



Instructions for Use – CADAVERIC ORAL FLUID

For testing of live patients using this device, please refer to the Instructions for Use entitled OraQuick® Ebola Rapid Antigen Test – Instructions for Use – Whole Blood.

For Use Under Emergency Use Authorization (EUA) Only

For in-vitro diagnostic use

NAME AND INTENDED USE

The OraQuick® Ebola Rapid Antigen Test for cadaveric oral fluid is a single-use immunoassay intended for the qualitative detection of antigens from Ebola Zaire virus (detected in the West Africa outbreak in 2014). The test is intended for use with oral fluid swab specimens from individuals with epidemiological risk factors for Ebola virus infection and suspected to have died of Ebola. The test is intended to aid in diagnosing Ebola Zaire virus infection as the cause of death in order to inform decisions on safe and dignified burial procedures to prevent transmission of the Ebola Zaire virus in the community. The test may also detect antigens from Sudan Ebola virus and Bundibugyo Ebola virus; however, it does not distinguish between these different Ebola virus species.

The OraQuick® Ebola Rapid Antigen Test for cadaveric oral fluid may be used with direct testing or in conjunction with recommended swabs in viral transport media.

The authorized OraQuick® Ebola Rapid Antigen Test for cadaveric oral fluid is not intended for use as a diagnostic test for oral fluid swabs of living individuals. This test is not intended 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 for cadaveric oral fluid is authorized for use by personnel who are adequately equipped, trained, and capable of testing for Ebola infection in laboratories, facilities, and in field surveillance and response teams acting under the direction of public health authorities.

In a limited number of cases, a negative result may not preclude Ebola virus disease. The OraQuick® Ebola Rapid Antigen Test was evaluated in a limited clinical study using retrospective clinical specimens from deceased 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), Tai Forest virus (TAFV), and Ebola virus (EBOV). 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 uses 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 cadaveric oral fluid. The OraQuick® Ebola Rapid Antigen Test is comprised of both a single-use test device and a 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 sample are captured by Ebola antibodies at the Test (T) Zone, which become immobilized on the nitrocellulose membrane and visualized by colloidal gold labeled 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.

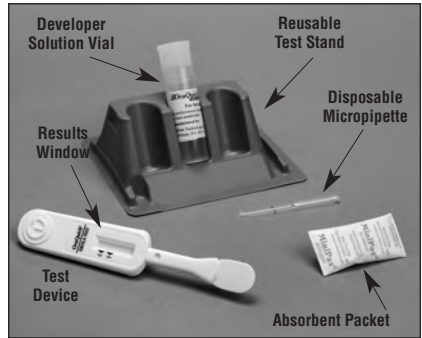
For cadaveric oral fluid specimens, there are two ways to collect specimens: 1. Swab the gum line or the soft pallet tissue in the back of the throat and then insert the device directly into the developer solution. 2. Swab the gum line or the soft pallet tissue in the back of the throat with a recommended swab and insert into a recommended viral transport media. Refer to the *Cadaveric Oral Fluid Procedure – Direct Collection or Cadaveric Oral Fluid Procedure – Transport Media* sections in this package insert for the specimen collection instructions. When using viral transport media, collect the specimen from the transport media tube by using the plastic micropipette included in the kit. Transfer the specimen to the device through the sample port. Insert the device into the developer solution. 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, no labeled complexes 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 minute 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
Reusable Test Stands	25	100
Plastic Micropipettes	30	120
Package Insert	1	1
Cadaveric Oral Fluid Quick Reference Guide	1	1



Note: An additional Package Insert and Quick Reference Guide for use of the device with Venipuncture, Whole Blood or Fingerstick Whole Blood may be present in the Kit.

