LightMix® Ebola Zaire rRT-PCR Test

Cat. No. 40-0666-96 Roche SAP n°07422423001

Kit with lyophilized reagents for 96 PCR reactions (20 µl) for detection of Ebola Zaire (2014) RNA

1. Intended Use

The ***LightMix® Ebola Zaire rRT-PCR Test*** is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of RNA from the Ebola Zaire virus (detected in the West Africa outbreak in 2014) on specified instruments in EDTA whole blood or TriPure-inactivated EDTA whole blood from individuals with signs and symptoms of Ebola virus infection in conjunction with clinical and epidemiological risk factors.

Testing with the ***LightMix® Ebola Zaire rRT-PCR Test*** should not be performed unless the individual meets clinical and epidemiologic criteria for testing suspect specimens.

Results are for the presumptive identification of Ebola Zaire virus. The definitive identification of Ebola Zaire virus requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of Ebola Zaire virus infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of Ebola Zaire virus.

The level of Ebola Zaire virus that would be present in EDTA blood from individuals with early systemic infection is unknown. Due to the difficulty in obtaining clinical specimens, this assay was not evaluated with blood from individuals with Ebola Zaire virus infection. Negative results do not preclude Ebola Zaire virus infection and should not be used as the sole basis for patient management decisions.

The ***LightMix® Ebola Zaire rRT-PCR Test*** is intended for use only under Emergency Use Authorization (EUA) by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or similarly qualified non-U.S. laboratories, and is limited to clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Notification of Public Health: Local, state and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection OR no detection EVD test result on the need for additional testing and appropriate transportation of specimens.

2. Test Principle

The ***LightMix® Ebola Zaire rRT-PCR Test*** is a real-time reverse transcription polymerase chain reaction test and designed to detect RNA from the Ebola Zaire virus (detected in the West Africa outbreak of 2014) in EDTA whole blood or TriPure-inactivated EDTA whole blood from patients.

The Ebola Zaire virus (detected in the West Africa outbreak of 2014) is a ssRNA Filovirus, causing severe disease typically associated with viral hemorrhagic fever (VHF). A 127-nt long fragment from the viral RNA-Polymerase (L protein) gene is amplified with specific primers and detected with a FAM-labeled hydrolysis probe.

As an internal control for the nucleic acid extraction and amplification processes, a second pair of primers amplifies a human mRNA of an endogenous human house-keeping gene, which is always expressed in the sample and detected with a R6G-labeled hydrolysis probe.

This one-step RT-PCR test is a one-tube assay in which specific RNA templates are first reverse-

transcribed into cDNA copies. This cDNA then undergoes PCR in which cyclic heating and cooling of the reaction logarithmically amplifies a specific region of DNA. The probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle.

3. Kit Contents

- 1 Vial **Ebola Zaire Mix** (**yellow** cap, 96 reactions)
  Contains: 1) lyophilized primer and FAM-labeled probe sequences that specifically detect Ebola Zaire virus in whole blood; and 2) lyophilized primer and R6G-labeled probe sequences that specifically detect an endogenous human house-keeping gene, used as an internal process control with each clinical specimen to indicate that nucleic acid has been adequately isolated from the clinical specimen and that PCR on the extracted nucleic acid has been successful.
- 1 Vial **Ebola Positive Control (RNA)** (**black** cap, 32 reactions)
  Contains lyophilized synthetic RNA, designed to react with the **Ebola Zaire Mix** to indicate whether the Ebola Zaire RT-PCR has been successful.

4. Storage and Shelf Life

- Store all kit components at 4-24℃ in the dark upon receipt. **Do <u>not</u> freeze** the lyophilized reagents.
- Do not use any kit component past the labeled expiration date.
- Reconstituted **Ebola Zaire Mix** reagent is stable for at least 2 weeks if stored at 4℃ protected from light.
- Reconstituted **Ebola Positive Control (RNA)** reagent should be stored frozen until the labeled expiration date. Avoid multiple freeze-thaw cycles.

5. Materials Required But Not Supplied

Note: All Catalog Numbers listed in this section are from Roche Diagnostics GmbH (Mannheim, Germany), unless otherwise specified.

<u>General</u>
- Clean pipettes/tips reserved for RNA work only
- RNase, DNase free-disposable plasticware
- Centrifuge capable of spinning microwell plates
- Vortex mixer

<u>Specimen inactivation</u>
- TriPure Isolation Reagent (Cat.-No. 11 667 157 001 *or* 11 667 165 001)
- Microfuge tubes, 1.5-ml, RNase-, DNase-free that can be spun at 12,000 x g
- Microfuge capable of spinning at 12,000 x g

<u>Nucleic acid extraction, Automated</u>
- MagNA Pure 96 Instrument (Cat.-No. 06 541 089 001)
- MagNA Pure 96 DNA and Viral NA Small Volume Kit (Cat.-No. 06 543 588 001)
- MagNA Pure 96 Tips (1000 µl) (Cat.-No. 06 241 620 001)
- MagNA Pure 96 Processing Cartridges (Cat.-No. 06 241 603 001)
- MagNA Pure 96 Output Plate (Cat.-No. 06 241 611 001)
- MagNA Pure 96 Sealing Foil (Cat.-No. 06 241 638 001)
- MagNA Pure 96 System Fluid (Internal) (Cat.-No. 06 430 112 001) *or* (External) (Cat.-No. 06 640 729 001)

<u>Nucleic acid extraction, Manual</u>
- High Pure Viral Nucleic Acid Kit (Cat.-No. 11 858 874 001)
- Standard tabletop microfuge capable of 13,000 x g centrifugal force (e.g., Eppendorf 5415C)
- Microfuge tubes, 1.5 ml, sterile

- Absolute ethanol
- Isopropanol

Amplification and Detection
- LightCycler® 480 II Instrument (Cat.-No. 05 015 278 001) *or* **cobas z** 480 Analyzer (Cat.-No. 05 200 881 001)
- LightCycler® Software Version 1.5 or higher *or* **cobas z** 480 Software Version 1.5 or higher with UDF Version 1.0 or higher
- LightCycler® 480 Multiwell Plate 96 white, with seals (Cat.-No. 04 729 692 001) *or* c4800 MWP (Cat.-No. 05 232 724 001)
- LightCycler® Multiplex RNA Virus Master (Cat.-No. 06 754 155 001).

