Instructions for Use

Idylla™ Ebola Virus Triage Test

For Use under Emergency Use Authorization (EUA) Only

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1 Name

Idylla™ Ebola Virus Triage Test
Short name: EBOV

2 Intended Use / Indications for Use

The Idylla™ Ebola Virus Triage 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 2014) in EDTA venous whole blood from individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors.

Testing with the Idylla™ Ebola Virus Triage Test should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens.

Results are for the presumptive identification of Ebola virus RNA. The definitive identification of Ebola virus RNA requires additional testing and confirmation procedures in consultation with public health or other authorities from whom reporting is required. The diagnosis of Ebola virus infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of Ebola virus RNA. Negative results do not preclude Ebola virus infection and should not be used as the sole basis for patient management decisions.

The level of the Ebola virus that would be present in blood from individuals with early symptomatic infection is unknown. Due to the difficulty in obtaining clinical specimens positive for Ebola virus, the Idylla™ Ebola Virus Triage Test was evaluated with limited numbers of contrived specimens spiked with live Ebola Zaire virus RNA. The test has not been evaluated with blood from individuals with Ebola Zaire virus infection.

The Idylla™ Ebola Virus Triage Test is for use only under Emergency Use Authorization (EUA) by laboratories in the United States certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests, and by laboratories in the United States certified under CLIA to perform high complexity tests, or in similarly qualified non-U.S. laboratories, by clinical laboratory personnel who have received specific training on the use of the Idylla™ Ebola Virus Triage Test on the Idylla™ system.

Notification of Public Health authorities: 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 or negative Idylla™ Ebola Test result on the need for additional testing and appropriate transportation of specimens.

3 Background

The Ebola Zaire virus is a Filovirus that causes a hemorrhagic fever disease in humans and is associated with significant mortality. It is transmitted through contact with infected blood or body fluids (e.g., urine, stool, and vomit). The incubation period ranges from 2 to 21 days. Since its discovery in 1976, five Ebola species have been described: Zaire, Sudan, Côte d'Ivoire (Tai Forest), Bundibugyo and Reston Ebola virus. Ebola Zaire virus is the cause of the 2014 West African outbreak. The West African outbreak is the largest Ebola virus outbreak known to date, and affected multiple African countries (Guinea, Liberia, Sierra Leone, and Nigeria). In addition, a small number of cases were imported into the USA, UK, Spain, Senegal and Mali. WHO reported, as of May 2016, there have been a total of 28,616 cases with 11,310 deaths.

4 Summary and Explanation of the Test

4.1 Principles of the Procedure

The Idylla™ System covers the entire process from sample-to-result with fully integrated sample preparation followed by rRT-PCR (real-time reverse transcriptase polymerase chain reaction) amplification and detection of target sequences.

The Idylla™ System consists of the Idylla™ Console connected to one or more Idylla™ Instruments. Samples are inserted into/added to the Idylla™ Cartridges. The Idylla Cartridges are designed for specific test applications and are run on the Idylla™ System using application specific Test Type Packages (TTP). The Idylla™ Ebola specific software (Ebola TTP) automatically processes the sample in the Idylla™ EBOV Test Cartridge and analyzes the obtained PCR data.

INFORMATION

For more information on the Idylla™ System, please refer to the Idylla™ Operator Manual.

The Idylla™ Ebola Virus Triage Test qualitatively detects viral RNA from the Ebola Zaire virus prese[illegible] EDTA venous w[illegible]le blood. To prevent erroneous reporting, a sample process control (SPC) and a human sample [illegible]trol (R[illegible]eP) are inclu[illegible]d. The total turnaround time from sample to result takes approximately 100 minutes.

The Idylla™ Ebola Virus Triage Test consists of four standardized, automated process[illegible]

- Sample homogenization, and cell lysis
- DNA/RNA Extraction
- rRT-PCR detection
- Data analysis and reporting

All reagents required to perform the Test are contained within the Cartri[illegible]ce the w[illegible]lood sample is added to the Cartridge and the Cartridge lid is closed, the sample materia[illegible]tained i[illegible] Cartridge and cannot be retrieved.