MATERIALS REQUIRED AND AVAILABLE AS AN ACCESSORY TO THE KIT

OraQuick® Ebola Rapid Antigen Oral Fluid 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

Foil Transfer Pouch 1001-0494

MATERIALS REQUIRED BUT NOT PROVIDED

Timer or watch capable of timing 30 minutes

Biohazard waste container

OPTIONAL MATERIALS NOT PROVIDED WITH KIT

BD Universal Viral Transport for Viruses, Chlamydia, Mycoplasmas and Ureaplasmas

Σ-Virocult® System (MW951S)

WARNINGS AND PRECAUTIONS

- 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 Precautions¹, Infection Control for Viral Hemorrhagic Fevers in the African Health Care Setting² 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 for cadaveric oral fluid testing.
- Follow the instructions carefully when performing the OraQuick® Ebola Rapid Antigen Test. Failure to do so may cause an inaccurate test result.
- The use of any viral transport media for cadaver testing other than BD Universal Viral Transport for Viruses, Chlamydia, Mycoplasmas and Ureaplasmas (220528) or Σ -Virocult® System (MW951S) has not been evaluated and has not been authorized under this EUA.
- This test kit is for use with venipuncture and fingerstick whole blood and cadaveric oral fluid swab specimens only. Please refer to the whole blood Package Insert for information on whole blood testing.
- 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.
- 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

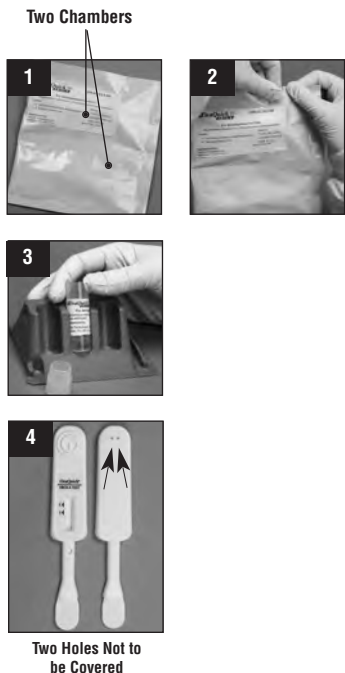
Store unused OraQuick® Ebola Rapid Antigen Tests unopened at 2°- 30°C (36°- 86°F). Do not open the Divided Pouch until you are ready to perform a test. If stored refrigerated, ensure that the Divided Pouch is brought to operating temperature (15°- 40°C, 59°- 104°F) before opening.

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*).

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 4*).

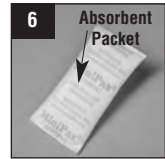
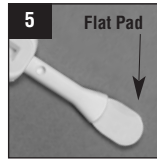


**CADAVERIC ORAL FLUID PROCEDURE – DIRECT COLLECTION
STEP 1: COLLECT**

1. Remove the Device from its Pouch. **DO NOT** touch the Flat Pad (see picture 5). Check to make sure that an Absorbent Packet is included with the Device (see picture 6). If no Absorbent Packet is present, discard the Device and obtain a new Pouch for testing.
2. Open the mouth of the cadaver and collect a sample from the posterior oral cavity by swiping the flat pad around the back of the soft palate (see pictures 7 and 8). If collection in this manner is not feasible due to rigor mortis, collection of cadaveric oral fluid can be taken from the gum line. To obtain a sample from the gum line, swipe the flat pad once around the top and once around the bottom of the gum line (see pictures 9 and 10).

NOTE: Both sides of the Flat Pad may be used during this procedure.

3. If the device cannot be placed immediately in the Developer Vial for Testing (Step 2 below), the device may be placed in a foil Transfer Pouch. **Only those pouches provided by OraSure Technologies as an accessory as part number (1001-0494) should be used to ensure proper stability of the sample.**
4. The sealed Transfer Pouch may be stored at 15°-40°C (59°-104°F) for 60 minutes or at 2°-8°C (36°-46°F) for up to 22 hours prior to testing.



STEP 2: TEST

1. If testing samples were stored on blue ice, remove the Transfer Pouch from storage and allow it to come to room temperature for 15 minutes prior to testing.
2. Insert the Flat Pad of the Device all the way into the Vial (see picture 11). 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 12).
3. Start timing the test (see picture 13). **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 14). Positive results may be interpreted as soon as lines are visible at the Test (T) Zone and Control (C) Zone. Negative results have to be read 30 minutes after inserting the device into the Developer Vial.
4. Refer to the *Test Result and Interpretation of Test Result* section in this Package Insert.



