EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR ORAQUICK EBOLA RAPID ANTIGEN TEST DECISION SUMMARY

A. De Novo Number:

DEN190025

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the OraQuick Ebola Rapid Antigen Test.

C. Measurand:

Ebolavirus Antigens

D. Type of Test:

Lateral flow immunoassay

E. Applicant:

OraSure Technology, Inc.

F. Proprietary and Established Names:

OraQuick Ebola Rapid Antigen Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.4002

2. Classification:

   Class II

3. Product code(s):

   QID

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The OraQuick Ebola Rapid Antigen Test is an in vitro diagnostic single-use immunoassay for the qualitative detection of antigens from viruses within the *Ebolavirus* genus but does not differentiate between these viruses. Testing with the OraQuick Ebola Rapid Antigen Test must only be performed when public health authorities have determined the need for this test. Testing for Ebola Virus Disease (EVD) must be performed in accordance with current guidelines provided by the appropriate public health authorities that address appropriate biosafety conditions, interpretation of test results, and coordination of testing, results and patient management with public health authorities. The OraQuick Ebola Rapid Antigen Test is intended for use with specimens from:

   - individuals with epidemiological risk factors with signs and symptoms of EVD or
   - recently deceased individuals with epidemiological risk factors who are suspected to have died of EVD.

   EVD is a nationally notifiable condition and must be reported to public health authorities in accordance with local, state, and federal regulations.

   The OraQuick Ebola Rapid Antigen Test is intended for use with venipuncture whole blood and fingerstick whole blood specimens as an aid in diagnosis of EVD in patients suspected of and with signs or symptoms consistent with EVD who have epidemiological risk factor(s) for *Ebolavirus* exposure (e.g., contact with a known or suspected case, travel to a geographic location at a time when *Ebolavirus* transmission was known or suspected to have occurred). Performance of the device with *Ebolavirus* positive fingerstick whole blood was established in a non-human primate model.

   The OraQuick Ebola Rapid Antigen Test is intended for use with cadaveric oral fluid collected from recently deceased individuals with epidemiological risk factors who are suspected to have died of EVD. Cadaveric oral fluid should be collected directly with the device or collected with oral swabs in viral transport media. The OraQuick Ebola Rapid Antigen Test is intended as an aid in the determination of EVD as the cause of death to inform decisions on safe handling of cadavers to prevent disease transmission.

   The OraQuick Ebola Rapid Antigen Test results are presumptive, definitive identification of EVD requires performing additional testing and confirmation procedures in consultation with public health and/or other authorities to whom reporting is required.

   Negative results were observed in individuals with low levels of circulating virus, therefore negative results do not preclude infection with viruses within the *Ebolavirus* genus.

The level of *Ebolavirus* antigens that would be present in EVD clinical specimens from individuals with early systemic infection is unknown. Test performance of the OraQuick Ebola Rapid Antigen Test is associated with the level of *Ebolavirus* antigens in the patient; therefore, the test is not intended for use in an asymptomatic population for mass-screening purposes (e.g., as the sole means of EVD control at airports or border-crossings) or for testing of individuals at risk of exposure without observable signs of infection.

The OraQuick Ebola Rapid Antigen Test is intended for use by experienced personnel who have documented device specific training offered by OraSure Technologies Inc., training in the correct use of recommended personal protective equipment (PPE) and expertise in infectious disease diagnostic testing, including the safe handling of clinical specimens potentially containing *Ebolavirus*. The test is intended for use by laboratory professionals or healthcare workers who have demonstrated availability of biosafety equipment, access to patient containment facilities, and established procedures (e.g., SOP) for coordinating testing, results and patient management with public health authorities consistent with state, local and federal recommendations and guidelines.

2. <u>Indication(s) for use:</u>

   Same as intended use.

3. <u>Special conditions for use statement(s):</u>

   For prescription use only.

   For *in vitro* diagnostic use only.

4. <u>Special instrument requirements:</u>

   Not applicable.

I. Device Description:

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, fingerstick whole blood and 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 single-use test devices consist of a plastic housing that protects the assay test strip with the reagents. The assay strip can be viewed through the test device result window. The test result window has indicated zones for the *Ebolavirus* specific Test Line and the Control Line of the test. The test consists of a sealed pouch with two separate compartments for each of the following two components:

1. OraQuick Ebola Rapid Antigen Test

2. Developer Vial

The OraQuick Ebola Rapid Antigen Test further contains the following materials for the sample processing:

1. Test Stand
2. Micropipettes capable of pipetting 20ul of sample

The OraQuick Ebola Rapid Antigen Test kit must be used with the OraQuick Ebola Rapid Antigen Test Kit Controls and a Visual Reference Panel (available separately). The OraQuick Ebola Rapid Antigen Test Kit Controls contain:

1. One Ebola positive control vial (orange capped)
2. One Ebola negative control vial (white capped)

The positive control contains 0.25mL of Ebola recombinant VP40 equivalent to a moderate positive result and is diluted in a defibrinated pool of normal human plasma negative for *Ebolavirus*. The negative control contains 0.25 mL of defibrinated pool of normal human plasma negative for *Ebolavirus*. The controls do not contain infectious *Ebolavirus* material.

The OraQuick Ebola Visual Reference Panel (VRP) is intended to assist new operators in becoming proficient at reading specimens with antigen levels near the limit of detection of the device. The devices in the VRP do not contain any infectious material. The VRP contains three Quick Ebola Rapid Antigen Test devices that have been designed to represent reading intensities representative of

1. A test result near the limit of detection (LoD)
2. A low positive test result
3. A negative test result

Additional materials required but not provided include

1. Timer

J. Standard/Guidance Document Referenced (if applicable):

EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

K. Test Principle:

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. *Ebolavirus* 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 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 or calibrated lab pipette both of which transfer 20 µl of blood to the device, followed by the insertion of the device into the developer vial.

Cadaveric oral fluid specimens can be collected in two ways:
1. Swabbing the gum line or the soft pallet tissue in the back of the throat with the flat pad of the device and then insert the device directly into the developer solution, or
2. Swabbing the gum line or the soft pallet tissue in the back of the throat with a recommended swab that will then be inserted into recommended viral transport media The following viral transport systems are recommended and were validated for use with the device: Σ- Virocult VTM Collection (K082472) and BD Universal Viral Transport Medium (K042970). When using recommended viral transport media, 20 µl specimen are collected from the transport media tube using a calibrated lab pipette and transferred to the device through the sample port. The device is then inserted into the developer solution.

For all sample types, 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 red to purple line. If the specimen does not contain *Ebolavirus*, no labeled complexes bind at the Test Zone and no line is observed. The intensity of the line color is not 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 red to purple line will appear at the Control (C) Zone during the performance of all valid tests whether or not the sample is positive or negative for *Ebolavirus* (refer to the Test Result and Interpretation of Test Result section in the package insert). Positive results may be interpreted as soon as red to purple lines are visible at the Test (T) Zone and Control (C) Zone. Negative results must be read 30 minutes after inserting the device into the Developer Vial.

The OraQuick Ebola Rapid Antigen Test must be used with the external OraQuick Ebola Rapid Antigen Test Kit Controls and the Visual Reference Panel (available separately) according to the Instructions for Use.

L. Performance Characteristics (if/when applicable):

Notes:

- The term '*Ebolavirus'* refers to the genus *Ebolavirus*. The term 'Ebola virus' specifically refers to the species Ebola Zaire, one member of the genus *Ebolavirus*. Where this specific reference is not desired the general term *Ebolavirus* is used.
- In the absence of sufficient amounts of cadaveric oral fluid analytical device validation studies were performed with oral fluid from living individuals. Contrived positive samples were derived by adding live or inactivated *Ebolavirus* or recombinant VP40 antigen to oral fluid from living individuals.
- Generally, the term contrived is used in this document for all non-natural samples. There are two ways *Ebolavirus* samples have been contrived for the studies described in this document: (1) negative matrix plus recombinant VP40 antigen and (2) negative matrix plus a live or inactivated *Ebolavirus* preparation. In both instances negative matrix is natural negative clinical matrix sourced from humans. No artificial matrices have been used in this de novo. The way samples were contrived is described for each study individually.

1. Analytical performance:

    a. Precision/Reproducibility:

    A reproducibility study was performed at three (3) testing sites, two external and one internal, with three (3) operators per site. Each of the three (3) operators per site, tested each of three panel members twice daily with each of three lots over the course of 5 days. Panel members derived from recombinant *Ebolavirus* VP40 protein (rVP40-Ag) added into negative matrix at each of three different reactivity levels. i.e., negative, low positive (2x LoD) and moderate positive (5x LoD). Sample testing was performed blinded. Three sub-studies were performed for each of three matrices: Direct Collect Oral Fluid, Viral Transport Media (VTM), and Whole Blood. In the absence of large enough amounts of cadaveric oral fluid, this study was performed with oral fluid from living individuals as a surrogate for cadaveric oral fluid. Panel members for each matrix were formulated based on the respective LoD in the specific matrix. Combined results were analyzed per lot, operator and site. Results across operators, runs, lots and days are shown below per site and overall in **Table 1** (Whole Blood), **Table 2** (direct collected Oral Fluid) and **Table 3** (VTM collected Oral Fluid).

Table 1: Reproducibility of Functional Test Results – Summary Whole Blood

	Negative		Low Positive		Moderate Positive	
	n/N	%CI	n/N	%CI	n/N	%/CI
Site 1	(b) (4)					
Site 2						
Site 3						
Overall Concordance	(b) (4)	99.6% (98.9, 99.9)	(b) (4)	99.9% (99.3, 100.0)	(b) (4)	100.0% (99.5, 100.0)

Table 2: Reproducibility of Functional Test Results – Summary Oral Fluid (Direct Collection)

	Negative		Low Positive		Moderate Positive	
	n/N	%CI	n/N	%CI	n/N	%/CI
Site 1	(b) (4)					
Site 2						
Site 3						
Overall Concordance	(b) (4)	98.1% (97.0, 99.0)	(b) (4)	95.2% (93.5, 96.5)	(b) (4)	99.4% (98.6, 99.8)

Table 3: Reproducibility of Functional Test Results – Summary Oral Fluid (VTM Collection)

	Negative		Low Positive		Moderate Positive	
	n/N	%CI	n/N	%CI	n/N	%/CI
Site 1	(b) (4)					
Site 2						
Site 3						
Overall Concordance	(b) (4)	99.9% (99.3, 100.0)	(b) (4)	99.8% (99.1, 100.0)	(b) (4)	100.0% (99.5, 100.0)

(b) (4)

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

<u>Controls</u>

Traceability: N/A

Expected values:

To monitor the assay performance, reagent performance, and procedural errors, positive and negative external controls must be run in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. External Controls are provided separate from the OraQuick Ebola Rapid Antigen Test Kit devices in the OraQuick Ebola Rapid Antigen Test Kit Control kit. The Ebola positive control consists of recombinant Ebola VP40 diluted into a defibrinated pool of *Ebolavirus* negative normal human plasma. The Ebola negative control vial consists of defibrinated *Ebolavirus* negative normal human plasma. The positive control is expected to give a positive signal, the negative control is expected to provide a negative signal.