During real-time reverse transcriptase polymerase chain [illegible]action, an [illegible]nzym[illegible]nverts the negative single stranded viral RNA into complementary DNA (cDNA). Once converted[illegible]e cDNA is spec[illegible]ally am[illegible]ified during the PCR. Using target specific probes for the Ebola Zaire strain, the amplifica[illegible]n reaction is mo[illegible]ored in real-time. The probes are labelled with fluorescent reporter dyes and quenchers.

The Cartridge contains five PCR chambers in whi[illegible]he rR[illegible]CR takes pl[illegible]e. For the Idylla™ Ebola Virus Triage Test, the chambers contain the reagents for the following P[illegible] reactions[illegible]scent labeled reporter dyes generated upon amplification are analyzed in each of the chambers:

CHAMBER A	CHAMBER B	CHA[illegible]	[illegible]HAMBER D	CHAMBER E
Ebola and SPC	Ebola a[illegible]PC	Ebola and SPC	[illegible]ola and SPC	RNaseP

SPC: Sample Processing Co[illegible]

4.2 Principles of the [illegible]st Specif[illegible] Software

The Idylla[illegible]BOV Test[illegible]xecution a[illegible]a interpretation is performed by Test specific software that is referred to as the **Test Ty[illegible] Package** ([illegible]). The Ebola TTP automatically analyzes the collected fluorescent signals. All obtained fluorescent signals ar[illegible]alu[illegible]d if they [illegible] the acceptance criteria for validity. The fluorescence signals of the controls are assessed as part of the[illegible]a interpretation algorithm. SPC signals are used to verify adequate processing of the sample. With a high Ebola viral load, [illegible] SPC may not be detected because of competitive inhibition. In these cases, the Ebola amplification will be positive. The En[illegible]nous Control (EC) detects whether a human sample (EDTA venous whole blood) has been added to the Cartridge. This sample control detects the human RNaseP gene, which is present in human cells. Finally, if all controls are valid, the presence of Ebola virus is determined and results are reported on the Console.

5 Product Contents

5.1 Materials provided

The following materials are provided:

Idylla™ Ebola Virus Triage Test Cartridges (box of six, Catalog No.: A1013/6). Idylla™ Ebola Virus Triage Test Cartridges are individually packaged in a sealed pouch. Each Cartridge contains the necessary reagents to perform a single Idylla™ EBOV Test. The Cartridge is sealed, preventing contact between the user handling the Test and the reagents inside the Cartridge.

Each Cartridge contains:

Component	Total Amount
Lysis buffer (containing Guanidinium Thiocyanate)	5 mL
Nuclease free water	2.2 mL
70% Ethanol solution	4.9 mL
Dried rRT-PCR reagents	4µL per PCR chamber

5.2 Materials Required But Not Provided

The following materials are required to perform an Idylla™ Ebola Virus Triage Test, but not provided with the Test kit:

- Idylla™ Instrument (Catalog No.: P0020) and Console (Catalog
- Calibrated pipette (e.g. Eppendorf 20 – 200 µL)
- Disposable filter pipette tips fitting the calibrated pipette (e.g. Eppendorf Catalog No.: 022491296)
- Cleaning agent (10% bleach solution)
- 70% Ethanol solution (EtoH)
- EBOV Test Type Package (TTP)

Information

For more information on obtaining, installing and updating the existing Ebola TPP, or downloading the Idylla™ Operator Manual, please refer to the Contact Information section. Instructions for installation of a TTP are included in the Operator Manual.

6 Warnings and Precautions

- For in vitro diagnostic use
- For use under Emergency Use Authorization only.
- All testing must be conducted under appropriate biosafety conditions in accordance with applicable country, state and local laws and within CDC guidelines.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.
- All results should be interpreted by a trained professional in conjunction with review of the patient's clinical signs and symptoms and history.
- Treat all biological specimens, including used Cartridges, as potentially infectious. Ebola specific guidelines are available from Centers for Disease Control and Prevention (CDC, Ref 6, 7). Guidelines for blood specimen handling, storage and disposal are available from the Clinical and Laboratory Standards Institute (Refs 1, 2, 3).