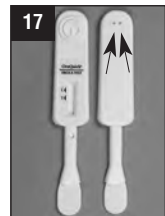
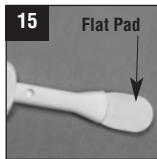
**CADAVERIC ORAL FLUID PROCEDURE – TRANSPORT MEDIA
STEP 1: COLLECT**

1. Cadaveric oral fluid specimens should be collected and handled following the instructions for use of the swab/viral transport media. Once a swab specimen is collected, it should be placed in the transport media vial. Specimens may be stored at 2°-40°C (36°-104°F) for up to 24 hours. If the sample cannot be tested within 24 hours of collection, the transport media can be frozen at -70°C (-94°F) with 3 freeze/thaw cycles.

STEP 2: TEST

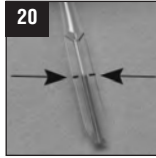
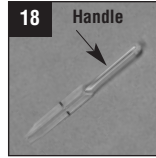
1. Prior to testing, mix the transport media tube gently by inversion several times to ensure a homogeneous sample.
2. Remove the Device from the Pouch. **DO NOT** touch the Flat Pad (see picture 15). Place the device on a flat clean surface. **(Note: to keep the collection pad clean, avoid contact with 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 16). 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 17).



Two Holes Not to be Covered

- Pick up an unused micropipette by the handle (see picture 18). Hold the micropipette, place it in the transport media tube, and slowly draw the sample up. Filling of the micropipette is automatic, do not squeeze while sampling (see picture 19). Make sure that the micropipette is filled up to the indicator line with the sample and there are no bubbles present (see picture 20). If a bubble is present, discard collected sample and obtain a new sample using a new micropipette.
- Deposit the cadaveric sample through the sample port on the device by compressing the micropipette directly above the sample port (see picture 21).
- Insert the Flat Pad of the Device all the way into the Vial (see picture 22). 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 23).
- Start timing the test (see picture 24). **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 25). Positive results may be interpreted as soon as lines are visible at the Test (T) Zone and Control (C) Zone. Negative results have to be read 30 minutes after inserting the device into the Developer Vial.
- Refer to the *Test Result and Interpretation of Test Result* section in this Package Insert.



GENERAL TEST CLEAN-UP

- 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.
- Change your gloves between each test to prevent contamination. Throw away the used gloves in a biohazard waste container.
- Use a freshly prepared 10% solution of bleach to clean up any spills.

QUALITY CONTROL PROCEDURES

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”) zone 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, cadaver 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 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 cadaver 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. 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 26)

A Negative test result is interpreted as Ebola antigen not detected in the specimen. The cadaver 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 27, 28, and 29).

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



In accordance with CDC and WHO recommendations cadavers with a positive result should be subjected to safe and dignified burial procedures and contacts of an Ebola positive cadaver should be identified. Follow up testing of possible contacts should be conducted in accordance with, *EMERGENCY GUIDELINE Implementation and management of contact tracing for Ebola virus disease* (http://apps.who.int/iris/bitstream/10665/185258/1/WHO_EVD_Guidance_Contact_15.1_eng.pdf?ua=1) issued by the World Health Organization (WHO) and the Centers for Disease Control (CDC).

INVALID

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

- **NO** purple line appears in the C Zone (see picture 30), or
- a purple background in the Result Window makes it difficult to read the result at 30 minutes (see picture 31), or
- any partial line on one side of the C or T Zones (see picture 32 and 33).

An **Invalid** test result means that there was a problem running the test, related either 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 results 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 cadaveric oral fluid samples.
4. Negative results do not preclude Ebola virus infection.
5. Potential cross reactivity of the OraQuick Ebola Rapid Antigen Test with Ebola vaccines and therapeutics has not been evaluated. Specimens from cadavers who have received therapeutics or vaccines against Ebola virus may exhibit false positive or other confounding test results.
6. Testing samples with concentrations of mucin above 15mg/mL may result in false positive results.
7. Cross-Reactivity with organisms other than those tested in the Cross Reactivity Study have not been assessed and may lead to erroneous results.