6. Precautions, Warning, and Material Safety Data (MSDS)

- For In Vitro Diagnostic Use under Emergency Use Authorization only.
- Local, state, and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection OR no detection (negative) EVD test result on the need for additional testing and appropriate transportation of specimens.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures on authorized instruments.
- Use of this product is limited to specified laboratories and clinical laboratory personnel who have been trained on authorized instruments.
- All personnel who are involved in collecting, processing, handling, or transporting specimens from a patient with suspected Ebola virus infection should take appropriate precautions following the procedures recommended by Centers for Disease Control and Prevention (CDC) (1, 2).
- Use appropriate laboratory and personal protective equipment when using this kit.
- Treat all specimens and waste as potentially infectious.
- Ensure that all samples have been inactivated with TriPure reagent prior to any testing.
- The laboratory work-flow must conform to standard practices. Due to the risk of contamination of PCR with previously generated PCR amplicons, PCR set-up and PCR amplification must be performed in physically separated areas.
- Lyophilized PCR positive control should be reconstituted and handled in a location designated for the handling of high-copy nucleic acid (e.g., designated dead air box) apart from the normal PCR setup area.
- Avoid microbial or nuclease contamination of the reagents while pipetting the aliquots. The use of disposable sterile tips is essential.
- Do not mix reagents from different lots.
- Do not use the reagents after the expiration date.
- Performance of this assay has only been established with the specimen types listed in the Intended Use. Performance with other specimen types has not been evaluated.
- Proper sample collection, storage, and transport are essential for correct results.
- Refer to the respective Operator's Manuals for all the operative and safety instructions of equipment used in the assay.
- Handle all reagents following the respective manufacturer's instructions for use.
- Do not allow TriPure reagent to come into contact with sodium hypochlorite (bleach) solution or acids. These mixtures produce a highly toxic gas.
- Dispose of the unused reagents and waste materials according to the applicable laws and regulations.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.

Material Safety Data (MSDS)

According to U.S. OSHA 29 CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

7. Sample Collection

- The ***LightMix® Ebola Zaire rRT-PCR Test*** is intended for detection of RNA from the Ebola Zaire virus in whole blood specimens.
- Specimens should be collected in EDTA tubes according to the manufacturer's instructions for the specimen collection device.
- Shipping should be performed according to the policies of the shipping performer, customs regulations, and the requirements of the receiving laboratory.
- Follow the recommended infection control precautions for Ebola or other hemorrhagic fever viruses in handling all specimens.

8. Testing Procedure

8.1 Specimen Inactivation (Optional)

Caution: *Protocol should be performed in a Class II or higher BioSafety Cabinet (BSC) or Glove Box using appropriate personal protective equipment, including phenol-resistant gloves.*

Refer to the following document for details:

- TriPure Isolation Reagent (Instructions for Use) (***Caution****: For the procedure below, the chloroform step, Step 2 of the Procedure 2 described in the Instructions for Use, should be omitted.*)

Blood samples potentially infected with Ebola virus (all species and strains) may be inactivated (i.e., rendered non-infectious), prior to nucleic acid extraction, by the addition of 5 parts TriPure with 1 part whole blood following the method below:

a. Add 1.0 mL of TriPure to a 1.5-mL microfuge tube.
b. Add 0.20 mL of whole blood sample to the microfuge tube containing TriPure.
c. Vortex the tube 5 seconds.
d. Incubate the tube at room temperature for 5 minutes.
e. Centrifuge the tube at 12,000 x g for 15 minutes. (***Caution****: Do not exceed 12,000 x g.*) After centrifugation, the sample should separate into two phases with an opaque interface *possibly* visible.
f. Remove the top (aqueous) phase to a new microfuge tube or other appropriate tube for handling and/or storage. Take care to avoid the interface and the lower (organic) phase. This aqueous phase (approximately 0.7 ml) will be used for nucleic acid purification.
g. These samples can now be handled outside the BSC, according to safety precautions defined by the testing laboratory. If not used immediately for nucleic acid extraction, these samples should be stored at -80ºC.

Caution: *While treatment with TriPure has been shown to be an effective method to disrupt viruses and to stabilize the target nucleic acid (3), all specimens should still be handled as if they were infectious and present a potential safety hazard.*

8.2 Nucleic Acid Extraction

Materials and equipment:
Refer to Section 5 for all materials and equipment required for nucleic acid extraction, but not provided with this kit.

Sample materials

- Appropriate specimens for nucleic acid extractions are EDTA whole blood or TriPure-inactivated EDTA whole blood.

  *(**Caution**: If EDTA whole blood samples without TriPure inactivation are used for the nucleic acid extraction and PCR amplification procedures described below, these procedures should be performed in a Class II or higher BioSafety Cabinet (BSC) or Glove Box using appropriate personal protective equipment, including phenol-resistant gloves.)*
- For each extraction batch, always include at least one Negative Control by replacing specimen with PCR-grade water. The Negative Control will serve as an external processing control for both extraction and amplification.

Extraction using MagNA Pure 96 System

Refer to the following document for details:

- MagNA Pure 96 System Operator's Guide
- MagNA Pure 96 DNA and Viral NA Small Volume Kit (Instructions for Use)

Perform the MagNA Pure 96 System run following the steps below:

- For each sample from which nucleic acid is to be extracted, place 200 µl of the TriPure-inactivated, aqueous phase into one well of a MagNA Pure 96 processing cartridge. This cartridge is hereafter referred to as the "source plate".
- Create a work order in the MagNA Pure 96 software with the following specifications:
  - Order Type: Purification
  - Kit: DNA/Viral NA SV
  - Protocol: Viral NA Universal SV (version may be 2.0 or higher)
  - Volume, Sample: 200 µl
  - Volume, Elution: 50 µl
  - Internal Control: None
  - Custom Source Plate: None
  - Target Plate: MP96 Output Plate
  - Sample names: (*Enter as appropriate in the Sample Table).*
- Save the work order by selecting the SAVE button; enter a file name for the order and click *Save*.
- Verify that the deck waste tip holders are free of tips and that there is a waste cover in place.
- Load the appropriate reagents and consumables onto the MagNA Pure 96 instrument deck.
- Load the source plate onto the instrument.
- Close the load flap and start the run.
- When the run is finished, open the load flap. Unload the MagNA Pure 96; carefully remove the output plate containing the eluates.