Stability:

Storage conditions for the control kit were evaluated in multipl (b) (4) real-time stability studies each of which tested one control kit lot. The main studie ted (b) (4) eplicates per time point for Shelf Life and open vial stability. Open vial stability s assessed based on kits that had undergone (b) (4) month Shelf Life. All visual observations were graded as (b) (4) by comparing each test result to an Ebola Attribute Chart that consists of devices for which the colored Test Lines represent reading scores of 0 (b) (4) The targeted average level for the Test Line of the positive control is (b) (4) but individual test replicates may have Test Lines of (b) (4) The targeted average level for the Test Line of the negative control i (b) (4) he data of each of the fours studies supports an expiration dating of 12 months for closed vials and 8 weeks for open vial when stored at 5°C ± 3°C.

In addition (b) (4) was evaluated as a storage condition. (b) (4) is not a recommended storage temperature due to (b) (4)
(b) (4)

<u>Calibrators</u>

This device does not contain calibrators.

Reagents

Traceability: N/A

Expected values: N/A

Stability:

Shelf Life (Realtime):

Multiple real-time and accelerated stability studies were performed for the OraQuick Ebola Rapid Antigen Test kits. In the main study, multiple lots (b) (4) of the pouched OraQuick Ebola Rapid Antigen Test kits are subjected to real-time stability testing at both (b) (4) (b) (4) . The test panel consisted of the Ebola positive control from the associated control kit, a negative control (*Ebolavirus* negative whole blood) and an additional Panel Member (PM1). Starting at (b) (4) an additional panel member at 4x LoD of recombinant VP40 diluted into whole blood was added. Whole blood as a testing matrix was included in the assessment because it was identified as the limiting factor to correct shelf life assignment. Testing in clinical matrix curtails the risk that shelf life is over assigned. All test panel members were evaluated at each time point in replicates of (b) each per lot. All visual observations were graded as (b) (4) by comparing each test result to an internal Ebola Attribute Chart that consists of devices for which the colored Test Lines represent reading scores of (b) (4)

While assay output (signal strength) decrease at higher temperatures, all (b) lots met the acceptance criteria over the entire course of the study at both 2-8°C and 0°C when tested with the positive control and PM1. With testing of the whole blood panel member at 4x LoD one lot met acceptance criteria up to month 11, one lot up to month 15 and the third lot up to 10 months.

Based on the combined data of three lots upon consideration of the 4x LoD whole blood test panel member the stability assessment for the pouched OraQuick Ebola Rapid Antigen Test kit supports a shelf life of (b) (4) when stored at (b) (4)

Shelf Life (Accelerated):

The OraQuick Ebola Rapid Antigen product had been subjected also to accelerated stability testing performed at (b) (4) Based on the low positive test sample used in the study the accelerated stability study supports a time limited storage at elevated temperatures for up to (b) (4)

Shipping Stability:

One of the lots subjected to reagent stability assessment underwent additional assessment for shipping stability. The shipping stability study evaluated exposure to cycling temperature extremes of frozen (i.e., -18ºC), tropical wet (i.e., 40ºC with 80% Relative Humidity [RH]) and ambient temperature ((b) (4) with (b) (4) RH). The data support shipping at ambient temperature with a transit time of up to 12 days.

Visual Reference Panel

Traceability: N/A

Expected values:

The OraQuick Ebola Visual Reference Panel is intended to assist new operators in becoming proficient at reading specimens with antigen levels near the limit of detection of the device. All new operators must be able to correctly interpret all devices provided within the OraQuick Ebola Visual Reference Panel prior to using the OraQuick Ebola Rapid Antigen Test since the failure to read at low intensities can result in the inability to detect low positive patient samples near the limit of detection of the OraQuick Ebola Rapid Antigen Test and may consequently result in false negative results.

The OraQuick Ebola Visual Reference Panel is comprised of OraQuick Ebola Rapid Antigen Test devices that have been designed to represent reading intensities of limit of detection, low positive, and negative test results.

Stability:

Shelf Life (Realtime):

Shelf life of the Visual Reference Panel (VRP) was assessed using (b) (4) lots of each of the devices contained in the VRP, i.e., Negative (Reading Level 0), LoD (Target Reading Level 1+) and Low Positive (Target Reading Level 3+).
The following conditions were tested in the real time stability study:

- Pouched devices at (b) (4)
- Pouched devices at (b) (4)
- Un-pouched devices at (b) (4)
- Un-pouched devices at (b) (4)
- Un-pouched devices (b) (4)

All three lots of VRP met all stability acceptance criteria for the Test and Control lines up to and including the month 13 time point for all conditions. There was no longitudinal degradation in signal intensity throughout the course of the study. This

study supports a pouched and un-pouched shelf life of 12 months when stored at 15°C to 30°C including normal in-use benchtop conditions during a standard 8-hour work shift.

Shelf Life (Accelerated):

An accelerated stability study was performed in which VRP panel members were tested at (b) (4) for each of the following conditions:

- Pouched
- Un-pouched
- Benchtop condition

In the accelerated stability study open, closed and benchtop conditions all three VRP lots demonstrated no degradation in signal intensity after being subjected to (b) (4) Accelerated stability testing supports temporary temperature deviations during the shelf life.

Sample Stability

Whole Blood Sample Stability:

Individual whole blood samples were collected in K3-EDTA and tested with the OraQuick Ebola Rapid Antigen Test after various storage conditions. Each unique whole blood sample was divided in half. One half was left untreated (control), to the other half a dilution of gamma irradiated Ebola virus (Strain E. Zaire Mayinga, NR-31807) was added that produced a low positive sample at 2.5 x Limit of Detection (LoD). The following test time points were assessed:

- Time 0 (immediate testing)
- (b) C storage testing conducted at hours (b) (4) °C storage testing conducted at hours (b) (4) and at days (b) (4)
- Three (3) freeze/thaw cycles at (b) (4) with testing conducted at 24, 48 and 72 hours; evaluation of the frozen storage and freeze/thaw was included to support the used of clinical whole blood and fingerstick specimens that were a mix of fresh and frozen whole blood samples.

Aliquots were evaluated for correct identification by the OraQuick Ebola Rapid Antigen Test after blinding and randomization. Results are listed below in **Table 4** for refrigerated and incubated storage and in **Table 5** for frozen storage and freeze/thaw cycles.

Table 4: Whole Blood Sample Stability

	% Correctly Identified at Indicated Storage Temperature			
Time (Days)	2-8°C		30°C	
	Low Positive	Negative	Low Positive	Negative
(b) (4)				

All samples were correctly identified throug (b) (4) ours of testing. The whole blood sample stability study supports a sample stab for 24 hours up to 30ºC.

In the initial round of fresh/frozen testing 2 samples were missed after the first freeze/thaw cycle even though aliquots of the same sample were correctly detected after two and three freeze/thaw cycles. An investigation was performed that concluded that samples had likely been tested before fully equilibrating to room temperature. Additional 30 samples were subjected to one freeze thaw cycle only. Generally, samples after a 2 and 3 freeze-thaw cycle exhibited no loss of analyte signal due to the freezing process and the study therefore supports the use of frozen whole blood specimens with up to three freeze/thaw cycles.

Table 5: Whole Blood Freeze/Thaw Sample Stability

Freeze/Thaw Cycles	% Correctly Identified at Indicated Storage Temperature	
	Low Positive	Negative
1	(b) (4)	
2		
3		

Oral Fluid Sample Stability – Direct Collection Method:

For oral fluid samples directly collected with the test device through swabbing the gumline of an individual 10 samples each were tested that were negative (untreated) and low positive at 2.5x LoD. The low positive sample was prepared by spiking a pool of saliva with gamma irradiated virus at 2.5x LoD. Testing was performed by pipetting 70 ul of prepared negative and low positive solution directly onto the flat pad. The devices were then stored as follows to validate immediate and delayed insertion of the devices into the developer vial provided with the kit; samples were read randomized and blinded:

(b) (4)

- Refrigerated (2-8°C): (b) (4)

All negative and low positive samples were correctly identified upon immediate testing (b) (4)
Upon storage of the sample loaded devices at (b) (4) the agreement for the low positive samples was 100% for all timepoints at 30, 60, and 90 minutes. The negative samples yielded expected results with (b) (4) concordance.
Upon storage of the sample loaded devices at 2-8°C one low positive sample each at (b) (4)

This study supports storage delayed insertion of the test device after the device with the oral fluid sample was stored at 2-8°C for up to (b) (4)

Oral Fluid Sample Stability – Viral Transport Media (VTM) Collection:

(b) (4)

(b) (4)

This study supports sample stability for oral fluid in both the BD VTM and the Σ-Virocult VTM for up to 48 hours from -70°C to 40°C including three freeze/thaw cycles.

d. Detection limit:

Multiple studies were conducted to establish the limit of detection (LoD) of the OraQuick Ebola Rapid Antigen Test using gamma irradiated Ebola Zaire virus and recombinant VP40 Antigen. Considering feasibility in the context of a (b) (4) analyte the testing procedure had to be slightly modified for some of the analytical studies; however, each analytical and clinical study that relied on contrived samples is supported by an LoD study specific for the material and procedure used.

Inactivation: To support the use of inactivated material in the LoD and other analytical studies parallel testing of live and inactivated Ebola Zaire material was performed as follows: Ebola Zaire virus stock (strain Mayinga) was diluted in PBS at a concentration of (b) (4) /mL and then diluted down to (b) (4) dilution series. Dilutions were aliquoted and frozen. For each of the dilutions inactivation was performed on one of the frozen aliquots using gamma irradiation at 5 x 10^6 RAD. Aliquots of each dilution of live Ebola Zaire virus and inactivated Ebola Zaire virus were tested with the OraQuick Ebola Rapid Antigen Test according to Package Insert instructions and were also subjected to nucleic acid extraction and VP40-PCR testing.

Results as summarized in **Table 6** below demonstrate that the reading score of the OraQuick Ebola Rapid Antigen Test is not impacted by the inactivation process as no difference between live and inactivated Ebola Zaire virus material was observed.

Table 6: PCR and OraQuick Ebola Antigen Test results for parallel testing of live and inactivated Ebola Zaire Virus

Dilution of virus	Replicate	Live		Inactive	
		OraQuick Visual Read	Ct value	OraQuick Visual Read	Ct value
(b) (4)					

Limit of Detection (LoD) in Whole Blood: To determine a tentative LoD, serial dilutions of inactivated Ebola Zaire virus (strain Mayinga) were made in a pool of *Ebolavirus* negative venous whole blood. Each dilution was tested in 10 or more replicates. The dilutions were randomized with *Ebolavirus* negative whole blood and blinded. Three operators each tested a subset of the randomized and blinded samples using the OraQuick Ebola Rapid Antigen Test. A Probit analysis was then used to determine a tentative LoD, defined as the concentration calculated to yield positive results 95% of the time.

To confirm the tentative LoD, a panel of 20 samples at the tentative LoD (1.2×10^6 $TCID_{50}$/mL), 20 negative samples, and 20 moderate positive samples (7.81×10^7 $TCID_{50}$/mL) were prepared in negative venous whole blood, randomized and tested by three blinded operators. **Table 7** summarizes the number of positive (LoD and Moderate Positive test devices) and negative results obtained for the inactivated virus positive and negative whole blood samples.