CADAVERIC ORAL FLUID CLINICAL PERFORMANCE

A total of 277 retrospective cadaveric specimens (collected in VTM) were tested in a study conducted by the World Health Organization; all samples were originally tested with the RealStar Filovirus Screen Test. Prior to testing with the OraQuick® Ebola Rapid Antigen Test, all samples were re-tested with the Xpert Ebola Assay (Cepheid) to confirm the original Ebola PCR result.

A total of 222 RealStar Filovirus Screen RT-PCR negative specimens were tested on the Xpert Ebola Assay (Cepheid). Of these specimens 29 gave an invalid result, possibly because of a failure of the human gene target internal control due to poor specimen quality, and were excluded. Therefore, a total of 193 PCR negative specimens were included in the RDT evaluation.

Of the 55 RealStar Filovirus Screen RT-PCR positive specimens tested on Xpert Ebola Assay (Cepheid), 51 specimens passed as positive; 3 were negative and 1 gave an invalid result and were therefore excluded. Of the 51 positive specimens, 16 were tested in a manner inconsistent with the instructions for use and were therefore excluded from the study. A total of 35 positive specimens were included in the performance evaluation.

Using the Xpert Ebola Test (Cepheid) as the comparator assay, the performance of the OraQuick® Ebola Rapid Antigen Test is shown in the following table:

	Xpert Ebola Test (Cepheid)	
	Positive Percent Agreement (PPA) (95% CI)	Negative Percent Agreement (NPA) (95% CI)
OraQuick® Ebola Rapid Antigen Test	34/35 97.1% (85.5 - 99.5)	193/193 100.00% (98.1 - 100)

Note: The study also included testing of 16 samples that were diluted 1:4 in aqua dest. The OraQuick® Ebola Rapid Antigen test correctly detected 14 out of these 16 samples as positive. The two samples that were incorrectly detected as being negative had high PCR Ct values representative of Ebola virus concentrations below the assay's LOD.

i. CDC Study – Sierra Leone

CDC in collaboration with the Ministry of Health (MOH) has conducted a Field Evaluation of New Rapid Diagnostic Tests for Ebola Virus Disease in Sierra Leone that included the testing of cadavers. A total of 111 samples were tested with the OraQuick® Ebola Rapid Antigen Test according to the instructions for use with cadaveric oral fluid using the direct sampling method. The 111 samples tested by OraQuick® RDTs were all negative by PCR (the 50 specimens collected in Kenema were tested with the Trombley PCR assay on the Cepheid Smartcycler II). Three samples initially produced invalid results and were re-tested according to the instructions for use; two of these samples were re-tested as negative. One of the invalid results could not be resolved by re-testing as it gave another invalid result and was therefore excluded from the performance calculation. The following table includes a summary of the field study data:

Date of testing	Region	RDTs tested	Reactive	Non-reactive	Invalid	PCR
January 10, 2016	Kenema	50	0	50 ^A	0	50 ^B
January 6 – 13, 2016	Western	61	1 ^C	59 ^D	1 ^D	61
	Total	111	1	109	1	111

- A) The control line on the OraQuick® device was not visualized and the test was interpreted as invalid. Upon repeat testing the OraQuick® test was non-reactive. A swab for PCR was collected and the samples were negative by PCR.
- B) PCR is currently performed in conjunction with the OraQuick® tests for all cadavers. The 111 samples tested by OraQuick® RDTs were all negative by PCR (Trombley PCR assay on the Cepheid Smartcycler II.)
- C) The OraQuick® results were reactive but PCR confirmation was negative.
- D) Two initial OraQuick® devices were interpreted as invalid. Upon repeat testing with OraQuick®, one was non-reactive and the other was invalid. The twice invalid sample was tested by PCR and was found to be negative.