Manual extraction using High Pure Viral Nucleic Acid Kit

Refer to the following document for details:

- High Pure Viral NA Nucleic Acid Kit (Instructions for Use) (***Caution****: For the procedure below, please note the highlighted steps which have been modified from the original Instructions for Use.*)

Perform the manual extraction following the steps below:

- Prepare the following working solutions following the instructions in the Instructions for Use of the kit:
  - Proteinase K Solution
  - Carrier RNA Solution
  - Working Binding Solution (must be made fresh for each use)
  - Inhibitor Removal Buffer Solution
  - Wash Buffer Solution
  - *Pre-warmed Elution Buffer (transfer 50µL per sample into a microfuge tube(s) and incubate at 70°C until the elution step.)*
- Extract nucleic acid from whole blood following the method below:

  Extraction
  - In a 1.5-ml microfuge tube, mix 200 µl of TriPure-inactivated whole blood with 200 µl of Working Binding Solution and 50 µl of Proteinase K Solution;
  - Incubate the tube at 72°C for 10mins;
  - *Add 250µL isopropanol into each sample and mix by inversion*;
  - Transfer the entire sample into the upper reservoir of a High Pure Filter Tube placed in a collection tube;
  - Centrifuge the assembly for 1 min at 8,000 x *g*;
  - *Remove the filter tube, discard the flowthrough in the collection tube, and place the filter tube back into the collection tube;*
  - *Centrifuge the assembly for 1 min at 8,000 x g;*
  - Remove the filter tube and place into a new collection tube.

  Inhibitor removal
  - Add 500 µl of Inhibitor Removal Buffer Solution into the upper reservoir of the filter tube;
  - Centrifuge the assembly for 1 min at 8,000 x *g*;
  - Remove the filter tube and place into a new collection tube.

  Washing
  - Add 450 µl of Wash Buffer Solution into the upper reservoir of the filter tube;
  - Centrifuge the assembly for 1 min at 8,000 x *g*;
  - Remove the filter tube and place into a new collection tube;
  - Add 450 µl of Wash Buffer Solution into the upper reservoir of the filter tube;
  - Centrifuge the assembly for 1 min at 8,000 x *g*;
  - Remove the filter tube, discard the flowthrough in the collection tube, and place the filter tube

back into the collection tube;
- Centrifuge the assembly for 10 seconds at ~13,000 x *g*;
- Remove the filter tube and place into a nuclease-free sterile 1.5mL microfuge tube;

Elution
- *Add 25µL of pre-warmed (70°C) Elution Buffer to the upper reservoir of the filter tube;*
- Incubate the assembly at room temperature for a minimum of 1 minute;
- Centrifuge the assembly for 1 min at 8,000 x *g*. The microfuge tube now contains viral nucleic acids and is ready for PCR amplification.

Handling of specimen extracts:
Keep the specimen extracts at 2-8°C until PCR amplification. If not used immediately, freeze the extracts at -80ºC. Do not freeze or thaw extracts more than once prior to PCR amplification.

8.3 PCR Amplification

Materials and equipment:
Refer to Section 5 for all materials and equipment required for PCR amplification, but not provided with this kit.

Sample materials
- Use the eluates from the Nucleic Acid Extraction procedure above.
- For each PCR run, always include one NC sample that has undergone the Nucleic Acid Extraction procedure above in the same extraction batch as the clinical specimens included in the PCR run.
- For each PCR run, always include a positive control by replacing specimen with the reconstituted **Ebola Positive Control (RNA)**.

PCR instrument
Amplification and detection can be accomplished by following the instructions below using either the LightCycler® II 480 Instrument *or* the **cobas z** 480 Instrument.

Refer to the following document for details:
- LightCycler® 480 Instrument Operator's Manual *or* **cobas z** 480 Operator Manual
- LightCycler® Multiplex RNA Virus Master (Instructions for Use)

Programming
Refer to the instrument operator's manual for details. Start programming before preparing solutions. The protocol consists of four program steps with the recommended parameters in Table 1 below.
1. Reverse Transcription of the viral RNA
2. Denaturation: sample denaturation and enzyme activation
3. Cycling: PCR-amplification
4. Cooling: cooling of the thermal block

For the LightCycler® II 480 Instrument, select Detection Formats **FAM** (Excitation 465 and Emission 510) and **Yellow 555** or **R6G** (Excitation 533 and Emission 580) with Quantification Factor **10**. For the **cobas z** 480 Instrument, the detection formats are the same, except for **Yellow 555/R6G** with Excitation 540.

Table 1 PCR amplification Programming Parameters

Program Step:	***RT Step***	***Denaturation***	***Cycling***		***Cooling***
Parameter					
Analysis Mode	None	None	Quantification mode		None
Cycles	*1*	1	45		1
Target [℃]	55	95	95	64	40
Hold [hh:mm:ss]	00:03:00	00:00:30	00:00:03	00:00:12	00:00:05
Ramp Rate [℃/s]	4.4	4.4	4.4	2.2	1.5
Acquisition Mode	None	None	None	**Single**	None

Preparation of Assay-Specific Reagents
- **Ebola Zaire Mix** (**yellow** cap):
  - **Add 50 µl** PCR-grade water to each vial. Mix the solution (vortex) and spin down.
  - **Use 0.5 µl** reconstituted reagent for a 20 µl PCR reaction.
  - Refer to Section 4 for proper storage of the reconstituted reagent.

- **Ebola Positive Control (RNA)** (**black** cap):
  - **Add 160 µl** PCR-grade water to the vial. Mix by pipetting the solution up and down 10 times.
  - **Use 5 µl** reconstituted reagent for a 20 µl PCR reaction.
  - Refer to Section 4 for proper storage of the reconstituted reagent.

Preparation of the reaction mix

- In a cooled reaction tube, prepare the reaction mix for single reactions (left) or one 96-well plate (right) (Table 2).

Table 2 Preparation of RT-PCR Reaction Mix

For use with the Roche LightCycler® Multiplex RNA Virus Master		
One reaction	**Component**	**100 reactions**
10.4 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	1040 µl
0.5 µl	Reconstituted **Ebola Zaire Mix**	50 µl
4.0 µl	**LightCycler® Multiplex RNA Virus Master** (see Roche kit manual)	400 µl
0.1 µl	**RT Enzyme** (provided with the Roche Master kit)	10 µl
15.0 µl	**Volume of Reaction Mix**	**1.500 µl**

- Mix gently and spin down.
- **Transfer 15 µl** per well.
- **Add 5 µl** of sample or control to each well for a final reaction volume of 20 µl. Seal the plate and centrifuge.
- Load the plate and start the run.

9. Interpretation of the Results

Upon completion of the PCR run, perform data analysis using the instrument software as described in the operator's manual. The amplification results are reported as Cp values. The instrument software also tentatively reports a reaction result as positive (a Red curve in the amplification plot from the software), negative (a Green curve in the amplification plot from the software), or uncertain (a Blue curve in the amplification plot from the software).

The results from the Negative Control added in the extraction step and the Positive Control added at the PCR step should be examined prior to interpretation of any clinical specimen result. If these controls are not valid, the clinical specimen results should not be interpreted.