Table 7: Summary of the confirmatory LoD study for the OraQuick Ebola Rapid Antigen Test with Whole Blood and inactivated Ebola Zaire virus (Mayinga)

Whole Dilution	Concentration [$TCID_{50}$/mL]	Total Number of Tests	Ct Value of inactivated viral stock at indicated concentration	Number of Positive Results	Number of Negative Results
LoD	1.64×10^6	20	(b) (4)	19	1
(b) (4)					

The same procedure (i.e., initial range finding study followed by a confirmatory study with 20 replicates) was followed for determining an LoD with recombinant VP40 antigen and a second Ebola Zaire virus stock from BEI Resources (NR-31807) in support of analytical studies that used these additional materials. **Table 8** below provides a summary of the LoD of the OraQuick Ebola Rapid Antigen Test with the different materials used in the analytical and contrived clinical studies.

Table 8: LoD of the OraQuick Ebola Rapid Antigen Test for Whole Blood when used with specified test materials.

	Ebola Zaire (Mayinga) [$TCID_{50}$/mL whole blood][1]	rVP40-Ag [ng/mL whole blood] [1]	E. Zaire Mayinga (NR-31807)] [Dilution] [1]
LoD	1.64 x 10^6	53ng/mL	(b) (4)

[1] LoD when 20 µl of this dilution in whole blood is applied to the sample port of the test device.

Limit of Detection (LoD) in Oral Fluid: A similar study, designed as the whole blood LoD study (above), was performed; i.e., an initial range finding study with at least 10 replicates per dilution was followed by a confirmatory LoD study that tested 20 replicates each of samples that were negative, moderate positive and concentrated at the tentative LoD. In support of the analytical studies that used contrived samples the LoD was established for inactivated virus as well as for recombinant VP40 antigen.

Both collection methods, i.e., (1) direct collection of oral fluid and (2) collection of oral fluid with a flocked swab/VTM (see section *K. Test Principle*) were validated for their respective LoD. For the flocked swab/VTM method two swab/VTM systems were validated, Σ- Virocult VTM and the BD Universal Transport Medium.

Oral fluid with each of the methods was obtained from 5 or more individuals using either the flat pad of the OraQuick device or the flocked swab of the respective VTM systems. After collection, 5µL of the inactivated virus solutions or of the recombinant VP40 antigen (rVP40-Ag) solutions were pipetted on either the collection pad (for the direct collection method) or onto the flocked swab (for the VTM methods). Negative samples were collected the same way but 5µl of *Ebolavirus* negative diluent was added. Collection and spiking were performed by an unblinded operator per a randomization schedule. Each of three blinded operators then tested and read a subset of the devices 30 minutes after placement of the device in the developer solution.

Results of the confirmatory study are summarized in **Table 9** below for inactivated Ebola Zaire Mayinga and in **Table 10** for rVP40-Ag. Note that the Ebola Zaire Mayinga (BEI Resources, NR-31807) used in the oral fluid studies was a different viral stock from the one used for the whole blood LoD study above; the BEI material was not provided with a titered concentration prior to inactivation and as such no concentration can be listed for the dilutions.

Table 9: Summary of the confirmatory LoD study for the OraQuick Ebola Rapid Antigen Test with Oral Fluid using gamma-irradiated E. Zaire Mayinga (BEI, NR-31807)

	Sample	Dilution of Viral Stock NR-31807	Total Number of Tests	Number of Positive Results	Number of Negative Results
	(b) (4)				

	(b) (4)
Direct Collection OraQuick	
Σ- Virocult VTM Collections	
BD Universal Transport Medium	

LR: Low positive based on range finding study
MR: Moderate positive based on range finding study
N/A: Not applicable; no inactivated virus was added onto the devices.

Table 10: Summary of the confirmatory LoD study for the OraQuick Ebola Rapid Antigen Test with Oral Fluid and rVP40-Ag

	Sample	**Concentration of added rVP40-Ag Solution (µg/mL)**	**Total Number of Tests**	**Number of Positive Results**	**Number of Negative Results**
Direct Collection OraQuick	LoD	(b) (4)	20	19	1
	Moderate		20	20	0
	Negative		20	0	20
Σ- Virocult VTM Collections	LoD		20	20	0
	Moderate		20	19	1
	Negative		20	0	20
BD Universal Transport Medium	LoD		20	20	0
	Moderate		20	20	0
	Negative		20	0	20

Table 11 below provides an overview over the oral fluid LoD obtained with all different oral fluid sample collection/processing methods and Ebola virus materials used in the analytical and contrived clinical studies and/or claimed per the intended use of the device.

Table 11: LoD of the OraQuick Ebola Rapid Antigen Test for Oral Fluid when used with different collection methods and test materials.

Collector	LoD [Amount of rAg per Test] [1]	LoD [Concentration of rVP40-Ag in Oral Fluid[1]]	LoD [Dilution of gamma-irradiated E. Zaire Mayinga (b) (4)
Direct Collection OraQuick	0.53 ng/test	7.6 ng/mL	(b) (4)
Σ-Virocult Transport System	3.20 ng/test	3200 ng/mL	
BD Universal Viral Transport Medium	0.22 ng/test	465 ng/mL	

[1] LOD concentration in oral fluid was calculated using the average volumes of oral fluid that are absorbed by each of the swabs/devices and the volume of VTM (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 50 µl 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.

[2] LoD when 5 µl of this dilution is applied to the swab or directly to the flatpad of the OraQuick test device.

e. *Analytical specificity:*

Reactivity (Inclusivity)

Analytical reactivity of the OraQuick Ebola Rapid Antigen Test was evaluated for additional strains of the *Ebolavirus* (**Table 12**). 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. All *Ebolavirus* strains were provided as inactivated viruses. Each viral strain was tested in three (3) replicates when added at a 1:2 and/or 1:10 dilution in whole blood at the concentrations listed in **Table 12** below.

The OraQuick Ebola Rapid Antigen Test is positive with Ebola Sudan (strains Boneface [1976] and Gulu [2000]) and with Ebola Bundibugyo (strain Uganda [2007]) but is negative with Ebola Ivory Coast [1994] and Ebola Reston [1989/90].

Table 12: Inclusivity with inactivated virus of different *Ebolavirus* species

Ebolavirus Strain	Concentration Tested	Positive Replicates	Reactivity (Positive (P)/ Negative (N))
Ebola Zaire Mayinga ZZXDK901/812094/VSP	1.5×10^6 $TCID_{50}$/mL	Positive as per LoD study	
Taï Forest (Ivory Coast, 11/27/94)	Unknown (1:10 dilution)	0/3	N
	Unknown (1:2 dilution)	0/3	N
Taï Forest (Ivory Coast)	1.36×10^7 VP/mL[1]	0/3	N
	7.5×10^7 VP/mL[1]		
Reston (119876 Pennsylvania)	3.16×10^6 pfu/mL	0/3	N
	1.58×10^7 pfu/mL	0/3	N
Reston (aka H28)	5.83 x 105 PFU/mL	0/3	N
Sudan (Boneface)	1×10^5 pfu/mL	0/3	N
	5×10^5 pfu/mL	3/3	P
Sudan (200011676 Gulu)	5.6×10^4 pfu/mL	2/3	P
	2.8×10^5 pfu/mL	3/3	P
Sudan (200011676 Gulu)	3.25×10^5 PFU/mL[1]	0/3	N
	5.95×10^5 PFU/mL [1]	0/3	N
	1.19×10^6 PFU/mL [1]	3/3	P
	1.79×10^6 PFU/mL [1]	3/4	P
Bundibugyo (200706291 Uganda prototype)	3.98×10^4 pfu/mL	2/3	P
	1.99×10^5 pfu/mL	3/3	P
Bundibugyo (Uganda)	3.73×10^4 PFU/mL [1]	0/3	N
	6.83×10^4 PFU/mL [1]	1/3	N
	1.37×10^5 PFU/mL [1]	3/3	P
	2.05×10^5 PFU/mL [1]	3/3	P
[1] preparation of the same strain from a different source			

Cross reactivity:

Cross reactivity of the OraQuick Ebola Rapid Antigen Test was initially evaluated by testing additional viral, bacterial, and parasitic pathogens according to the same protocol and study design that was outlined under reactivity (above). Testing was performed with live organisms unless otherwise indicated. In the study, three (3) replicates were tested with the pathogens when added to venous whole blood at the concentrations listed in **Table 13** below. None of the tested organisms produced false positive results in the OraQuick Ebola Rapid Antigen Test at the concentration tested. Potential cross reactivity with Dengue Virus was not assessed. HIV-2 cross reactivity was evaluated with HIV-2 positive samples from the Ivory Coast.

Table 13: Cross reactivity

Virus/Bacteria/Parasite	Type/Strain	Concentration Tested	Reactivity
Marburg[1]	RAVN	Log 5.53 $TCID_{50}$/mL	None
	Musoke	3.16 x10^6 PFU /mL	None
		3.73 x 10^6 PFU/mL	None
	200501379 Angola prototype	1.44 x 10^6 PFU/mL	None
	VOEGE	3.6 x10^6 PFU /mL	None
Crimean Congo Hemorrhagic Fever[1]	OMAN199809166 #811466	5.6 x10^4 $TCID_{50}$/mL	None
Lassa[1]	Josiah	3.16 x 10^5 PFU /mL	None
		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[1]	ZH-501	3 x10^6 PFU/mL	None
		5.43 x 10^6 PFU/mL	None
Yellow Fever[1]	Vaccine Strain #806588	Unknown	None
	Asibi	1.88 x 10^6 PFU/mL	None
Chikungunya virus	ATCC VR-64	3.0 x 10^8 LD_{50}/mL	None
Dengue virus	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 34150	2.12 x 10^5 PFU/mL	None
Influenza A	A/Wisconsin/10/1998	2.3 x 10^5 $TCID_{50}$/mL	None
Influenza B	B/Florida/04/06	4.6 x 10^5 $TCID_{50}$/mL	None
Rotavirus	ATCC VR-899	6.4 x 10^6 $TCID_{50}$/mL	None
Adenovirus	Type 5 ATCC VR-5	2.0 x 10^5 $TCID_{50}$/mL	None
RSV	ATCC VR-26	9.0 x 10^5 $TCID_{50}$/mL	None
Enterovirus	Enterovirus 71 ATCC VR-1432	5.1 x 10^5 $TCID_{50}$/mL	None
Salmonella	*S. enterica* ATCC 10708	3.8 x 10^7 CFU/mL	None
Salmonella typhi	*S. typhi* ATCC 6539	5.4 x 10^5 CFU/mL	None
Shigella	*S. dysenteriae* ATCC 9361	4.0 x 10^6 CFU/mL	None
Pseudomonas aeruginosa	*P. aeruginosa* ATCC 15442	3.4 x 10^8 CFU/mL	None
Vibrio Cholera	*V. cholera* ATCC 39050	6.7 x 10^5 CFU/mL	None
Streptococcus pneumonia	*S. pneumonia* ATCC 6303	2.1 x 10^8 CFU/mL	None
Hemophilus influenza (meningitis)	*H. influenzae* ATCC 33930	3.0 x 10^7 CFU/mL	None
Leptospira genus	*L. biflexa* ATCC 23582	~ 9.1 x 10^4 CFU/mL	None
Neisseria meningitides	*N. meningitides* ATCC 13090	3.5 x 10^6 CFU/mL	None
Yersinia enterocolitica	*Y. enterocolitica* ATCC 23715	7.0 x 10^6 CFU/mL	None
Plasmodium falciparum (malaria)	*P. falciparum* ATCC 30932	0.26% parasitemia	None