This study provided a Negative Percent Agreement (NPA) for the OraQuick® Ebola Rapid Antigen Test of 109/110 = 99.1% with a 95% Confidence Interval of 95.0 - 100%.

ii. CDC Study – Guinea

In Guinea 334 oral swab samples were taken from cadavers and tested in parallel with PCR and with the OraQuick® Ebola Rapid Antigen Test. Samples were collected between June 2015 and August 2015. All samples were negative by PCR and by the OraQuick® Ebola Rapid Antigen Test. This study provided a Negative Percent Agreement (NPA) for the OraQuick® Ebola Rapid Antigen Test of 334/334 = 100% with a 95% Confidence Interval of 98.9 - 100%.

iii. CDC Study – Liberia

Additionally, 97 specimens from cadavers in Liberia were tested with the OraQuick® Ebola Rapid Antigen Test. Samples were parallel tested with either the CDC or the DOD EUA PCR. Samples were collected between May and June 2015 and tested with the OraQuick® Ebola Rapid Antigen Test according to the instructions for use with cadaveric oral fluid. All samples tested were negative by PCR; three of these PCR negative samples resulted in an invalid test result with the OraQuick® Ebola Rapid Antigen Test. The samples were not retested with the OraQuick® Ebola Rapid Antigen Test and were therefore excluded from the performance calculation below. All other PCR negative samples also resulted in a negative OraQuick® Ebola Rapid Antigen Test result. This study provided a Negative Percent Agreement (NPA) for the OraQuick® Ebola Rapid Antigen Test of 94/94 = 100% with a 95% Confidence Interval of 96.1 to 100%.

LIMIT OF DETECTION – LIVING DONORS

A Limit of Detection (LoD) range finding study was conducted with oral fluid collected from living donors with the BD Universal Transport System (3mL medium), the Σ-Virocult Transport System (1mL medium), and directly collected with the OraQuick® Ebola Rapid Antigen Test. For the direct collection with the OraQuick® Ebola Rapid Antigen Test recombinant VP40 antigen was spiked directly on to the collection pad of the OraQuick® Ebola Rapid Antigen Test after oral fluid collection and prior to insertion in to the Developer Vial. Recombinant VP40 antigen was spiked on the swab of the respective transport system after oral fluid collection and prior to placement in the transport medium. A total of 20µL was then placed in the sample port of the OraQuick® Ebola Rapid Antigen Test prior to insertion of the device into the Developer Vial.

The tentative LoD for oral fluid directly collected from living donors with the OraQuick® Ebola Rapid Antigen Test was 0.53 ng/test with a VP40 antigen concentration in oral fluid of 7.6 ng/mL. This concentration was confirmed as the LoD by 19 out of 20 replicates testing positive with the same recombinant VP40 antigen at this concentration.

The tentative LoD for oral fluid collected from living donors with the BD Universal Transport System was 0.217 ng/test with a VP40 antigen concentration in oral fluid of 465 ng/mL. This concentration was confirmed as the LoD by 20 out of 20 replicates testing positive with the same recombinant VP40 antigen at this concentration.

The tentative LoD for oral fluid collected from living donors with the Σ-Virocult® Transport System was 3.2 ng/test with a VP40 antigen concentration in oral fluid of 3200 ng/mL. This concentration was confirmed as the LoD by 20 out of 20 replicates testing positive with the same recombinant VP40 antigen at this concentration.

Collector	LoD/Test [Amount of rAG per Test]	LoD [Concentration of rAG in Oral Fluid [®]]
Direct Collection OraQuick	0.53 ng/test ^A	7.6 ng/mL
BD Universal Viral Transport for Viruses	0.217 ng/test	465 ng/mL
Σ-Virocult® Transport System	3.20 ng/test	3200 ng/mL

^ARecombinant antigen, live and inactivated virus were tested in parallel with the OraQuick® Ebola Rapid Antigen Test for use with whole blood. With whole blood the LoD of 0.53ng/test corresponded to an LoD of 1.64×10^6 TCID₅₀/mL.

^BLoD concentration in oral fluid was calculated using the average volumes of oral fluid that is absorbed by each of the swabs/devices (i.e., 70 µl for the OraQuick® Ebola Rapid Antigen Test flat pad, 70 µl for the BD swab diluted into 3 mL of VTM and 50µl for the Σ-Virocult® swab diluted in 1 mL VTM) and the subsequent volume of 20µl of the VTM solution that was transferred to the device.