- Use personal protective equipment (PPE) consistent with current guidelines including safety goggles and / or face shields, masks or respiratory equipment, disposable gowning, boots, and gloves. Users performing this Test should be appropriately trained of the donning and doffing of personal protective equipment. Wash hands thoroughly after handling specimens.
- Spills must be handled according to the instructions described in the Idylla™ Operator Manual.
- Samples must not be inactivated using Trizol or AVL prior to loading sample onto the Idylla™ Ebola Virus Triage Test as inactivation reduces Test performance and may lead to erroneous results.
- Improper collection, storage, or transport of specimens may lead to erroneous results and the need for re-testing and loss of the Test specimen.
- When processing more than one sample at a time, open only one Cartridge; add the sample and close the Cartridge before processing the next sample. Change gloves between samples.
- Do not exceed the allowed amount of specimen: 200 µl of EDTA whole blood. Overloading the Cartridge could lead to an erroneous or invalid result. Using a smaller amount of whole blood may cause the Test to be less sensitive.
- Use of this assay should only be for trained personnel.
- Check the expiration date on the Cartridge pouch before use. The expiration date reflected is the last date on which a Cartridge may be used.
- Use the Cartridge within 1 hour after opening the pouch.
- Do not use a Cartridge if its pouch is pierced, or shows other signs of damage.
- Do not use a Cartridge that shows any visible damage. Do not use a Cartridge that has been dropped or shaken. Shaking or dropping the Cartridge may yield invalid results.
- Cartridges and samples need to be treated on a clean and decontaminated
- Do not open the Cartridge lid until you are ready to perform a Test.
- During dispensing, make sure the pipette tip is positioned deep enough in the Cartridge opening to avoid liquid spills.
- Make sure you do not touch the lysis pad with the pipette, pipette tip or your fingers.
- Immediately safely dispose of the used pipette tip as hazardous waste in accordance with local procedures.
- Once the sample is inserted in the Cartridge, keep the Cartridge leveled.
- Do not try to reopen the Cartridge lid after inserting a sample and having closed the lid, nor after the Test run.
- Make sure the Cartridge is dry before loading into the Instrument.
- Do not reuse processed tests. Tests are for single use only.

7 Storage and Handling of Cartridges

Store the Idylla™ Ebola Virus Triage Test Cartridges at ambient temperature (15-30°C, 59-86°F). Make sure that Cartridges have reached a temperature of 15 to 30°C (59-86°F) before use. Unused Cartridges will be stable at ambient temperatures until the expiration date printed on the label, if stored in their sealed pouch under the recommended storage conditions.

CAUTION

- Use the Cartridge within 1 hour after opening the pouch.
- Do not use a Cartridge if its pouch is pierced, or shows other signs of visible damage.
- Do not use a Cartridge that has been dropped or that shows any visible damage.
- Do not open the Cartridge lid until you are ready to perform a Test.

8 Specimen Type, Storage and Preparation

Collect the venous whole blood specimens according to standard phlebotomy procedures. Guidelines for collection, transport, preparation and storage of specimens for molecular methods are available from the Clinical and Laboratory Standards Institute (Ref 3), WHO Guidance on Ebola specimens (Ref 4) and CDC guidance (Refs 5, 6).

8.1 Specimen Requirements

The Idylla™ Ebola Virus Triage Test is designed to process 200 µL of EDTA whole blood obtained by venipuncture.

CAUTION

Using a smaller amount of whole blood may cause the Test to be less accurate.

Using a larger amount of sample may cause a Test failure.