Virus/Bacteria/Parasite	Type/Strain	Concentration Tested	Reactivity
Plasmodium viva (malaria)	*P. vivax* ATCC 30151	6.8 x 10^5 cells/mL	None
Trypanosoma	*T. cruzi* ATCC 30013	3.1 x 10^7 CFU/mL	None
Rickettsia	R. africae (protein) BEI NR-42992	11.6 mg/mL	None
Bacteroides fragilis	VPI 2553 [EN-2; NCTC 9343] ATCC 25285	1.0 x 10^8 CFU/mL	None
Klebsiella pneumoniae	[CIP 104216, NCIB 10341] ATCC 4352	5.8 x 10^7 CFU/mL	None
Enterococcus faecium	NCTC 7171 [DSM 20477, JCM 8727, NCDO 942] ATCC 19434	7.6 x 10^7 CFU/mL	None
Escherichia coli	AMC 198 ATCC 11229	4.5 x 10^7 CFU/mL	None
Vesicular Stomatitis Virus	Rhabdovirus Indiana Lab [V-520-001-522] ATCC VR-158	7.9 x 10^6 $TCID_{50}$/mL	None
Human Immunodeficiency Virus-1	Clinical Sample ZeptoMetrix 022117B1D	5.1 x 10^6 $TCID_{50}$/mL	None
Hepatitis A Virus	Clinical Sample University of Ottawa 021916B1C-1D	4.5 x 10^7 $TCID_{50}$/mL	None
Cytomegalovirus	Herpesviridae AD-169 ATCC VR-1788	2.9 x 10^5 $TCID_{50}$/mL	None
Epstein-Barr Virus	Herpesviridae, Lymphocryptovirus P-3 ATCC VR-602	1.44 x 10^5 copies/mL[2]	None
Hepatitis B Virus	Clinical Sample DLS13-01459	1.2 x 10^7 IU/mL	None
West Nile Virus	Flaviviridae, Flavivirus B 956 [V-554-001-522] ATCC VR-1267	2. 3 x 10^8 $TCID_{50}$/mL	None
Hepatitis C Virus	Clinical Sample DLS14-08008	4.7 x 10^6 IU/mL	None
Mumps Virus	Paramyxoviridae, Rubulavirus Jones ATCC VR-1438	7.1 x 10^5 $TCID_{50}$/mL	None
Measles	Paramyxoviridae, Morbillivirus Edmonston ATCC VR-24	1.5 x 10^6 $TCID_{50}$/mL	None
Rubella	Togaviridae, Rubivirus M33 ATCC VR-315	1.5 x 10^5 $TCID_{50}$/mL	None
Streptococcus pneumoniae	Slovakia 14-10 [29055] ATCC 700677	4.6 x 10^4 CFU/mL	None

Virus/Bacteria/Parasite	Type/Strain	Concentration Tested	Reactivity
Borrelia hermsii[3]	HS1 Serotype 26 ATCC BAA-2821 Lot 6020	1:11 Dilution of purchased unquantified culture	None
Yersinia pseudotuberculosis[3]	IP2666 BEI NR-4371 Lot 57852108	1:11 Dilution of purchased unquantified culture	None
Rickettsia australis[3]	JC BEI NR-10454	8.1 x 10^5 $TCID_{50}$/mL	None

[1] = Organisms and strains marked with an asterisk were tested inactivated
[2] = determined by digital droplet PCR in plasma
[3] = Due to the unavailability of Yersinia pestis, Borrelia recurrentis, Rickettsia prowazekii, and Rickettsia typhi, surrogate organisms Borrelia hermsii, Yersinia pseudotuberculosis, and Rickettsia australis were utilized.

Interference:

Exogenous and endogenous interfering substances were evaluated for interference by adding the potential interfering substances to *Ebolavirus* negative matrix and to rVP40 antigen containing matrix (positive). Recombinant VP40 antigen was spiked at 2 x LoD; some substances were repeated at 4 x LoD (see footnotes in the tables below).

Endogenous interferents testing for Bilirubin, Hemoglobin, Protein and HAMA was completed in venous whole blood. Three whole blood samples were each tested in two replicates; for Cholesterol 4 whole blood samples were each tested in 3 replicates; for HAMA; Rheumatoid Factor (RF) testing was performed on 3 serum samples, that were each tested in two dilutions with 2 replicates. Dilutions for RF were made in serum. The concentrations of RF that were tested ranged from 525 IU/mL to 11,900 IU/mL.

For testing oral fluid specific potential interferents, mucin and leukocytes were added into *Ebolavirus* negative and rVP40 antigen containing positive expectorated oral fluid from living individuals. Additionally, subjects used toothpaste prior to direct oral fluid collections with the test device.

Results of the interference testing are summarized in **Table 14** and **Table 15** below for endogenous (in venous whole blood and oral fluid as indicated) and exogenous (tested in venous whole blood only) interfering substances, respectively.

Table 14: Testing of potentially interfering endogenous substances specific to whole blood and oral fluid

Interfering Substance	Target Testing Concentration	n	rVP40 Ag Negative Samples		rVP40-Ag Positive Samples	
			Positive	Negative	Positive	Negative
whole blood-specific potential interferents						
Bilirubin	25 mg/dL	6	0	6 (100%)	6 (100%)	0
Hemoglobin	20 g/dL	6	0	6 (100%)	6 (100%)	0
Protein	5 g/dL	6	0	6 (100%)	6 (100%)	0
HAMA	2464 ng/mL	6	0	6 (100%)	6 (100%)	0
Rheumatoid Factor (KB82487)[2]	11,900 IU/mL	6	6 (100%)	0	6 (100%)	0
	2000 IU/mL	6	5 (83%)	1 (17%)	6 (100%)	0
Rheumatoid Factor	2920 IU/mL	2	2 (100%)	0	2 (100%)	0
	1460 IU/mL	4	4 (100%)	0	4 (100%)	0
	1050 IU/mL	2	0	2 (100%)	2 (100%)	0
Cholesterol	13 mmol/L	12	0	12 (100%)	12 (100%)	0
Antinuclear Antibodies	1:160 titer	12	0	12 (100%)	11 (91.7%)	1 (8.3%)[1]
	1:120 titer	3			3 (100%)	0
	1:160 titer at 4.0 x LoD	12			12 (100%)	0
oral fluid-specific potential interferents						
Toothpaste	N/A	(b) (4)				
Mucin	20 mg/mL					
	15 mg/mL					
Leukocytes	6.12×10^9 cells / L					

[1] Considering the technology (i.e., manually performed and visually read test), the total number of failed replicates in the entirety of this study is consistent with the statistical failure rate of a low positive sample near the LoD. Therefore, those interfering substances that produced occasional false negative results in the low positive rVP40-Ag containing samples (2x LoD) were repeated in the presence of slightly higher rVP40-Ag concentration (4x LoD) to distinguish random statistical distribution of measurements on a low positive sample from true interference of the test substance.

(b) (4) was a RF positive plasma and testing was performed in plasma not in whole blood

Interference (i.e., false positive results) was observed when testing RF and high concentrations of Mucin (>15mg/mL). Titration indicated that this effect disappeared when concentrations fell to 1050 IU/mL for RF and to 15 mg/mL for mucin. This observation is noted in a limitation described in the package insert.

Table 15: Testing of potentially interfering exogenous substances (commonly used drugs) in whole blood

Interfering Substance	Target Testing Concentration	n	rVP40-Ag Negative Samples		rVP40-Ag Positive Samples	
			Positive	Negative	Positive	Negative
Acetylsalicylic Acid	3.62 mmol/L	12	0	12 (100%)	12 (100%)	0
Salicylic Acid	4.34 mmol/L	12	0	12 (100%)	12 (100%)	0
Sulfamethoxazole	1.58 mmol/L	12	0	12 (100%)	12 (100%)	0
Ribavirin	11.04 µg/mL	12	0	12 (100%)	11 (91.7%)	1 (8.3%)[1]
	5.52 µg/mL	3	NT	NT	3 (100%)	0
	11.04 µg/mL at 4.0 x LoD	12	NT	NT	12 (100%)	0
Emtricitabine	7.5 µg/mL	12	0	12 (100%)	11 (91.7%)	1 (8.3%)[1]
	3.75 µg/mL	3	NT	NT	3 (100%)	0
	7.5 µg/mL at 4.0 x LoD	12	NT	NT	12 (100%)	0
Efavirenz	16.6 µg/mL	12	0	12 (100%)	12 (100%)	0
Ritonavir	33.6 µg/mL	12	0	12 (100%)	10 (83.3%)	2 (16.7%)[1]
	16.8 µg/mL	6	NT	NT	6 (100%)	0
	33.6 µg/mL at 4.0 x LoD	12	NT	NT	12 (100%)	0
Elvitegravir	5.61 µg/mL	12	0	12 (100%)	12 (100%)	0
Chloroquine	17.95 µg/mL	12	0	12 (100%)	12 (100%)	0
Atovaquone / Proguanil	89.1/2.8 µg/mL	12	0	12 (100%)	12 (100%)	0
Ibuprofen	2425 µmol/L	12	0	12 (100%)	12 (100%)	0
Acetaminophen	1324 µmol/L	12	0	12 (100%)	12 (100%)	0

Interfering Substance	Target Testing Concentration	n	rVP40-Ag Negative Samples		rVP40-Ag Positive Samples	
			Positive	Negative	Positive	Negative
Quinine	148 µmol/L	12	0	12 (100%)	11 (91.7%)	1 (8.3%)[1]
	74 µmol/L or 24 µg/mL	3	NT	NT	3 (100%)	0
	148 µmol/L at 4.0 x LoD	12	NT	NT	12 (100%)	0
Rifampin	78.1 µmol/L	12	0	12 (100%)	12 (100%)	0
Amoxicillin	206 µmol/L	12	0	12 (100%)	12 (100%)	0
Tetracycline	34 µmol/L	12	0	12 (100%)	12 (100%)	0
Erythromycin	81.6 µmol/L	12	0	12 (100%)	12 (100%)	0
Biotin	3.6 µg/mL	12	0	12 (100%)	12 (100%)	0

[1] Considering the technology (i.e., manually performed and visually read test), the total number of failed replicates in this study is consistent with the statistical failure rate of a low positive sample near the LoD. Therefore, those interfering substances that produced occasional false negative results in the low positive rVP40-Ag containing samples (2x LoD) were repeated in the presence of slightly higher rVP40-Ag concentration (4x LoD) to distinguish random statistical distribution of measurements on a low positive sample from true interference of the test substance.

Occasional false negative results in the presence of rVP40-Ag were observed for some endogenous interferents (i.e., mucin and ANA) and some drugs (i.e., Ribavirin, Emtricitabine, Ritonavir and Quinine). However, considering the low spiking concentration of 2x LoD, the total number of rVP40 positive samples included in the study and that this test is a manually performed and visually read test, this observation was likely due to the random distribution of a very low positive sample. Therefore, testing was repeated at a slightly higher but still low positive concentration (i.e., 4x LoD). The additional testing confirmed that the observation of occasional false negative results with a very low positive sample were likely due to random statistical distribution of a low positive sample and not true interference of the substance.

f. Sensitivity with the WHO Reference Reagent:

There is currently no International Standard for *Ebolavirus* Antigens available. However, multiple candidate EBOV recombinant protein preparations were evaluated to serve as WHO International Reference Reagents (IRR) for monitoring the performance of *Ebolavirus* Antigen based tests to be used for presumptive diagnosis of EVD. This study used the 1st Reference Reagent 2016, lyophilized Ebola VP40 Antigen; Item# 2016.2302 (refer to Wilkinson et al. 2016, WHO collaborative study to assess the suitability of a WHO International Reference Panel for Ebola virus VP40 antigen; WHO/BS/2016.2302), obtained from the National Institute for Biological Standards and Control (NIBSC). Only '*Sample 3*' of the WHO Reference Reagent was used; it is a low positive sample based on freeze-dried recombinant VP40 (full-length) expressed in *E.coli*. The VP40 sequence is based on a 2014 isolate of Ebola Zaire (i.e., Makona-Kissidougou-C15; GenBank KJ660346).

