens tested in a Ct range 15-34 (Refer to the table containing the Percent Agreement for Select Ct Ranges)

Percent Positive Agreement for select Ct Ranges

PCR Ct Ranges	Percent Agreement	95% CI*
15 – 24	100% (16/16)	86.77% - 100.0%
15 – 29	90.5% (19/21)	69.62% - 98.83%
15 – 34	84.0% (21/25)	63.92% - 95.46%

*Calculated using Clopper-Pearson exact method

OraQuick® Ebola Rapid Antigen Test compared to the Comparator

		Comparator RT-PCR Results									
		15- 20		21-24		25-29		30-34		Overall	
		+	-	+	-	+	-	+	-	+	-
OQ Ebola Results	+	7		9		3		2		21	
	-		0		0	2	0	2	0	4	0

Comparator RT-PCR Results	
Ebola not detected	
+	-
	1
0	49

FINGERSTICK WHOLE BLOOD SPECIFICITY STUDY

To support a fingerstick whole blood claim, a specificity study was conducted testing two (2) fingerstick whole blood specimens from 26 subjects in the United States. For two of the samples the device was read out of the 30 minute read window and the samples were excluded from the analysis. A total of 50 samples were included in the analysis. Fifty (50) out of fifty (50) specimens tested were negative resulting in percent negative agreement with the expected negative results of 100% (95% CI 92.9% - 100.0%).

ANALYTICAL SENSITIVITY

LIMIT OF DETECTION

A Limit of Detection (LoD) range finding study in venous whole blood identified $1.64x10^6$ $TCID_{50}$/mL as the tentative LoD for Zaire Ebola Inactivated Virus (Virus Stock: Ebola Zaire Mayinga ZZXDK901 / 812094 / VSP / $2.5x10^8$ $TCID_{50}$/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.

Additionally, a LoD range finding study was performed using recombinant VP40 antigen spiked into pooled venous whole blood. This LoD range finding study determined the tentative LoD of the OraQuick® Ebola Rapid Antigen Test in venous whole blood with recombinant antigen to be 53,000 pg/mL or 1.06 ng/test. This tentative LoD was confirmed as the LoD by 20 out of 20 replicates testing positive with the same recombinant VP40 antigen at this concentration.

ANALYTICAL REACTIVITY (INCLUSIVITY)

Analytical reactivity of the OraQuick® Ebola Rapid Antigen Test was evaluated for additional strains of the Ebola virus. Testing of three (3) replicates was performed using negative venous whole blood as the testing sample matrix. The OraQuick Ebola Rapid Antigen Test also reacts with E. Sudan and E. Bundibugyo in addition to reacting with E. Zaire.

Ebola Strain	Inactivated or Live	Concentration Tested	Reactivity (Positive (P)/Negative (N))
Ebola Zaire Mayinga ZZXDK901/812094/VSP	Inactivated	$1.5x10^6$ $TCID_{50}$/mL	P[B]
Ivory Coast (COTE D'IVOIRE 11/27/94)	Inactivated	Unknown (1:10 dilution) Unknown (1:1 dilution)	N N
Reston (119876 Pennsylvania)	Inactivated	3.16×10^6 pfu/mL $3.16 x10^7$ pfu/mL	N N
Sudan (BONEFACE)	Inactivated	10^5 pfu/mL 5×10^5 pfu/mL	N P
Sudan[E] (200011676 GULU)	Inactivated	$5.6 x10^4$ pfu/mL 2.8×10^5 pfu/mL	P[A] P
Bundibugyo[F] (200706291 UGANDA prototype)	Inactivated	3.98×10^4 pfu/mL 1.99×10^5 pfu/mL	P P
Sudan Gulu[E] (2000011676)	Inactivated	3.25×10^5 PFU/mL 5.95×10^5 PFU/mL 1.19×10^6 PFU/mL 1.79×10^6 PFU/mL	N N P P[C]
Bundibugyo[F] (Uganda)	Inactivated	3.73×10^4 PFU/mL 6.83×10^4 PFU/mL 1.37×10^5 PFU/mL 2.05×10^5 PFU/mL	N P[D] P P
Taï Forest (aka Ivory Coast)	Inactivated	1.36×10^7 VP/mL 7.5×10^7 VP/mL	N N
Reston (aka H28)	Inactivated	5.83×10^5 PFU/mL	N

[A] Two of three replicates tested Positive and acceptance criteria met. [B] Testing completed in LoD study.