Examination of control results

- The reactivity of the Negative Control for the internal process control (IPC) assay (channel 580) should be considered negative if the reaction is reported as negative (Green curve). In all other cases, the Negative Control reaction for the IPC assay should be considered failed.
- The reactivity of the Negative Control for the Ebola assay (channel 530) should be considered negative if the reaction is reported as negative (Green curve) AND its corresponding Cp value is ≥35, as estimated from the curve. In all other cases, the Negative Control reaction for the Ebola assay should be considered failed.
- The Positive Control should not be assessed for the IPC assay result.
- The reactivity of the Positive Control for the Ebola assay should be considered positive if the reaction is reported as positive (Red curve) AND its corresponding Cp value is <35. In all other cases, the Positive Control reaction for the Ebola assay should be considered failed.
- In order for a test run to be valid, the Negative Control reaction within the run must be negative for both the Ebola assay and the IPC assay.
  - If the Negative Control fails for either the Ebola assay or the IPC assay, the entire run is invalid and potential sources of contamination should be identified and corrected. Re-test the extracted specimen and controls, and re-analyze. If the Negative Control fails again, re-test with all specimens re-extracted.
- In order for a test run to be valid, the Positive Control reaction within the run must be positive for the Ebola assay.
  - If the Positive Control fails for the Ebola assay, the entire run is invalid and potential sources of the PCR process failure should be identified and corrected. Re-test the extracted specimen and controls, and re-analyze. If the positive control reaction fails again, re-test with fresh aliquots of

extracted specimens or all specimens re-extracted.

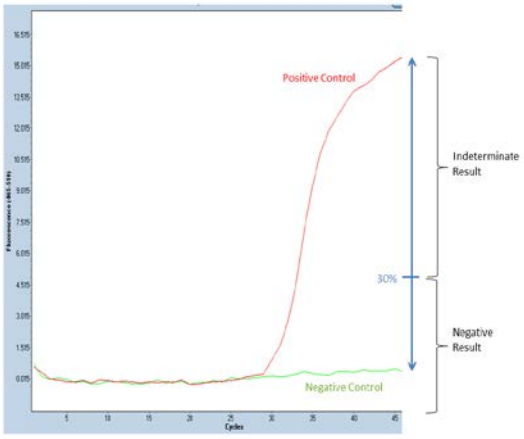
Examination of clinical specimen results

- The reactivity of a clinical specimen for the IPC assay should be considered positive if the reaction is reported as positive (Red curve) AND its corresponding Cp value is <35. The reactivity of a clinical specimen for the IPC assay should be considered negative if the reaction is reported as negative (Green curve). In all other cases, the result of the specimen for the IPC assay should be considered invalid. A positive IPC reaction from a clinical specimen indicates sufficient recovery of acceptable quality target sequence from the specimen. A negative IPC reaction could indicate absence of sufficient human cellular material in the specimen, improper nucleic acid extraction, low recovery of nucleic acid, improper PCR reaction setup, PCR inhibition, or reagent or equipment malfunction.
- The reactivity of a clinical specimen for the Ebola assay should be considered positive if the reaction is reported as positive (Red curve) AND its corresponding Cp value is <35. In all other cases, its respective fluorescence amplitude in relation to those of the Positive and Negative Controls should be further evaluated as detailed below.
- A clinical specimen is considered **Positive** for Ebola Zaire if the Ebola reaction is positive, regardless of the result of the IPC assay from the specimen.
- A clinical specimen is considered **Negative** for Ebola Zaire, if:
  - The IPC reaction is positive; AND
  - The Ebola reaction is reported as negative (Green curve); AND
  - The fluorescence amplitude for the Ebola assay at the last read (cycle 45) is < 30% of the amplitude difference between the Positive Control and the Negative Control above the Negative Control level.
- A clinical specimen is considered **Indeterminate** for Ebola Zaire, if the results do not fit either **Positive** or **Negative** as described above, which may include the following scenarios:
  a) The Ebola reaction is reported as uncertain (Blue curve); OR
  b) The Ebola reaction is reported as positive (Red curve), but with its corresponding Cp value is ≥35; OR
  c) The Ebola reaction is reported as negative (Green curve) AND the fluorescence amplitude for the Ebola assay at the last read (cycle 45) is ≥30% of the amplitude difference between the Positive Control and the Negative Control above the Negative Control level (see Figure 1 below for such examples); OR
  d) The Ebola reaction is reported as negative (Green curve) AND the IPC reaction is not positive.
- A clinical specimen with an **Indeterminate** result (Scenario a, b, or c above) requires re-testing the extracted specimen and controls, and re-analyzing. If the **Indeterminate** result is repeated, re-test with the specimens re-extracted or request a new sample.
- A clinical specimen with negative results for both the Ebola and IPC assays (Scenario d above) requires the extracted specimen to be re-tested, 1) undiluted and 2) at a 1:10 dilution (using nuclease-free PCR-grade water).
  - Valid positive or negative re-test results from the undiluted specimen will be taken as the final result.
  - Valid positive Ebola assay results from the 1:10 dilution will also be considered the final result.
  - Any negative IPC assay result or negative Ebola assay result from the 1:10 dilution requires the original clinical specimen to be re-extracted and re-tested.

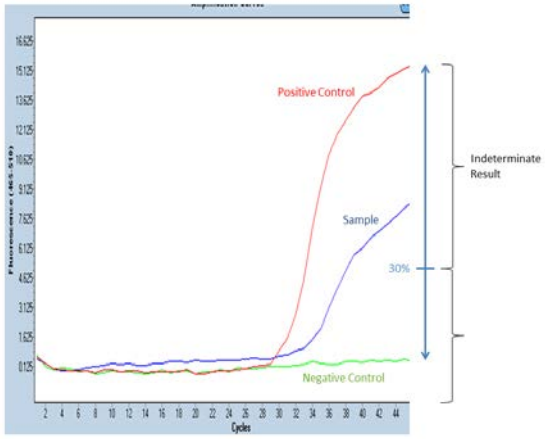
To facilitate in calling **Indeterminate** and **Negative** results using the fluorescence amplitude values, Figure 1 below provides: a) the definition of the amplitude difference between the Positive Control and the Negative Control above the Negative Control level; b) an example of an Indeterminate result due to a Blue curve; c) another example of an Indeterminate result due to a Blue curve; however, even if the software reports a blue curve, when the fluorescence amplitude value is near the 30% boundary, users should rather err on the side of calling the result Indeterminate; and d) an example of a Negative result due to a green curve and the fluorescence amplitude value is clearly below the 30% boundary.