Additional testing has established that the analytical LoD in cadaveric oral fluid and live donor derived oral fluid is equivalent.

CROSS REACTIVITY

Cross reactivity of the OraQuick® Ebola Rapid Antigen Test was evaluated by testing additional viral and bacterial pathogens. In this study, three (3) replicates were tested with the pathogens spiked into Ebola negative oral fluid 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	Inactivated or Live	Type/Strain	Concentration Tested	Reactivity
Herpes Simplex Virus Type 1	Live	ATCC VR-260	2.7×10^6 TCID ₅₀ /mL	None
Herpes Simplex Virus Type 2	Live	ATCC VR-734	1.0×10^6 TCID ₅₀ /mL	None
<i>Actinomyces viscosus</i>	Live	ATCC 43146	2.9×10^6 CFU/mL	None
<i>Candida albicans</i>	Live	ATCC 18804	6.1×10^6 CFU/mL	None
<i>Staphylococcus aureus</i>	Live	ATCC 6538	3.6×10^7 CFU/mL	None
<i>Staphylococcus epidermis</i>	Live	ATCC 12228	3.9×10^6 CFU/mL	None
<i>Streptococcus pyogenes</i>	Live	ATCC 19615	1.4×10^7 CFU/mL	None
<i>Streptococcus salivarius</i>	Live	ATCC 7073	7.7×10^6 CFU/mL	None
<i>Streptococcus mutans</i>	Live	ATCC 25175	2.0×10^6 CFU/mL	None
<i>Lactobacillus johnsonii</i>	Live	ATCC 33200	1.9×10^6 CFU/mL	None
<i>Porphyromonas gingivalis</i>	Live	ATCC 49417	1.7×10^6 CFU/mL	None
<i>Mycobacterium tuberculosis</i>	Live	ATCC 25177	1.0×10^6 CFU/mL	None

Additional cross reactivity with the following organisms was performed for the OraQuick Ebola Rapid antigen test for use with whole blood and no cross reactivity was observed at the tested concentration: Marburg, Crimean Congo Hemorrhagic Fever, Lassa, Rift Valley Fever, Yellow Fever (Vaccine Strain), Chikungunya virus, Influenza A, Influenza B, Rotavirus, Adenovirus, RSV, Enterovirus, Salmonella enterica, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio cholera, Streptococcus pneumonia, Haemophilus influenza (meningitis), Leptospira, Neisseria meningitides, Yersinia enterocolitica, Plasmodium falciparum (malaria), Plasmodium vivax (malaria), Trypanosoma cruzi, Rickettsia africae (protein), Dengue. However, the test reacts with Ebola Sudan and Ebola Bundibugyo. Please refer to the instructions for use for the OraQuick rapid antigen test for whole blood for further information.

INTERFERING SUBSTANCES









The OraQuick® Ebola Rapid Antigen Test was evaluated with the following interfering substances present in negative oral fluid and oral fluid 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 EP17-A2⁴. For Toothpaste, Mucin and Leukocytes testing was completed on three (3) oral fluid samples each tested at n=2 replicates for each condition.

The concentration for toothpaste could not be analytically quantified, but duration of interference use was two (2) minutes and then a thirty (30) minute wait before direct collection with the OraQuick® Ebola Rapid Antigen Test. At the Mucin 20mg/mL test concentration there was one (1) false positive in negative oral fluid test group and (1) false negative in the rAg spiked oral fluid test group. No interference was noted at the 15mg/mL test concentration.

Interfering Substances	Target Testing Concentration	Reactivity
Toothpaste	n/a	None
Mucin	20 mg/mL	Reactive
	15 mg/mL	None
Leukocytes	6.12×10^9 cells / L	None

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EXPLANATION OF SYMBOLS			
 LOT	Batch Code	 IVD	<i>In Vitro</i> Diagnostic Medical Device
 REF	Catalog Number		Manufacturer
	Caution, Consult Accompanying Documents	 PN	Part Number
	Use By		Temperature Limitation

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- 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).