This study was designed to test the sensitivity of the OraQuick Ebola Rapid Antigen Test with the Ebola Reference Reagent described above. Only 5 replicates per dilution were tested because of the limiting amount of material available. The study was performed with one lot of the OraQuick Ebola Rapid Antigen Test and one lot of the OraQuick Ebola Rapid Antigen Test Kit Controls. A total of three vials of the reference material were combined and diluted with the following dilution factors: 1:3, 1:6, 1:9, 1:12, 1:24 and 1:48. Dilutions were performed using the most challenging matrix, i.e., venous whole blood and dilutions and replicates were randomized for testing. Each dilution was tested in five replicates. Negative venous whole blood was tested in three replicates as a negative control. Results were graded visually as 0 to 5+ or >+5 by comparing each test result to an internal Ebola Attribute Chart that consists of devices for which the colored Test Lines represent reading scores of 0 to >+5. Results are summarized in **Table 16** below.

Table 16: Testing of the WHO Reference Reagent

Dilution	Test Replicate					Final Result
	1	2	3	4	5	
1:3	(b) (4)					
1:6						
1:9						
1:12						
1:24						
1:48						
P: Positive; LP: Low Positive; N: Negative						

The reconstituted Ebola rVP40-Ag resulted in high positive visual grades (≥ 4+) when tested undiluted with the OraQuick Ebola Rapid Antigen Test. The visual grade intensity decreased to low positive (≤ 3+) or negative when diluted 1:6 in whole blood.

g. High Dose Hook Effect study

Performance of the OraQuick Ebola Rapid Antigen Test was evaluated utilizing high concentrations of Ebola specific rVP40-Ag. A single operator utilizing a single lot of reagents tested samples corresponding to rVP40-Ag concentrations of (b) (4) µg/mL. Line intensity increased from 2+ at (b) µg/mL to 5+ at (b) µg/mL and remained at 5+ at the higher testing concentrations. There was no visible decline in band intensity when going from (b) (4) µg/mL analyte concentrations.

h. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

Clinical performance as outlined below was assessed in multiple studies using samples and results from routine testing performed as part of the response to the 2014 Ebola Zaire outbreak in West Africa. All natural clinical study samples were collected from patients of the intended use population, i.e., patients with signs and symptoms of EVD with epidemiological risk factors or individuals who recently died and were suspected to have died of EVD with epidemiological risk factors. Studies were performed according to the instructions for use of the OraQuick Ebola Rapid Antigen Test that was under emergency use authorization (EUA) at that time. Data collection for the West African studies was not based on a pre-defined study protocol developed by Orasure Technologies but was instead collected as part of the routine testing. Demographic study information was not available for these studies and patients and samples were not individually identifiable.

Studies performed by OraSure, such as the U.S. based specificity studies were IRB approved and samples collected with informed consent where required. Study specific enrollment criteria and demographics for the specificity studies in a U.S. based population are provided for each of the studies in their respective sections below.

Samples were tested, and results were read per instructions for use; i.e., positive sample results were read as soon as a signal appeared, negative sample results were read at 30 minutes (unless otherwise noted) and within a 3 minutes maximal time window. Clinical

studies were performed upon use of the control kit and visual reference panel (VRP) as follows:

External Controls

During the conduct of the clinical trial protocols, external control testing was performed at the following time points:

- Each new operator;
- Each new lot of test kits;
- Each new shipment of test kits;
- Test kit storage temperature was outside the range of 2°C - 40°C or 36°F - 104°F; and
- Testing area temperature was outside the range of 15°C - 40°C or 59°F - 104°F
- For the new de novo specificity cohort controls were also run when testing was repeated for invalid results.

Visual Reference Panels

Clinical sites and participating organizations were provided with visual reference panels to assist new operators in becoming proficient at reading specimens with antigen levels near the limit of detection of the device.

The following table provides a summary of all studies that support clinical performance:

Table 17: Summary clinical studies

	Sensitivity			Specificity		
	Venous Whole Blood	**Finger stick Blood**	**Cadaveric Oral Fluid**	**Venous Whole Blood**	**Finger stick Blood**	**Cadaveric Oral Fluid**
Endemic	25	NHP:10	35 (VTM)	50		193 (VTM; West Africa) 50 (Direct Collect; Sierra Leone) 97 (Direct Collect; Liberia)
Non-endemic				226	249	126 (VTM)
Contrived	25	N/A	20	N/A	N/A	N/A
Total	**50**	**10**	**55**	**276**	**249**	**463**

a. Clinical sensitivity:

VENOUS WHOLE BLOOD

Performance of the OraQuick Ebola Rapid Antigen Test with retrospective venous whole blood samples collected during the West Africa Outbreak 2014/15

A total of 75 retrospective remnant venous whole blood samples from routine *Ebolavirus* testing collected from patients in West Africa (Sierra Leone) during the 2014-2015 Ebola Zaire virus outbreak were tested with the OraQuick Ebola Rapid Antigen Test. Samples were characterized as positive and negative using an Emergency Use authorized Ebola virus VP40 Real-time RT-PCR Assay. Samples were stored frozen at ≤ -70°C. 25 Ebola positive samples were selected for testing with the OraQuick Ebola Rapid Antigen Test based on the Ct value of the comparator so that samples from low to high Ct values indicative of the full spectrum of Ebola viral loads were represented. 50 Ebola negative samples were randomly selected. All samples were randomized and tested blinded by three operators each of whom tested a subset of the samples. No replicate testing was performed. Twenty two out of the 50 negative samples were tested with deviations from the instructions for use (i.e., at 20 minutes) but were retained in the study cohort because they were known to be negative with a more sensitive and equally specific test (i.e., PCR).

Table 18 below summarizes the results of all 25 Ebola positive and 50 Ebola negative samples tested with the OraQuick Ebola Rapid Antigen Test in comparison to the results generated by Ebola Virus VP40 Real-time RT-PCR Assay (Comparator). Error! Reference source not found. shows the positive percent agreements (PPA) and negative percent agreement (NPA) across the entire study cohort (Ct 15 to 34 for PPA, and Ct >40 for NPA) as well as stratified by Ct values of the comparator (indicative of viral loads below and above the LoD of the OraQuick Ebola Rapid Antigen Test).

Table 18: Percent agreement against the RT-PCR comparator – overall and stratified by Ct

	PCR Ct Ranges	Percent Agreement	95% CI[1]
PPA	15 – 23	100% (16/16)	79.41% - 100.0%
	24 – 34[2]	55.6% (5/9)	21.20% - 86.30%
	15 – 34 (overall)	84.0% (21/25)	63.92% - 95.46%
NPA	>40	98.0% (49/50)[3]	89.35% - 99.95%

[1] Calculated using Clopper-Pearson exact method

[2] Cts in this range are representative for Ebola virus concentrations around and below the LoD of the OraQuick Ebola Rapid Antigen Test.

[3] This false positive sample tested repeat reactive (positive) in a re-test.

Figure 1 below summarizes the positive and negative results of all 25 Ebola virus positive samples tested with the OraQuick Ebola Rapid Antigen Test dependent on the Ct value of the PCR. Note that not every Ct value has a sample which was tested. Ct values for which no sample was tested are marked as N.T.

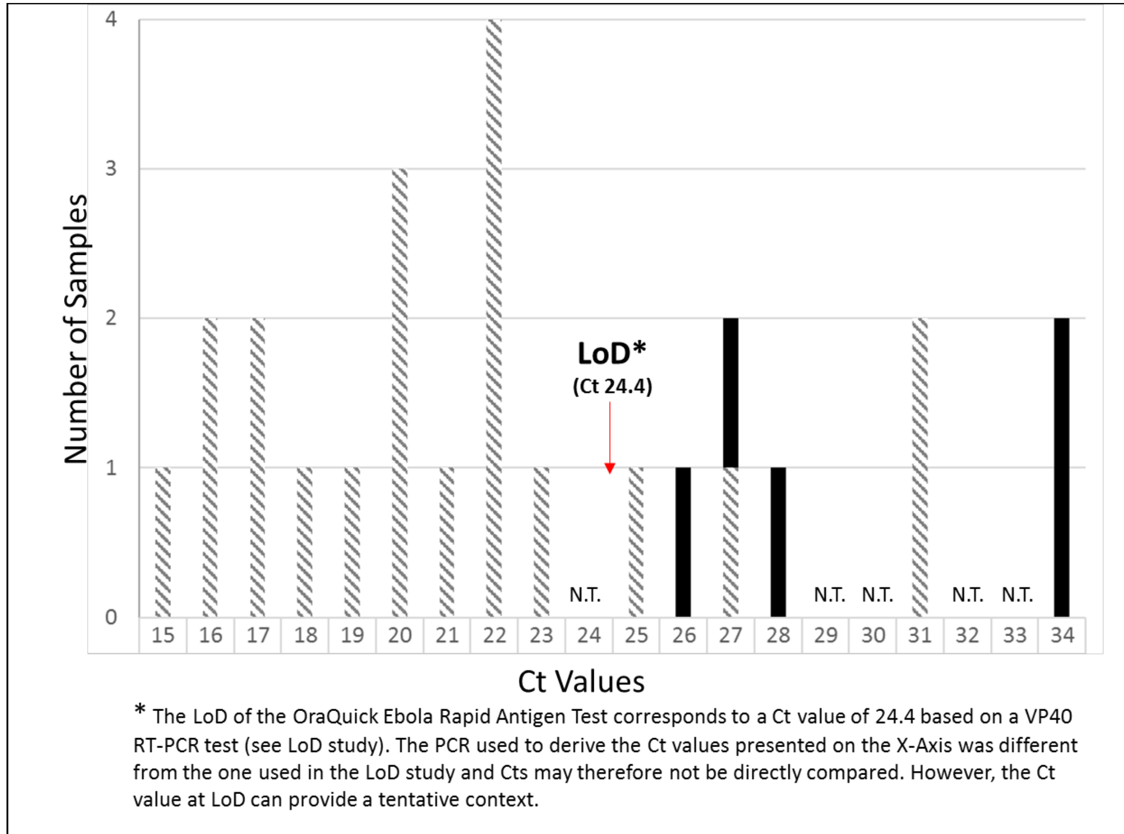


Figure 1: Results of the OraQuick Ebola Rapid Antigen Test with RT-PCR positive samples by Ct value; N.T.=Not Tested; hashed bars: samples returning a positive result with the OraQuick Ebola Rapid Ag Test; filled black bars: samples returning a negative results with the OraQuick Ebola Rapid Ag Test.