Figure 1 Examples of Negative and Indeterminate Results

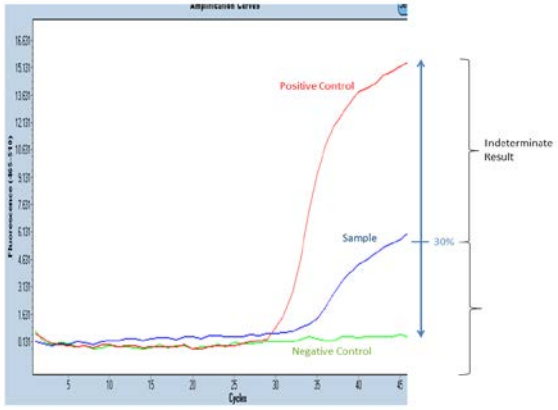
a)



b)



c)



d)

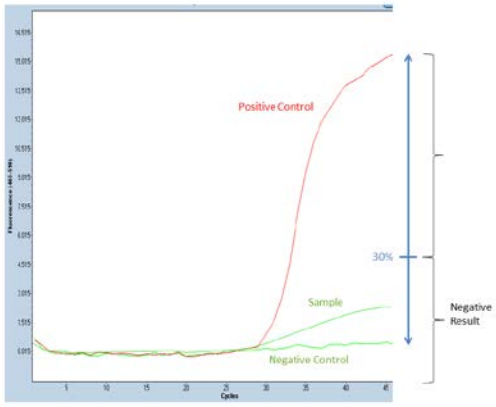


Table 3 below summarizes the steps for the interpretation of the test results.

Table 3 Summary of Result Interpretation

(*Note: Follow evaluation in this table in the order as listed.*)

Controls / Specimens	**Ebola Assay (Channel 530)**	**IPC Assay (Channel 580)**	**Interpretation**
Negative Control	-	-	Continue to evaluate Positive Control results.
	+	N/A*	Invalidate the run. Re-test from extracted specimen.
	N/A	+	
Positive Control	+	N/A	Continue to evaluate run results from clinical specimens.
	-		Invalidate the run. Re-test from extracted specimen.
Clinical specimens	+	N/A	Report as (+) for Ebola Zaire.
	-**	+	Report as (-) for Ebola Zaire.
	-***	+	Indeterminate result for the specimen. Re-test extracted specimen.
	-	-	Indeterminate result for the specimen. Re-test extracted specimen undiluted and 1:10 diluted.
Clinical specimens (1:10 diluted)	+	N/A	Report as (+) for Ebola Zaire.
	-	N/A	Re-extract and re-test the specimen.

* N/A: Not applicable;

** Verify the fluorescence amplitude value as detailed above (<30% of the amplitude difference between the Positive Control and the Negative Control above the Negative Control level);

*** Verify the fluorescence amplitude value as detailed above (≥30% of the amplitude difference between the Positive Control and the Negative Control above the Negative Control level).

10. Assay Limitations

- All results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
- The test does not detect strains of Ebola other than the Zaire strain.
- Interpretation of results from the ***LightMix® Ebola Zaire rRT-PCR Test*** must account for the possibility of false-negative and false-positive results.
- Negative results do not preclude infection with Ebola virus and should not be the sole basis of a patient treatment/management or public health decision.
- False positive results may occur from cross-contamination by target organisms, their nucleic acids or amplified product.
- Failure to follow the assay procedures may lead to false negative results.
- Improper collection, storage, or transport of specimens may lead to false negative results.
- Inhibitors present in the samples may lead to false negative results.
- The test is not validated as a quantitative test for treatment monitoring.
- Performance of this assay has only been established for EDTA whole blood and TriPure-inactivated EDTA whole blood. Performance with other specimen types has not been evaluated.
- This test should not be used to test specimens from asymptomatic individuals.
- Potential mutations within the target regions of the virus genome covered by the primer and/or probes of the test may result in failure to detect the presence of the pathogen.

11. Performance Characteristics

Analytical Sensitivity/Limit of Detection (LoD)

The analytical sensitivity of the ***LightMix® Ebola Zaire rRT-PCR Test*** was assessed by determination of limit of detection (LoD) through a series of three steps, i.e., an initial estimate, a refinement, and a final verification. Serial dilutions of inactivated viral nucleic acid purified from Ebola Zaire virus was spiked into pooled normal EDTA-whole blood. Viral nucleic acid was extracted from the spiked blood sample using the automated MagNA Pure 96 System and amplified on the LightCycler® 480 instrument following the Instructions for Use of the test kit. The viral material used in the study (Ebola Zaire, BEI Cat. No. DD-856) was obtained from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) via BEI, which was titered by plaque assay and then inactivated by cobalt irradiation.

For the initial estimation, eleven levels of spiked concentrations of the Ebola viral nucleic acid were tested in triplicates. The results of the initial testing are shown in Table 4 below. The lowest concentration at which all replicates were positive (4,781 PFU/mL) was treated as the tentative LoD.

Table 4 Limit of Detection, Initial Estimation

Concentration (PFU/mL)	Ebola Positive Result	Hit Rate	IPC Assay Positive Result
76,500	2/2*	100%	2/2*
38,250	3/3	100%	3/3
19,125	3/3	100%	3/3
9,562	3/3	100%	3/3
4,781	**3/3****	**100%**	**3/3**
2,390	2/3**	66%	3/3
1,195	0/3	0%	3/3
598	0/3	0%	3/3
299	0/3	0%	3/3
149	0/3	0%	3/3
75	0/3	0%	3/3
0	0/3	0%	3/3

* One of the triplicates was lost during the extraction process; therefore, there was only two valid data points for this concentration level.

** Includes one curve called by the instrument software as "uncertain", but considered positive for analysis purposes.

For the refinement, six levels of spiked concentrations of the Ebola viral nucleic acid were tested in five replicates. The results of the refinement testing are shown in Table 5 below. The lowest concentration at which all replicates were positive was 9,562 PFU/mL.

Table 5 Limit of Detection, Refinement

Concentration (PFU/mL)	Ebola Positive Result	Hit Rate	IPC Assay Positive Result
19,125	5/5	100%	5/5
9,562	**5/5**	**100%**	**5/5**
4,781	3/5	60%	5/5
2,390	0/5	0%	5/5
1,195	0/5	0%	5/5
598	0/5	0%	5/5
0	0/5	0%	5/5

For the verification, only two levels (4,781 PFU/mL and 9,562 PFU/mL) of spiked concentrations of the Ebola viral nucleic acid were tested in twenty replicates. The results of the verification testing are shown in Table 6 below. The lowest concentration at which at least 95% of the replicates were positive was chosen as the final LoD.