Do not use an inactivated sample (using Trizol or AVL) in the Idylla™ EBOV Test as it produces performan

8.2 Sample Storage and Preparation

The following statements apply to specimens that are used in the Idylla™ Ebo irus Triag est:

- Specimens should be collected in EDTA tubes, and stored according the man cturer's instructions for the specimen collection device and as adequate for the detection RNA (
- Shipping, if required, should be performed according to the po es of the s ing pe mer, customs regulations, and the requirements of the receiving laboratory (See Referen 6 or the follo - http://www.cdc.gov/vhf/ebola/healthcare-us/labora es/sp mens.html).
- Follow the recommended infection control precautions Ebo or othe rrhagic fever viruses in handling all specimens, e.g., WHO guidelines (Ref. 4) or loca uidelines

WARNING

Improper collection, storage, or transport specimens may d to the need for re-testing and loss of the Test specimen.

9 Perform a Test

9.1 Test Proced e

INFORMATI

Please refe he a™ Operat Manual for more extended information

The inst s belo ume tha e Idylla™ Instrument and Console are switched on, at least one Instrument is avail e for p essing, a the er has logged on to the Console.

T erform a T t, follow the steps:

	A N
1	ar open the Cartridge pouch and take out the Cartridge. **CAUTION** Process the Cartridge within 1 hour from opening the sealed pouch. Do not use Cartridges that have been dropped or are visually damaged Cartridges and samples need to be treated on a clean and decontaminated surface.
2	Press **New Test** on the Console.
3	Scan the barcode of the sample container using the Console barcode scanner. -OR- Manually fill in the sample ID in the corresponding field.

STEP	ACTION *(CONTINUED)*
4	Scan the barcode on top of the Cartridge using the Console barcode scanner.
5	Optionally, enter a comment to include in the Test request and Test result report.
6	Press **Confirm** to finalize the Test request.
7	Hold the Cartridge by its body and pull the lid open.
8	Remove the Cartridge clip.
9	Use a calibrated pipette to dispense **200 µL of the sample** into the Cartridge opening. Dispense the sample carefully **onto** the lysis pad which is located at the bottom of the Cartridge opening. CAUTION During dispensing, make sure the pipette tip is positioned deep enough through the Cartridge opening to avoid liquid spills. Make sure you do not touch the lysis pad with the pipette, pipette tip or your fingers. Do not exceed the allowed amount of specimen: 200 µl of EDTA whole blood. Overloading the Cartridge could lead to an erroneous or invalid result. Immediately safely dispose of the used pipette tip as hazardous waste in accordance with local procedures. Once the sample is inserted in the Cartridge, keep the Cartridge leveled.
10	Close the Cartridge by pushing the lid tight to ensure correct sealing. Gently pull the lid to confirm that it is locked. CAUTION Do not try to reopen the Cartridge lid after inserting a sample and closing the lid, nor after the Test run.
11	Wipe the surface of the Cartridge with a 10% bleach solution followed by a 70% EtOH solution to remove the remainders of the bleach solution. CAUTION Make sure the Cartridge is dry before loading it into the Instrument.
12	Choose an Instrument that is available for processing. TIP A blinking white light around the tray indicates the suggested Instrument for the Test run.
13	Open the Instrument tray by pushing the **open/close**-button on the tray.
14	Place the Cartridge on the tray.
15	Close the Instrument tray by pushing the open/close button on the tray. The Test starts automatically. The white light ring on the Instrument is constantly on.
16	Clean re-usable materials in the work environment that has come into contact with the sample using a tissue wetted with 10% diluted house-hold bleach, followed by wiping with a tissue wetted with 70% EtoH.
17	On the Console, the processing time is shown. INFORMATION The processing time for an Idylla™ Ebola Virus Triage Test is about 100 minutes.
18	When the Test is finalized, view the Test results on the Console.
19	Dispose of the used Cartridge in accordance with your laboratory's procedures. CAUTION Treat all biological specimens, including used Cartridges, as potentially infectious. Ebola specific guidelines are available from the Centers for Disease Control and Prevention (CDC, Ref 6). Guidelines for blood specimen handling, storage and disposal are available from the Clinical and Laboratory Standards Institute (Refs 1, 2, 3).