This study demonstrates that as viral load increases (i.e., low Ct ranges up to 23) the test PPA approaches 100%. The study also demonstrates that samples with lower viral loads (i.e., as indicated by Ct values at or above 24) as may be encountered early during the course of Ebola virus disease, there is a risk of false negative results.

This study supports the use of the OraQuick Ebola Rapid Antigen Test with venous whole blood from symptomatic individuals with epidemiological risk factors.

Sensitivity of the OraQuick Ebola Rapid Antigen Test with positive contrived venous whole blood samples

The objective of this study was to perform additional validation of venous whole blood samples when contrived with low concentration of Ebola virus (i.e., concentration around the LoD of the OraQuick Ebola Rapid Antigen Test) to supplement the low number of low positive samples in the above clinical study. Whole blood was obtained from febrile individuals and gamma-irradiated Ebola Zaire virus (strain Mayinga, BEI NR-31807) was added. A total of 25 individual negative samples were collected and Ebola Zaire virus was added as follows: to twelve (12) samples virus was added at 1.5 x Limit of Detection (LoD) and to thirteen (13) samples virus was added at 5 x LoD. Additional negative samples were used to randomize and blind the sample testing (because the negative samples for blinding were pooled they were not used for performance calculation). All samples were tested at n=1 and read at 30 minutes. Testing was performed at one site.

All sample test results (positive and negative) were concordant with expected results. Positive Percent Agreement for venous whole blood was 100% (95% CI 86.28% - 100.00%).

This study supports the use of the OraQuick Ebola Rapid Antigen Test with venous whole blood from symptomatic individuals with epidemiological risk factors.

FINGERSTICK

Non-Human-Primate (NHP) Study testing animal matched venous and fingerstick whole blood at each time point

Please refer to section M. *c. Other clinical supportive data (when a. and b. are not applicable*) of this document. This study supports the use of the OraQuick Ebola Rapid Antigen Test with finger stick whole blood from symptomatic individuals with epidemiological risk factors.

CADAVERIC ORAL FLUID

Multiple studies were performed with cadaveric oral fluid samples collected from individuals suspected to have died of *Ebolavirus* in the 2014 West African Ebola Zaire virus outbreak. The OraQuick Ebola Rapid Antigen Test result of the samples was compared to different RT-PCR tests. PCR tests on oral fluid were performed as part of the outbreak response to the 2014 West African Ebola Outbreak. Performance of EUA PCR tests was validated with whole blood, but oral fluid specific validation data of the PCR could not be obtained. It is likely that the analytical sensitivity of the PCR tests in oral fluid may be different from the analytical sensitivity in whole blood and that the clinical performance of the PCR test with these two matrices may differ. However, molecular detection of *Ebolavirus* RNA subject to prior purification of

RNA (as used in all of these PCRs) will remain significantly more sensitive than antigen detection despite the anticipated matrix related differences in analytical and clinical performance. Therefore, these PCR tests were accepted as a comparator for cadaveric oral fluid and whole blood.

Performance of the OraQuick Ebola Rapid Antigen Test with prospectively and retrospectively collected cadaveric oral fluid from West Africa

A study was conducted by the World Health Organization (WHO). Oral fluid swab specimens for the study were retrospectively (archived) and prospectively collected from cadavers in West Africa as part of the '*Safe and Dignified Burial*' procedures that were in place in West Africa during the 2014/15 Outbreak. Specimens were collected with the Σ-Virocult System viral transport media. Prior to entering the study all samples were re-tested by a comparator PCR test that received FDA emergency use authorization and Emergency Use Assessment and Listing (EUAL) of WHO. The comparator PCR was different from the test used for initial characterization.

A total of 277 specimens with prior PCR characterization were available. Of these, 222 samples were previously RT-PCR negative and 55 were RT-PCR positive. All samples were retested by the comparator PCR after storage. Twenty-nine (29) out of the 222 initially negative specimens gave invalid results due to failure of the internal control (human gene target) and were excluded. In addition, of the 55 initially RT-PCR positive three samples retested as negative with the comparator PCR, one sample gave an invalid result with the comparator PCR, and sixteen (16) specimens were of low volume and were therefore diluted 1 in 4 prior to PCR and RDT testing. These 20 samples were excluded from the final performance calculations. Note that dilution of samples for Ebola testing is not recommended for use with the OraQuick Ebola Rapid Antigen Test. Consequently, the performance estimates as presented in **Table 19** below are based on a total of 228 including 35 comparator PCR positive and 193 comparator PCR negative samples. Samples were tested by one operator with the OraQuick Ebola Rapid Antigen Test according to the instructions for use and compared to the comparator PCR result of the same sample.

Table 19: Performance of the OraQuick Ebola Rapid Antigen Test with retrospective cadaveric oral fluid samples from West Africa

		PCR Comparator		
		Positive	**Negative**	**Total**
OraQuick Results	**Positive**	34	0	34
	Negative	1	193	194
Total		**35**	**193**	**228**
Percent Agreement (n/N)	**PPA**	**97.1% (34/35)** (95% CI*: 85.5% - 99.5%)		
	NPA	**100% (193/193)** (95% CI*: 98.1% - 100%)		

The OraQuick Ebola Rapid Antigen Test correctly identified all PCR negative samples as negative. It also identified 34/35 PCR positive samples as positive. The test failed to detect one of the PCR positive samples that had a Ct indicative of a concentration below the LoD of the OraQuick Ebola Rapid Antigen Test.

This study supports the use of the OraQuick Ebola Rapid Antigen Test with cadaveric oral fluid.

Sensitivity of the OraQuick Ebola Rapid Antigen Test with Ebola Zaire virus positive contrived cadaveric oral fluid samples

Since the number of positive samples in the prospective West African studies (immediately above) were low, the scope of this study was to supplement the prospective studies with contrived positive samples. Twenty-five (25) saliva samples were collected from a non-endemic population and gamma-irradiated Ebola Zaire virus (Mayinga, BEI NR-31807) was added at 1.5x LoD and 5x LoD. Five (5) samples were left untreated to provide Ebolavirus negative samples for blinding the study. Samples were freshly prepared, randomized and blinded by an unblinded technician on the study day and then tested and read by a blinded operator. Results were read at 30 minutes.

Results are summarized in **Table 20** below. There were no invalid results and all Ebola Zaire virus negative samples tested concordant negative. The contrived sample study yielded a PPA of 95% (19/20). One sample at 1.5x LoD was recorded as false negative.

Table 20: Performance of the OraQuick Ebola Rapid Antigen Test with contrived Ebola Zaire positive oral fluid samples

	OraQuick Results	
	Positive	**Negative**
Contrived 1.5 x LoD	9	1
Contrived 5 x LoD	10	0
Negative Sample	0	5
Percent Agreement (n/N)	**95% (19/20) (95% CI*: 75.13% - 99.87%)**	

*Confidence Interval (CI) calculated using Clopper-Pearson (exact) method.

The contrived oral fluid study is supporting a claim for direct collected cadaveric oral fluid.

b. *Clinical specificity:*

Studies in this section were performed to assess specificity in cohorts with low prevalence (endemic, tail end of the 2014/15 West African Ebola Outbreak) and no

prevalence (non-endemic, U.S.) of *Ebolavirus* infection. For clinical specificity in the context of an outbreak setting, please refer to section *M.a. Clinical Sensitivity* (above).

For all studies performed in this section, samples were read at 30 minutes within a ±3 minutes maximal time window.

Specificity of the OraQuick Ebola Rapid Antigen Test with venous and fingerstick whole blood samples from a non-endemic population

Specificity of the OraQuick Ebola Rapid Antigen Test with prospectively collected venous and fingerstick whole blood was tested using samples from febrile and non-febrile individuals of a non-endemic U.S. population collected at five different sites. The study was approved by an IRB and subjects provided informed consent. Samples were randomized with contrived positive samples and tested blinded.

Enrollment Inclusion/Exclusion Criteria

Subjects included in the study:

- Were at least 7 years of age (any race);
- Were able to provide Informed Consent, and if applicable, Assent in English;
- Agreed to provide demographic information;
- Agreed to provide clinical data relative to febrile status (when applicable); for febrile samples individuals have/had a body temperature of ≥100.4 F within 12 hours of collection.
- Agreed to provide venous whole blood, fingerstick whole blood and/or oral fluid samples and agreed to testing with the OraQuick Ebola Rapid Antigen Test.

Subjects were excluded from participation in this study, if they:

- Gave a history of an *Ebolavirus* exposure risk;
- Were sponsor or investigator site employees or immediate family members of sponsor or investigator site;
- Were in the judgment of the investigator unable to complete the study or unlikely to comply with the study protocol.

Study Demographics

A total of 494 individuals were prospectively enrolled across 4 sites in the United States. One individual withdrew consent and was not tested. 493 subjects (247 venous and 246 fingerstick whole blood) were randomized. Samples of 40 non-febrile individuals were used to contrive positive samples for blinding purposes out of which 2 individual samples were excluded. From the remaining 452 individuals 2 individual samples were excluded from the analysis due to protocol deviations. Of these 450 samples 226 samples were venous whole blood (21 febrile and 204 non-febrile) and 224 samples were fingerstick (21 febrile and 203 non-febrile). Fever was defined by a body temperature of > 100.4°F.

In addition, fingerstick whole blood specimens from 26 subjects had been collected and tested at one additional clinical site for the OraQuick Ebola Rapid Antigen Test that was previously under Emergency Use Authorization (EUA). Out of these 26 samples data from one individual was excluded due to out of window read of the device. Data of the remaining 25 subjects was included and combined with the non-febrile dataset of the specificity cohort described above.

Each individual was accounted for with one fingerstick/venous sample per individual. The non-endemic per protocol population cohort for specificity has the demographics and baseline characteristics shown in **Table 21** below.

Table 21: Clinical study demographics and baseline characteristics

Variable	Estimate						
	New Specificity Cohort						Previous EUA Specificity Cohort
	Venous			Fingerstick			Fingerstick
	Non-febrile	Febrile	All	Non-febrile	Febrile	All	All[1]
N	(b) (4)						
<18 years	(b) (4) (7.3)	(14.3)	(8.0)	(10.3)	(9.5)	(10.3)	(0)
18-59 years	(b) (4) (82)	(81)	(81.9)	(79.3)	(85.7)	(79.9)	(92)
> 60 years	(b) (4) (10.7)	(4.8)	(10.2)	(10.3)	(4.8)	(9.8)	(8)
Mean	36.5	34.2	36.3	35.8	35.0	35.7	45
SD	16.43	16.00	16.37	16.41	14.96	16.25	13
Median	33.0	35.0	33.0	34.0	36.0	34.0	50
Min	7	7	7	7	10	7	23
Max	77	71	77	83	67	83	64
Gender (n, %)							
Female	(b) (4) (						(88)
Male	(b) (4) (40.0)	(47.6)	(40.7)	(49.8)	(47.6)	(49.6)	(12)
Race (n, %)							
Caucasian	(b) (4) (71.7)	(47.6)	(69.5)	(66.5)	(61.9)	(66.1)	(92)

African American	(b) (4) (22.4)		(20.4)	(28.1)	(14.3)	(26.8)	1 (4)
Hispanic/Latino	(b) (4) (2.4)	(47.6)	(6.6)	(1.0)	(19.0)	6 (2.7)	
Other[2]	(b) (4) (2.4)		(2.2)	(2.5)	1 (4.8)	6 (2.7)	1 (4)
Declined to answer	(b) (4) (1.0)	(4.8)	(1.3)	(2.0)	0 (0)	4 (1.8)	

[1] All subjects in this study were non-febrile

[2] Other is comprised of Alaska Native Americans, Asian, Hawaiian and Mixed races

Results

The final Per Protocol Population was defined as all randomized subjects for whom there existed an associated blood sample (venous or fingerstick) collected from the subject for analysis and who had no protocol violations of Inclusion/Exclusion criteria or study conduct. There were no invalid results in this study. Results for the Per Protocol Population are summarized **Table 22** below.