Table 6 Limit of Detection, Verification

Concentration (PFU/mL)	Ebola Positive Result	Hit Rate	IPC Assay Positive Result
9,562	20/20	100%	20/20
4,781	**20/20**	**100%**	**20/20**
0	0/20	0%	20/20

The claimed 95% Limit of Detection of the assay is 4,781 PFU/mL. This estimate may be higher than the true LoD of the assay since some of the viral nucleic acid template may not have been amplifiable due to the gamma radiation used for inactivation.

Analytical Reactivity

The analytical reactivity of the ***LightMix® Ebola Zaire rRT-PCR Test*** with different Ebola virus species and strains was assessed by: 1) testing available Filovirus samples, and 2) conducting *in silico* analysis on the Ebola Zaire primers and probes from the test kit for such reactivity.

Filovirus sample testing: All Filovirus samples (RNA or inactivated culture supernatants) that were available from BEI were tested. Sample concentrations were determined by BEI, and expressed in terms of copies, genome equivalents or plaque forming units. Note that some samples were provided at low concentration, limiting the amount of template in the PCR reaction. All samples were tested in triplicates using the ***LightMix® Ebola Zaire rRT-PCR Test***. Viral nucleic acid was extracted, if not provided already extracted, using the automated MagNA Pure 96 System and amplified on the LightCycler® 480 instrument following the Instructions for Use of the test kit.

In silico analysis: The primer and probe set of the ***LightMix® Ebola Zaire rRT-PCR Test*** was designed to amplify and detect Ebola Zaire only. NCBI BLAST analysis of the primer/probe sequences confirmed a 100% match to all circulating Ebola Zaire virus strains. The forward primer has single base mismatches to a group of Ebola Zaire sequences published in the year 2006 (Genbank acc. DQ205411-15), which are not related to the 2014 outbreak strain. The detection probe consists of a mixture of two individual sequences covering all circulating sequence variants. SequenceQuest analysis of the probe sequences counted 168 full matches and 25 sequence entries with a single mismatch.

The results of both the sample testing and the *in silico* analysis are summarized in Table 7. From the BLAST analysis all circulating Ebola Zaire strains are expected to be detected. Most other Filovirus tested or analyzed are expected to be negative, except Ebola Yambio, Ebola Maleo, and Ebola Zaire Chiro whose reactivity with the assay is uncertain.

Table 7 Analytical Reactivity

Filovirus	LightMix® Ebola Zaire rRT-PCR Testing				*In Silico* Result*
	Preparation Type	BEI Cat. No.	Concentration (Per Reaction)	PCR Result	
Ebola Zaire	RNA	NR-31806	$2x10^8$ copies	Positive (3/3)	Positive (No mismatch)
Ebola Bundibugyo	RNA	NR-31812	45 copies**	Negative (0/3)	Negative (R-Primer: 4 mismatches, Probe: 1 mismatch
Ebola Sudan	Culture supernatant	NR-31809	120 PFU**	Negative (0/3)	Negative (R-Primer: 4 mismatches, Probe: 1 mismatch)
Ebola Ivory Coast	Culture supernatant	R-43715	$2.08x10^7$ genomic equivalents	Negative (0/3)	Negative (R-Primer: 4 mismatches, Probe: 2 mismatches)
Ebola Reston	Culture supernatant	R-43875	$1.77x10^4$ PFU	Negative (0/3)	Negative (R-Primer: 3 mismatches, Probe: 10 mismatches)
Ebola Yambio					Uncertain (R-Primer: 3 mismatches, Probe: 3 mismatches)
Ebola Maleo					Uncertain (R-Primer: 3 mismatches, Probe: 3 mismatches)
Ebola Zaire Chiro					Uncertain (F-Primer: 2 mismatches, DQ205411-5, 2006)
Marburg Angola					Negative (R-Primer: 10 mismatches)
Marburg Ci67					Negative (R-Primer: 11 mismatches)
Marburg Musoke					Negative (R-Primer: 11 mismatches)
Marburg Ravn					Negative (R-Primer: 9 mismatches)

* The Forward Primer (F-Primer) is generic for all Filovirus. The Reverse Primer (R-Primer) is selected to be specific for Ebola Zaire. Primers with 4 or more mismatches to the target are expected to yield a negative result. Probes with more than 3 mismatches are expected to report negative results.

** The concentration of the available virus stock was lower than the planned testing level at $1x10^4$ copies or PFU/rxn. The actual concentration tested is indicated.

Analytical Specificity

The analytical specificity of the ***LightMix® Ebola Zaire rRT-PCR Test*** was assessed by testing pathogens known to cause Ebola-like clinical symptoms such as diarrhea, as well as pathogens which are prevalent in West Africa, in particular Malaria, and viruses causing hemorrhagic fever.

The ***LightMix® Ebola Zaire rRT-PCR Test*** has been tested with extracts containing genomic DNA or RNA from various pathogens using **cobas z** 480 instrument for amplification and detection. The results of this testing are summarized in Table 8 below. All tested pathogens showed negative reactivity with the assay.

Table 8 Analytical Specificity

Species	Concentration (copies/reaction)	Result
Gastrointestinal Virus (7)		
Norovirus GG2	>1.0E05	Negative
Norovirus GG1	>1.0E05	Negative
Rotavirus A	>1.0E05	Negative
Adenovirus 40	>1.0E08	Negative
Astrovirus	>1.0E03	Negative
Sapovirus	>1.0E03	Negative
Enterovirus	>1.0E05	Negative
Gastrointestinal Bacteria (10)		
Campylobacter jejuni	1.0E03	Negative
Salmonella typhi	1.0E04	Negative
Shigella flexneri	1.0E04	Negative
E.coli EIEC	1.0E04	Negative
Yersinia enterolytica	1.0E03	Negative
Helicobacter pylori	1.0E03	Negative
Plesiomonas shigelloides	>1.0E02	Negative
Aeromonas hydrophila	1.0E04	Negative
Clostridium difficile	1.0E04	Negative
Vibrio cholerae	1.0E03	Negative
Other Bacteria (3)		
Pseudomonas aeruginosa	1.0E03	Negative
Coxiella burnetii	1.0E03	Negative
Borrelia recurrentis	N/A*	Negative
Gastrointestinal Parasites (5)		
Giardia lamblia	1.0E03	Negative
Cryptosporidium	1.0E03	Negative
Dientamoeba	1.0E03	Negative
Entamoeba histolytica	1.0E02	Negative
Blastocystis spp	1.0E03	Negative
Malaria (4)		
Plasmodium falciparum	1.0E02	Negative
Plasmodium malariae	1.0E02	Negative
Plasmodium vivax	1.0E02	Negative
Plasmodium ovale	1.0E02	Negative
Influenza and Other Viruses (9)		
Influenza A H1N1	>1.0E04	Negative
Influenza A H5N1	>1.0E04	Negative
RSV-A	>1.0E04	Negative
RSV-B	>1.0E04	Negative
Dengue	>1.0E03	Negative
Yellow Fever	>1.0E03	Negative
Crim Congoe	>1.0E03	Negative
Chikungunya Virus	>1.0E03	Negative
Lassa	>1.0E03	Negative

* By *in silico* analysis only.