[C] A 4th replicate was added to confirm original non-reactive results for one replicate – 3 out of 4 replicates reactive.

[D] One of three replicates was reactive and criteria was not met.

[E,F] The two tested materials from the same strain have been obtained from a different source. Differences in preparation methods can lead to differences in the number of plaque forming units even if the starting material is the same.

HIGH DOSE HOOK EFFECT

A high-dose hook effect is a false negative result due to very high concentrations of the target analyte. Antigen at excessive concentrations could quench the specific antibodies in the assay decreasing complexes between the antibody on the gold conjugate and the biotinylated antibody. Insufficient binding of the gold conjugate could occur at the test line and the result interpreted as a false negative result.

An analytical study was conducted and the results demonstrate acceptable performance of the OraQuick® Ebola Rapid Antigen Test when evaluating samples at excessive antigen concentrations. There is no decrease in visual intensity at the test line for a VP40 antigen concentration up to 10,000 times the established LoD.

ANALYTICAL SPECIFICITY

INTERFERING SUBSTANCES

The OraQuick® Ebola Rapid Antigen Test was evaluated with the following interfering substances present in negative whole blood and whole blood spiked with recombinant antigen (rAg) at 2.0 X the LoD in order to assess their potential effect on the assay performance as per CLSI guidelines EP7-A2[3]. For Bilirubin, Hemoglobin, Protein and HAMA testing was completed on three (3) whole blood samples tested at n=2 replicates for each condition. Rheumatoid Factor testing was performed on three serum samples, that were each tested in two dilutions with n=2 replicates. The concentrations of Rheumatoid Factor that were tested ranged from 525 IU/mL to 11,900 IU/mL.

Interfering Substances	Target Testing Concentration	Reactivity
Bilirubin	25 mg/dL	None
Hemoglobin	20 g/dL	None
Protein	5 g/dL	None
HAMA	2464 ng/mL	None
Rheumatoid Factor	2920 IU/mL	Positive
	1460 IU/mL	Positive
	1050 IU/mL	None

No interference was observed for Bilirubin, Hemoglobin, Protein and HAMA at the levels tested. Rheumatoid Factor caused false positive results in the OraQuick® Ebola Rapid Antigen Test at all tested concentrations greater than 1050 IU/mL. Concentrations of Rheumatoid Factor equal or less than 1050 IU/mL do not cause interference in the assay.

CROSS REACTIVITY

Cross reactivity of the OraQuick® Ebola Rapid Antigen Test was evaluated by testing additional viral, bacterial, and parasitic pathogens. In this study three (3) replicates were tested with the pathogens spiked into venous whole blood at the concentrations listed below. None of the tested organisms produced false positive results in the OraQuick® Ebola Rapid Antigen Test at the concentration tested.