Table 22: Specificity of the OraQuick Ebola Rapid Antigen Test with venous and fingerstick whole blood samples from a non-endemic population – stratified by sample matrix and febrile status

Sample Type	Febrile/ Non-Febrile	Negative Percent Agreement (n/N)	95% CI*
Venous Whole Blood	febrile	100% (21/21)	83.9% - 100.0%
	non - febrile	100% (205/205)	98.2% - 100.0%
	overall	**100% (226/226)**	**98.4% - 100.0%**
Fingerstick Whole Blood	febrile	100% (21/21)	83.9% - 100.0%
	non-febrile	99.6% (227/228)	97.6% - 100.0%
	overall	**99.6% (248/249)**	**97.8% - 99.9%**
Combined	**febrile**	**100% (42/42)**	**91.6% - 100.0%**
	non-febrile	**99.8% (407/408)**	**98.6% - 100.0%**

n = number in agreement, N = sample size

* Confidence Interval (CI) calculated using Clopper-Pearson (exact) method.

There was no statistically significant difference in performance between venous and capillary whole blood for either febrile or non-febrile patients. The minor difference between the matrices observed for non-febrile subjects is not statistically significant since the confidence intervals overlap. The NPA for venous whole blood is 100%, the NPA for fingerstick blood is 99.6%.

Specificity of the OraQuick Ebola Rapid Antigen Test with direct collected cadaveric oral fluid from an endemic population/outbreak situation (West Africa)

Cadaver buccal swabs were collected and tested in Sierra Leone (Kenema) and Liberia at the tail end of the 2014/15 West African Ebola Outbreak. Samples were collected between May and August 2015 with the direct collection method using the flat pad of the OraQuick Ebola Rapid Antigen Test devices. (b)(4) samples were collected from Sierra Leone (Kenema) and (b)(4) samples were collected in Liberia.

In Sierra Leone a field evaluation of Rapid Diagnostic Tests for Ebola Virus Disease was conducted. Fifty (50) of the cadaveric oral fluid samples that were tested during the study were collected in Kenema using the direct sampling procedure with the OraQuick Ebola Rapid Antigen Test. Samples were tested according to the instructions for use with the OraQuick Ebola Test and compared to a validated RT-PCR. All samples were negative by PCR.

Additionally, (b)(4) oral fluid samples were collected from cadavers in Liberia. Samples were tested in parallel with an FDA EUA RT-PCR test and with the OraQuick Ebola Rapid Antigen Test according to the instructions for use with cadaveric oral fluid. All samples tested negative by PCR. Three of the PCR negative samples resulted in an invalid test result with the OraQuick Ebola Rapid Antigen Test and were excluded from calculation because no test results were obtained (the samples were not retested). **Table 23** below summarizes the performance of the test from both studies.

Table 23: Specificity of the OraQuick Ebola Rapid Antigen Test with direct collected archived cadaveric oral fluid samples from Sierra Leone

Region	N	Positive	Negative
Sierra Leone (Kenema)	50	0	50 [1]
Liberia	97 [2]	0	94
Total	**147**	**0**	**144**
Percent Agreement (n/N)	**PPA**	**N/A**	
	NPA	**100% (144/144) (95% CI*: 97.5% - 100%)**	

[1] One sample initially tested invalid (no Control Line) but repeat tested as negative. A swab for PCR was collected and the samples were negative by PCR.
[2] Three samples with invalid OraQuick Ebola Rapid Antigen Test results were excluded from the result calculation.

All PCR negative samples resulted in a negative OraQuick Ebola Rapid Antigen Test result. The study provided a Negative Percent Agreement (NPA) for the OraQuick Ebola Rapid Antigen Test of 100% for each of the sub-cohorts and for the combined study cohorts (95% Confidence Interval of 97.4% to 100%).

This study supports the use of the OraQuick Ebola Rapid Antigen Test with cadaveric oral fluid.

Specificity of the OraQuick Ebola Rapid Antigen Test with VTM collected cadaveric oral fluid from a non-endemic population

Sixty-three (63) matched sets of vendor-sourced cadaveric oral fluid samples collected in each VTM, Becton Dickinson (BD) Universal Viral Transport for Viruses and Σ-Virocult Transport System, were tested for evaluating specificity. All samples were collected within 96 hours of intake of the cadaver, shipped frozen and stored at -80°C. For blinding purpose (b)(4) contrived samples were prepared for each VTM system using oral fluid collected from live individuals (as a surrogate for cadaveric oral fluid). Immediately after collection of the oral fluid the swabs (b) (4) dilution was added to the swab. Samples were then randomized and tested blinded.

For the enrollment inclusion and exclusion criteria please refer to the study description provided in the study *Specificity of the OraQuick Ebola Rapid Antigen Test with venous and fingerstick whole blood samples from a non-endemic population* in the clinical specificity section b. (above). Demographics are provided in **Table 24** below.

Table 24: Demographics specificity study for VTM collected cadaveric oral fluid

Age at death in years (n, %)	
<50	5
50 - 69	33
>70	25
Min	31
Max	92
Median	68
Mean	67
SD	14.3
Gender (n, %)	
Female	29 (46)
Male	34 (54)

Results are summarized in **Table 25** below.

Table 25: Specificity of the OraQuick Ebola Rapid Antigen Test with VTM collected cadaveric oral fluid (non-endemic population)

Sample Type	VTM	Negative Percent Agreement (n/N)	95% CI*
Cadaveric Oral Fluid (non-endemic)	BD Universal VTM	100% (63/63)	94% - 100.0%
	Σ-Virocult	100% (63/63)	94% - 100.0%

All 63 samples in each of the VTM were negative resulting in a NPA of 100%. This study supports the use of the OraQuick Ebola Rapid Antigen Test with cadaveric oral fluid.

c. *Other clinical supportive data (when a. and b. are not applicable):*

Sensitivity of the OraQuick Ebola Rapid Antigen Test with animal and time matched venous and fingerstick whole blood - Non-Human Primate Study

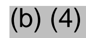
The purposes of the NHP study was to evaluate (1) concordance of OraQuick Ebola Rapid Antigen device results between venous whole blood (venous) and capillary blood (fingerstick) and (2) concordance of OraQuick Ebola Rapid Antigen results with a reference RT-PCR assay under EUA.

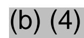
The NHP study was partially supported by National Institute of Allergy and Infectious Diseases (NIAID) Interagency agreement (b) (4) The non-human primate work completed at the NIAID Integrated Research Facility was supported by NIAID Division of Intramural Research and NIAID Division of Clinical Research and was performed under Battelle Memorial Institute Contract (No. (b) (4). The study was performed in accordance with animal study protocols approved by an Animal Care and Use Committee of NIAID Division of Clinical Research, part of the National Institutes of Health. The protocol is compliant with the US Department of Agriculture Animal Welfare Act regulations and the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals and adhere to the recommendations stated in The Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). All work with infectious virus was conducted in a biosafety level 4 laboratory a (b) (4) that is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

(b) (4)

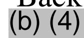
Animal matched venous and fingerstick whole blood specimens were collected from all animals under anesthesia at the following time points:

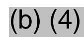
- twice prior to inoculation (Baseline 1 and 2)
- 3, 5, and 7 days post-exposure
- Upon reaching clinical endpoint criteria





Background of the NHP model:





Positive Percent Agreement (PPA) of the OraQuick Ebola Rapid Antigen Test when compared to the qRT-PCR and when compared to the animals infected status was calculated by day post challenge and is provided in **Table 28** below; 95% CI are not provided because of the low number of animals:

Table 28: Sensitivity of the OraQuick Ebola Rapid Antigen Test in NHP by Day post challenge

	PPA (compared to Infected Status)		PPA (compared to PCR)	
	Fingerstick	**Venous**	**Fingerstick**	**Venous**
Day 3	20% (1/5)	0 % (0/5)	N/A	N/A
Day 5	100% (5/5)	60% (3/5)	100% (5/5)	60% (3/5)
Day 7 to 8	100% (4/4)	100% (5/5)	100% (4/4)	100% (5/5)

PPA of the OraQuick Ebola Rapid Antigen Test when compared to PCR testing is 100% for fingerstick

PPA of the OraQuick Ebola Rapid Antigen Test compared to the animal infected status varies with respect to the time of challenge. PPA is low (0% for venous whole blood and 20% for fingerstick blood) during early stages of infection (i.e., Day 3) when symptoms are mild or not present. However, PPA was observed to increase with progressing disease as symptoms begin to present and when PCR turns positive (100% (b) (4) for fingerstick at and after day 5, (b) (4) for venous whole blood at and after day 5 and 100% [5/5] at and after day 7).

NPA of the OraQuick Ebola Rapid Antigen Test was calculated using the animals baseline samples prior to viral challenge. NPA was 100% (10/10 samples) for venous whole blood and fingerstick.

One fingerstick sample taken at post challenge day 3 tested as 'false positive' when compared to PCR but would be considered a true positive sample compared to the animal infected status.

(b) (4) As such this device should not be used to test asymptomatic individuals. This study therefore supports performance of the device with venous and fingerstick whole blood from individuals with signs and symptoms of Ebola Virus Disease and epidemiological risk factors.

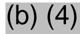
Performance of the OraQuick Ebola Rapid Antigen Test with cadaveric oral fluid from an endemic population

One study in Liberia, and two studies in Guinea, were performed as part of the outbreak response to the 2014/15 West African Ebola Outbreak. Study results were precluded from performance calculations in the clinical study section above (***Clinical sensitivity*** and ***Clinical specificity***) because these studies were performed either with major deviations from the instructions for use or used a comparator PCR method that could not be clearly identified. However, the data provide limited real-world evidence that is informative to the public with respect to the performance of OraQuick Ebola Rapid Antigen Test.

In the Liberian study (b) (4) cadaveric oral fluid samples were collected from Western, Liberia with the direc[redacted]llect method. The samples tested negative by PCR with an unidentified RT-PCR test. (b) (4)

For the first Guinea study 334 cadaveric oral fluid swab samples were collected between June 2015 and August 2015 from cadavers in Guinea using one of the

validated methods for oral fluid collection (VTM or direct collection). The samples were tested in parallel with an unknown RT-PCR. All samples were negative by PCR and by the OraQuick Ebola Rapid Antigen Test.



These studies support performance of the device with cadaveric oral fluid of an endemic population.

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   Not Applicable

M. Instrument Name:

Not applicable. The device does not utilize an instrument for result generation.

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ________ or No ____X____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes ________ or No ___X_____

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ________ or No ____X____

The device does not contain any software or instrument components.

3. Specimen Identification:

Not applicable.

4. Specimen Sampling and Handling:

See Instructions for Use.

5. Calibration:

Not applicable.