Interfering substances: To exclude interferences with other substances and specimen matrices, total nucleic extracts from EDTA fresh blood, frozen blood, plasma, stool, saliva and liquor were tested. All specimens spiked with in-vitro transcribed RNA were tested positive. All unspiked specimens were PCR-negative. In addition, purified human genomic DNA (20 µg/mL) was also tested negative.

Mock Clinical Study

To predict clinical performance of the ***LightMix® Ebola Zaire rRT-PCR Test***, EDTA-whole blood was obtained from 100 unique uninfected donors. Fifteen random specimens, selected from the 100-donor pool, were spiked with the inactivated Ebola viral nucleic acid at ~2.5x LoD (12,096 PFU/mL), at ~3.0x LoD (15,000 PFU/mL), and at a high concentration (MAX; 187,500 PFU/mL). All the spiked and unspiked specimens (145) were blinded and randomized. The viral material used in the study (Ebola Zaire, BEI Cat. No. DD-856) was obtained from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) via BEI, which was titered by plaque assay and then inactivated by cobalt irradiation.

All the samples were tested using the ***LightMix® Ebola Zaire rRT-PCR Test*** following the Instructions for Use of the test kit with the automated MagNA Pure 96 System for nucleic acid extraction and the LightCycler® 480 instrument for amplification and detection. The blinded spiking key was unmasked after the results were complete.

Table 9 below provides the results of the individual samples. Table 10 below provides a summary of the results. The assay had a nonreactive result for 100 out of 100 unspiked samples (Negative Percent Agreement = 100%, 95% CI: 96.3% - 100%). The assay detected 44 of 45 spiked samples (Positive Percent Agreement = 97.8%%, 95% CI: 88.4% - 99.6%). The remaining sample was an indeterminate curve. Re-testing of that sample yielded a positive result as expected.

Table 9 Mock Clinical Study, Sample Result Listing

Plate	Position	Description and Sample ID	Ebola Assay Cp	IPC Assay Cp
1	A1	Neg 92	NEG	28.43
1	A2	Pos (MAX) 13	31.46	29.25
1	A3	Neg 69	NEG	29.04
1	A4	Neg 31	NEG	28.93
1	A5	Neg 47	NEG	30.1
1	A6	Neg 29	NEG	28.72
1	A7	Pos (3xLOD) 11	33.9	32.12
1	A8	Neg 21	NEG	29.49
1	A9	Neg 98	NEG	29.49
1	A10	Neg 65	NEG	28.95
1	A11	Neg 5	NEG	30.34
1	A12	Neg 40	NEG	28.79
1	B1	Neg 13	NEG	29.19
1	B2	Pos (MAX) 4	31.57	30.3
1	B3	Neg 4	NEG	29.46
1	B4	Neg 10	NEG	29.12
1	B5	Neg 11	NEG	29.12
1	B6	Neg 16	NEG	28.96
1	B7	Neg 1	NEG	29.7
1	B8	Neg 37	NEG	30.05
1	B9	Pos (MAX) 14	31.55	28.79
1	B10	Neg 72	NEG	29.3
1	B11	Neg 28	NEG	29.81
1	B12	Neg 87	NEG	28.73
1	C1	Neg 15	NEG	30.11
1	C2	Neg 12	NEG	29.29
1	C3	Neg 74	NEG	28.95
1	C4	Neg 53	NEG	30.17
1	C5	Pos (MAX) 15	31.61	29.84
1	C6	Neg 80	NEG	29.04
1	C7	Pos (2.5xLOD) 7	34.34	28.73
1	C8	Neg 44	NEG	28.63
1	C9	Neg 67	NEG	29.07
1	C10	Neg 33	NEG	29.78
1	C11	Pos (MAX) 10	31.56	30.03
1	C12	Pos (2.5xLOD) 2	33.59	28.75
1	D1	Pos (3xLOD) 9	34.04	29.01
1	D2	Pos (MAX) 3	31.57	29.82

1	D3	Neg 2	NEG	28.7
1	D4	Pos (3xLOD) 1	34.05	30.78
1	D5	Neg 52	NEG	30.31
1	D6	Pos (2.5xLOD) 11	34.01	32.02
1	D7	Neg 39	NEG	30.22
1	D8	Pos (3xLOD) 6	34.02	28.68
1	D9	Neg 99	NEG	28.72
1	D10	Neg 48	NEG	29.69
1	D11	Neg 56	NEG	29.47
1	D12	Pos (MAX) 8	31.63	28.46
1	E1	Neg 23	NEG	30
1	E2	Neg 83	NEG	29.76
1	E3	Neg 81	NEG	28.82
1	E4	Neg 66	NEG	28.5
1	E5	Neg 26	NEG	29.55
1	E6	Neg 90	NEG	29.24
1	E7	Neg 36	NEG	29.81
1	E8	Neg 51	NEG	29.73
1	E9	Pos (3xLOD) 15	34.25	29.93
1	E10	Pos (MAX) 11	31.66	31.69
1	E11	Neg 58	NEG	30.6
1	E12	Pos (MAX) 6	31.35	28.69
1	F1	Neg 14	NEG	28.98
1	F2	Pos (2.5xLOD) 6	33.84	28.98
1	F3	Pos (MAX) 12	31.68	28.65
1	F4	Neg 22	NEG	29.64
1	F5	Neg 55	NEG	30.76
1	F6	Neg 94	NEG	29.43
1	F7	Pos (MAX) 1	31.52	30.93
1	F8	Neg 54	NEG	29.7
1	F9	Neg 75	NEG	29.67
1	F10	Neg 89	NEG	30.42
1	F11	Neg 79	NEG	29.95
1	F12	Pos (2.5xLOD) 14	33.36	28.97
1	G1	Pos (3xLOD) 14	33.67	28.75
1	G2	Neg 3	NEG	30.43
1	G3	Pos (3xLOD) 3	33.58	29.76
1	G4	Neg 63	NEG	28.75
1	G5	Neg 6	NEG	29.54
1	G6	Neg 32	NEG	30.62
1	G7	Pos (3xLOD) 13	IND	29.33
1	G8	Neg 70	NEG	28.98
1	G9	Neg 82	NEG	28.9
1	G10	Neg 30	NEG	28.88
1	G11	Neg 24	NEG	30
1	G12	Neg 50	NEG	30.17
1	H1	Neg 8	NEG	28.34
1	H2	Neg 41	NEG	29.23
1	H3	Pos (3xLOD) 8	33.96	28.63