Virus/Bacteria/Parasite	Inactivated or Live	Type/Strain	Concentration Tested	Reactivity
Marburg	Inactivated	RAVN	Log 5.53 $TCID_{50}$/mL	None
		Musoke	3.16 x 10^6 pfu/mL	None
		Lake Victoria (aka Musoke)	3.73 x 10^6 PFU/mL	None
		200501379 Angola	1.44 x 10^6 PFU/mL	None
		VOEGE	3.6 x10^6 pfu/mL	None
Crimean Congo Hemorrhagic Fever	Inactivated	OMAN199809166 #811466	5.6 x10^4 $TCID_{50}$/mL	None
Lassa	Inactivated	Josiah	3.16 x 10^5 pfu/mL	None
		Josiah	1.88 x 10^5 PFU/mL	None
		Macenta (aka Z-136)	1.19 x 10^7 PFU/mL	None
		Pinneo	2.00 x 10^6 PFU/mL	None
Rift Valley Fever	Inactivated	ZH-501	3 x10^6 pfu/mL	None
			5.43 x 10^6 PFU/mL	None
Yellow Fever	Inactivated	Vaccine Strain #806588	Unknown	None
		Asibi	1.88 x 10^6 PFU/mL	None
Chikungunya virus	Live	ATCC VR-64	3.0 x 10^8 LD_{50}/mL	None
Influenza A	Live	A/Wisconsin/10/1998	2.3 x 10^5 $TCID_{50}$/mL	None
Influenza B	Live	B/Florida/04/06	4.6 x 10^5 $TCID_{50}$/mL	None
Rotavirus	Live	ATCC VR-899	6.4 x 10^6 $TCID_{50}$/mL	None
Adenovirus	Live	Type 5 ATCC VR-5	2.0 x 10^5 $TCID_{50}$/mL	None
RSV	Live	ATCC VR-26	9.0 x 10^5 $TCID_{50}$/mL	None
Enterovirus	Live	Enterovirus 71 ATCC VR-1432	5.1 x 10^5 $TCID_{50}$/mL	None
Salmonella	Live	*S. enterica* ATCC 10708	3.8 x 10^7 CFU/mL	None
Salmonella typhi	Live	*S. typhi* ATCC 6539	5.4 x 10^5 CFU/mL	None
Shigella	Live	*S. dysenteriae* ATCC 9361	4.0 x 10^6 CFU/mL	None
Pseudomonas aeruginosa	Live	*P. aeruginosa* ATCC 15442	3.4 x 10^8 CFU/mL	None
Vibrio Cholera	Live	*V. cholera* ATCC 39050	6.7 x 10^5 CFU/mL	None
Streptococcus pneumonia	Live	*S. pneumonia* ATCC 6303	2.1 x 10^8 CFU/mL	None
Hemophilus influenza (meningitis)	Live	*H. influenzae* ATCC 33930	3.0 x 10^7 CFU/mL	None
Leptospira genus	Live	*L. biflexa* ATCC 23582	~ 9.1 x 10^4 CFU/mL	None
Neisseria meningitides	Live	*N. meningitides* ATCC 13090	3.5 x 10^6 CFU/mL	None
Yersinia enterocolitica	Live	*Y. enterocolitica* ATCC 23715	7.0 x 10^6 CFU/mL	None
Plasmodium falciparum (malaria)	Live	*P. falciparum* ATCC 30932	0.26% parasitemia	None
Plasmodium vivax (malaria)	Live	*P. vivax* ATCC 30151	6.8 x 10^5 cells/mL	None
Trypanosoma	Live	*T. cruzi* ATCC 30013	3.1 x 10^7 CFU/mL	None
Rickettsia	Protein Only	R. africae (protein) BEI NR-42992	11.6 mg/mL	None
Dengue	Inactivated	Serotype 1, strain WP74	6.20 x 10^4 PFU/mL	None
		Serotype 2, strain 16803	7.18 x 10^5 PFU/mL	None
		Serotype 3, strain CH53489	1.52 x 10^4 PFU/mL	None
		Serotype 4, strain 341750	2.12 x 10^5 PFU/mL	None

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1. CDC, Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Bloodborne Pathogens in Health-Care Settings. MMWR 1988; 37(24):377-388.
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3. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.* CLSI Document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

EXPLANATION OF SYMBOLS

LOT	Batch Code	IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number		Manufacturer
	Caution, Consult Accompanying Documents	PN	Part Number
	Use By		Temperature Limitation

For Technical or Customer Service within the United States, phone (800) ORASURE (800-672-7873).
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Critical reagents in the OraQuick® Ebola Rapid Antigen Test are being supplied by:

- the Viral Hemorrhagic Fever Consortium, or "VHFC" (www.VHFC.org). The VHFC reagents were developed with the support of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health ("NIH/NIAID"). VHFC members Autoimmune Technologies LLC and Zalgen Labs LLC manufacture the critical reagents.
- the Biological Defense Research Directorate at the United States Navy Medical Research Center (NMRC).

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