6. Quality Control:

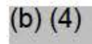
The OraQuick Ebola Rapid Antigen Test contains an internal procedural control (i.e., the Control Line) on the test device that must be visible for the test to be valid. If the control Line is not present the test is invalid and the sample must be retested.

The OraQuick Ebola Rapid Antigen Test is cleared with the OraQuick Ebola Rapid Antigen Control kit. The external controls are described in the Device Description (section I) above. It contains a positive (recombinant VP40 Antigen) and a negative (negative human plasma) control. Frequency for external controls are descried in the package insert.

O. Other Supportive Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Due to the nature of the *Ebolavirus* and the regions impacted by the most recent outbreaks, it was recognized that the test may be used in non-laboratory settings outside the US. To mitigate risks associated with this use of the device, robustness and human factors studies (usability) were performed as follows.

Robustness Studies:


(b) (4)

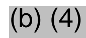
- **Residual substances on hands/gloves:** This study was designed to determine if the performance of the OraQuick Ebola Rapid Antigen Test is affected by exposing the collection pad to substances potentially found on hands. (b) (4)

- **Shaken developer solution:** This study was designed to determine if the performance of the device is affected by (1) constant shaking (not to induce spillage) and (2) shaking of the developer vial prior to inserting the device. No shaking occurred in the control. (b) (4)

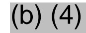
- **Reading distance on correct reading:** This study determined if the results of the OraQuick Ebola Rapid Antigen Test can be properly read when devices are read at various distances. (b) (4)

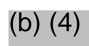
- **Sample placement on the device:** This study determines whether the placement of the sample affects the performance of the OraQuick Ebola Rapid Antigen Test. (b) (4)

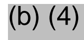
(b) (4)

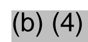


Usability Testing
Two studies were performed to confirm that intended use operators in their intended use environment could use the OraQuick Ebola Rapid Antigen Test effectively (i.e. users can use the product to deliver an accurate indication when evaluating a patient or cadaver sample for Ebola) and safely (i.e., users can use the product with minimal to no use errors, and with minimal risk of harm to both themselves and the patient). The human factor studies were performed in PPE, i.e., jumpsuit (no pressurized suit), hoods with visors, face shields, and double gloves.




(b) (4)





P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device. The labeling consists of the instructions for use, a quick reference guide and Fact Sheets for Healthcare providers and Patients.

Q. Identified Risks to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
Risk of false results	Certain analytical performance data, clinical performance data, and Certain device information in labeling, Certain limitations on distribution, Certain validation procedures and studies, Collection device specification
Failure to correctly interpret test results	Certain device information in labeling, Certain limitations on distribution, Certain results interpretation information in labeling, Certain risk mitigation strategies and end user trainings, Certain validation procedures and studies
Failure to correctly operate the device	Certain results interpretation information, Certain device description, Certain device information in labeling, Certain limitations on distribution, Certain risk mitigation strategies and end user trainings, Certain validation procedures and studies

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

The primary benefit associated with use of the OraQuick Ebola Rapid Antigen test is rapid detection of *Ebolavirus* antigens directly from patient specimens. Although testing of clinical specimens revealed that the OraQuick Ebola Rapid Antigen Test is less sensitive than assays that detect nucleic acids, the clinical and analytical validation studies indicate that there may be substantive benefits to patients and healthcare providers associated with use of the OraQuick assay through rapid diagnosis of infected patients and cadavers. Individuals treated at healthcare facilities in which a nucleic acid assay is not immediately available may particularly benefit from use of the OraQuick assay, as infected patients can be rapidly identified using the OraQuick Ebola Rapid Antigen test, and appropriate infection control and laboratory biosafety procedures can be initiated to reduce disease spread.

Summary of the Assessment of Risk

The primary risks associated with use of the OraQuick Ebola Rapid Antigen Test are false negative and false positive results. Additional risks include incorrect interpretation of the assay results or failure to correctly operate the device which could also result in erroneous results. False negative results may result in delayed diagnoses of a potentially fatal infection and less vigorous infection control measures with potential increased risk of transmission, while a false positive result may result in an unnecessary public health response. As treatments for *Ebolavirus* are currently being developed, in the future, erroneous results may result in delayed antiviral therapy with a subsequent increase in morbidity and mortality, or unnecessary exposure to treatment with possible adverse events.

Summary of the Assessment of Benefit-Risk

The probable benefits of the OraQuick Ebola Rapid Antigen Test outweigh the probable risks in light of the listed special controls and the general controls. The proposed special controls are necessary to ensure safe use of the assay and mitigate the risks associated with a highly virulent pathogen such as *Ebolavirus*. The validation data suggests that false positive results will be uncommon, and risks associated with false negative results can be mitigated by use of the device in association with other diagnostic tests for patients suspected of *Ebolavirus* infection. The risks of patient harm are further mitigated by the proposed special controls, including product labeling and distribution criteria for clinical laboratories, which will ensure that results are less likely to be incorrectly interpreted and appropriate biosafety precautions are utilized.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

S. Conclusion

The information provided in this De Novo submission is sufficient to classify this device into class II under regulation 21 CFR 866.4002 FDA believes that the stated special controls, in combination with the general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code(s): QID
Device Type: Device to detect antigens of biothreat microbial agents in human clinical specimens
Class: II (special controls)
Regulation: 21 CFR 866.4002

(a) Identification:

A device to detect antigens of biothreat microbial agents in human clinical specimens is identified as an in vitro diagnostic device intended for the detection of antigens of microbial agents in specimens collected from patients with signs and symptoms of infection with biothreat microbial agents and who have been exposed to these agents or are suspected or at risk of exposure. The device can include antibodies for immobilization and detection of the analyte. This device may also be used for cadaver testing to prevent human diseases for which cadavers constitute a source of human-to-human transmission.

(b) Classification: Class II (special controls). The special controls for this device are:

1. The distribution of these devices is limited to laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, coordination of testing, results and patient management with public health authorities and that have personnel that have completed device specific training required under paragraph (b)(5)(vi) of this section.

2. If sample collection devices are used, any sample collection device used must be FDA-cleared, -approved, or -classified as 510(k) exempt (standalone or as part of a test system) for the collection of human specimens; alternatively, the sample collection device must be cleared in a premarket submission as a part of this device.

3. Labeling must include an intended use statement that includes the following:

    i. What the device detects;

    ii. The type of results provided to the user;

    iii. The sample types with which it is intended for use;

    iv. Whether the measurement is qualitative, semi-quantitative, or quantitative;

    v. The clinical indications appropriate for the test use; e.g., in conjunction with patient history, epidemiological information, clinical observations, and other laboratory evidence to make patient management decisions;

    vi. The intended use population(s) (e.g., signs and symptoms and epidemiological risk factors);

    vii. If applicable, that use of the device is limited to an outbreak, suspected exposure of the subject, or to a situation where public health authorities have determined the need for the test;

    viii. A statement that testing should be performed and reported in accordance with current guidelines provided by the appropriate public health authorities;

    ix. A statement that use of this device is limited to laboratories, laboratory professionals or healthcare workers that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results and coordination of testing, results and patient management with public health authorities, and that consist of personnel who completed device specific training provided by [insert name of the manufacturer].

4. The labeling required under 21 CFR 809.10(b) must include:

   i. A detailed explanation of the interpretation of results, including acceptance criteria for evaluating the validity of results.

   ii. For any devices intended solely for the presumptive identification of an antigen(s) of biothreat microbial agents, a warning statement that the test results alone do not conclusively establish infection and that additional testing and confirmation procedures may be necessary in consultation with the appropriate public health or other authorities to whom reporting is required.

   iii. Limiting statements indicating, as applicable:

      a. Situations where the device has been demonstrated to fail or may not perform at its expected performance level (e.g., any disease specific circumstances or circumstances identified by human factors or robustness studies);

      b. That device results are intended to be followed up according to the latest professional guidelines (e.g., recommendations from the Centers for Disease Control and Prevention);

      c. That specimens can result in false negative results if collected outside of the indicated window for testing as recommended by public health authorities;

      d. A statement that this is a nationally notifiable disease or other condition caused by a biothreat microbial agent that should be reported to public health authorities in accordance with local, state, and federal law.

      e. Any specific circumstances that pose significant risk to public health, and for which the device has not been validated, including testing of matrices and patient populations that are not identified in the intended use such as individuals without signs and symptoms of infection including mass infection screening (such as airport or border screening) that is not limited to individuals who have signs and symptoms and who have been exposed to biothreat microbial agents or are suspected or at risk of such exposure.

      f. A description of the recommended training for safe use of the device and to minimize the risk of false results.

   iv. For any devices intended for use in a point of care setting, labeling must include a brief reference sheet for healthcare professionals that accompanies the device and that includes, at a minimum, the name and intended use of the

test, step-by-step instructions of all control and sample testing procedures for the claimed sample types, the result(s) interpretation, warning and limitation statements, and information for troubleshooting or technical assistance with the device.

5. Design verification and validation must include, as applicable:

   i. A detailed device description, including the principle of device operation and test methodology from obtaining a sample to the test result, all pre-analytical methods for specimen processing, and a rationale for target selection and selection of reagents.

   ii. Detailed documentation and results from analytical performance studies, including: Limit of Detection (LoD), analytical sensitivity determined using a standardized reference material that FDA has determined is appropriate (e.g., a World Health Organization reference material or international standard), analytical reactivity (i.e., species and/or strain inclusivity), analytical specificity (cross-reactivity and microbial interference), interference of endogenous and exogenous substances, specimen stability, reproducibility, precision, and other studies as applicable to the technology (e.g., linearity). Results for multiple lots must be included in the LoD study and in the precision or reproducibility studies.

   iii. For any devices intended for use in a point of care setting, detailed documentation and results that demonstrate the robustness of the device, including the entire testing procedure from sampling to result interpretation (e.g., human factors or flex studies).

   iv. For any devices that detect the presence of an analyte directly from specimen, detailed documentation and results from a shelf-life assessment that includes panel members formulated in the most complex clinical matrix identified in the device's intended use.

   v. Detailed documentation and results from either a clinical study or, when determined to be acceptable by FDA, a study with an equivalent data set. Documentation from this study must include study reports, testing results, and results of all statistical analyses, including line data of all test samples, and, if applicable, an appropriate justification describing how the sample set is representative of the intended use population. This study must compare the device performance to results obtained from a reference or comparator method that FDA has determined is appropriate and must be conducted with multiple reagent lots. If applicable, this study must use prospectively (sequentially) collected samples for each indicated specimen type that are collected from subjects representative of the intended use population (if the number of prospective clinical samples is insufficient to characterize the performance of the device, as determined by FDA, then additional characterized clinical

samples must be evaluated to supplement the study). This study must include specimens that contain relevant organism concentrations applicable to the indicated specimen type(s) and the targeted analyte(s), if applicable.

vi. As part of the risk management activities, an appropriate device specific end user training program must be offered as an effort to mitigate the risks of false results, failure to correctly operate the device, and failure to correctly interpret test results, and as applicable, operator exposure to biothreat microbial agents that may be present in test specimens and control materials.

vii. As part of the risk management activities, if the labeling includes hyperlinks to documents from public health authorities regarding sampling, sample shipment, sample testing or clinical management of patients suspected of being infected; or if the labeling includes direct contact information for any such public health authority, then the hyperlinks and contact information must be reviewed at least annually and updated to reflect any changes to those hyperlinks or contact information.