1	H4	Pos (2.5xLOD) 3	33.74	29.63
1	H5	Neg 42	NEG	29.64
1	H6	Neg 46	NEG	29.06
2	i1	Neg 97	NEG	27.92
2	i2	Pos (MAX) 7	30.93	28.22
2	i3	Neg 19	NEG	29.69
2	i4	Neg 34	NEG	28.95
2	i5	Pos (3xLOD) 5	32.83	28.78
2	i6	Pos (MAX) 5	31.11	28.85
2	i7	Pos (MAX) 2	30.84	28.47
2	i8	Neg 64	NEG	29.18
2	i9	Pos (2.5xLOD) 1	32.57	30.13
2	i10	Pos (MAX) 9	30.71	28.76
2	i11	Neg 95	NEG	29.44
2	i12	Neg 7	NEG	29.21
2	J1	Neg 45	NEG	29.08
2	J2	Neg 57	NEG	29.01
2	J3	Neg 76	NEG	29.03
2	J4	Pos (2.5xLOD) 5	33.25	28.87
2	J5	Neg 59	NEG	29.02
2	J6	Neg 77	NEG	28.87
2	J7	Neg 86	NEG	30.03
2	J8	Neg 18	NEG	28.56
2	J9	Neg 85	NEG	28.27
2	J10	Pos (2.5xLOD) 13	32.99	28.91
2	J11	Neg 96	NEG	30.98
2	J12	Neg 78	NEG	28.7
2	K1	Pos (2.5xLOD) 9	32.91	28.63
2	K2	Neg 60	NEG	30.78
2	K3	Pos (3xLOD) 2	32.87	28.57
2	K4	Neg 9	NEG	30.31
2	K5	Neg 68	NEG	30.49
2	K6	Neg 84	NEG	28.77
2	K7	Pos (2.5xLOD) 10	33.12	29.71
2	K8	Neg 61	NEG	28.87
2	K9	Neg 25	NEG	28.49
2	K10	Neg 49	NEG	28.74
2	K11	Neg 43	NEG	28.85
2	K12	Neg 93	NEG	28.21
2	L1	Neg 73	NEG	28.9
2	L2	Neg 27	NEG	28.83
2	L3	Pos (3xLOD) 10	32.91	29.74
2	L4	Pos (3xLOD) 12	33.02	28.45
2	L5	Pos (2.5xLOD) 15	33.46	29.73
2	L6	Neg 35	NEG	29.86
2	L7	Pos (2.5xLOD) 12	33.49	28.5
2	L8	Neg 71	NEG	28.43
2	L9	Pos (2.5xLOD) 8	32.98	28.57
2	L10	Neg 100	NEG	29.51

2	L11	Neg 20	NEG	29.12
2	L12	Pos (2.5xLOD) 4	32.74	29.69
2	M1	Neg 91	NEG	28.46
2	M2	Pos (3xLOD) 7	32.94	28.3
2	M3	Pos (3xLOD) 4	32.88	29.88
2	M4	Neg 88	NEG	29.24
2	M5	Neg 17	NEG	29.22
2	M6	Neg 38	NEG	28.68
2	M7	Neg 62	NEG	28.28

Table 10 Mock Clinical Study, Summary Statistics

Sample Type	Concentration (PFU/mL)	Positive	Indeterminate	Negative
Negative	0	0	0	100
2.5x LOD	12,096	15	0	0
3x LOD	15,000	14	1	0
MAX	187,500	15	0	0
Total (145)		44	1*	100
				95% CI
Positive Percent Agreement		44/45	97.8%	88.4% - 99.6%
Negative Percent Agreement		100/100	100%	96.3% - 100%

* Re-testing of the sample yielded a positive result as expected.

Performance Using Alternative Methods

The mock clinical study described above was conducted using TriPure-inactivated whole blood sample with automated MagNA Pure 96 System for nucleic acid extraction and the LightCycler® 480 instrument for amplification and detection. The performance of the ***LightMix® Ebola Zaire rRT-PCR Test*** was also assessed using alternative methods, including: 1) nucleic acid extraction using a manual method, 2) amplification and detection using **cobas z** 480 Analyzer, and 3) using whole blood samples without TriPure inactivation.

Twenty replicates of one pooled normal whole blood and twenty replicates of the same pooled whole blood, but spiked with ~2.5x LoD (12,096 PFU/mL) were prepared for each of the alternative methods. The viral material used in the study (Ebola Zaire, BEI Cat. No. DD-856) was obtained from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) via BEI, which was titered by plaque assay and then inactivated by cobalt irradiation.

For the alternative nucleic extraction method, the spiked and unspiked samples were tested using the ***LightMix® Ebola Zaire rRT-PCR Test*** following the Instructions for Use of the test kit, including sample inactivation with TriPure, using the High Pure Viral Nucleic Acid Kit for manual nucleic acid extraction, and using the LightCycler® 480 instrument for amplification and detection.

For the alternative PCR instrument, the spiked and unspiked samples were tested using the ***LightMix® Ebola Zaire rRT-PCR Test*** following the Instructions for Use of the test kit, including sample inactivation with TriPure, using the automated MagNA Pure 96 System for nucleic acid extraction, and using the **cobas z** 480 Analyzer for amplification and detection.

For testing samples without inactivation, the spiked and unspiked samples were tested using the ***LightMix® Ebola Zaire rRT-PCR Test*** following the Instructions for Use of the test kit using the automated MagNA Pure 96 System for nucleic acid extraction and the LightCycler® 480 instrument for amplification and detection, except the TriPure inactivation step was skipped before the nucleic acid extraction.

Performance of all the alternative methods is shown to be comparable to that of the reference method (TriPure inactivation, automated nucleic acid extraction, and the LightCycler® 480 instrument for amplification and detection) (Table 11).

Table 11 Performance Using Alternative Methods

Alternative Method	Ebola Result, Spiked Samples			Ebola Result, Unspiked Samples		
	Positive	Indeterminate	Negative	Positive	Indeterminate	Negative
Manual extraction (vs. automated extraction)	18/20	1*/20	1*/20	0/20	0/20	20/20
cobas z 480 (vs. LightCycler® 480)	18/20	1*/20	1*/20	0/20	0/20	20/20
No sample inactivation (vs. TriPure inactivation)	20/20	0/20	0/20	0/20	0/20	20/20

* Re-testing of the sample yielded a positive result as expected.

12. References

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13. Version History

V141219 Release version

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