9.2 Quality Control

Each Idylla™ Ebola Virus Triage Test includes two integrated internal controls: a Sample Processing Control (SPC) and an Endogenous Control (EC). The SPC and EC are interpreted by the Ebola TTP software included data interpretation algorithm. Only if both controls pass the systems acceptance criteria will a result be provided, otherwise the sample will be called invalid.

INFORMATION

Refer to Summary and Explanation of the Test.

External Controls are not provided with the Test. External controls should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable. Negative whole blood patient specimens can be used as External Negative Controls. For clinical laboratories that need to verify test performance periodically with external controls, the following commercial Ebola reference material is available and may be used with the Test:

- Armored RNA reference materials for Ebola Zaire virus are available from Asuragen [illegible] (Austin, T[illegible]
- AccuPlex rEbola GP/NP Reference Material #0505-0001 is available from SeraCare [illegible]e Sciences. (Acc[illegible]ng to information provided by SeraCare, AccuPlex rEbola GP/NP Reference Material is desig[illegible]d to the 2014 Z[illegible]re strain of the virus. It is recombinant virus that includes sequences from the glyco[illegible]tein (GP), [illegible]eoprotein [illegible]P) and VP24 region (http://www.seracare.com/Products/AccuPlex%E2%84%A2rEbolaG[illegible] Referenc[illegible]aterial/tab[illegible]42/Default.aspx).)

For information on how to obtain optional external positive control ma[illegible]rials, cont[illegible] Biocartis [illegible]stomer Service at customerservice@biocartis.com or visit www.biocartis.com.

10 Interpretation of Results

The Test result output is qualitative and offers t[illegible]e possible re[illegible]s:

- Detected (EVD)
- Not detected (NEVD)
- Invalid

The Idylla™ System automatically interprets [illegible]e Idylla™ EBOV Test results and makes them available for viewing on the Console as follows:

DISPLAYED TEST RES[illegible]	INTERPR[illegible]N
Ebola Virus Dete[illegible]d	Ebola virus specific RNA detected by rt-PCR.
No Ebola Virus D[illegible]cted	Ebola virus specific RNA not detected by rt-PCR.
Invalid Rem[illegible]k: Repe[illegible] Test wi[illegible] n[illegible] Cartridge	Ind[illegible]tes that results obtained with the sample deviate from expectations. This may be [illegible]used by a variety of reasons such as: incorrectly stored Cartridges, Cartridges used that exceeded their in-use period after removal from the pouch, or Cartridge malfunctioning. If result is Invalid, no result can be reported and it is recommended to repeat the Test with a new Cartridge and a new sample of the same specimen.

11 Limitations

The following limitations apply to the Idylla™ Ebola Virus Triage Test:

- Testing with the Idylla™ Ebola Virus Triage Test should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens.
- Test results are for the presumptive identification of Ebola virus. The definitive identification of Ebola virus requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of Ebola virus must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of Ebola virus.
- Negative results do not preclude Ebola virus infection. Negative Test results should not be used as the sole basis for patient management decisions.
- The Idylla™ Ebola Virus Triage Test has been verified for use on the Idylla™ System only.
- The Idylla™ Ebola Virus Triage Test should be used in accordance with these instructions for use.
- To ensure reliable results, the Idylla™ System should be maintained as described by the manufacturer in the Operator Manual.
- The validated sample type for the Idylla™ Ebola Virus Triage Test is EDTA whole blood obtained by venipuncture originating from patients with signs and symptoms of Ebola virus infection in combination with clinical and epidemiological risk factors. Performance Characteristics with other sample types has not been established.
- Inactivation of the sample using Trizol or AVL prior to loading sample into the Idylla™ Ebola Virus Triage Test may lead to erroneous results.
- Deviation from EDTA whole blood as sample type may reduce test performance or lead to an erroneous or invalid result.
- The claimed specimen amount for the Idylla™ Ebola Virus Triage Test is 200µl +/-5% of whole blood. For samples that do not meet these criteria, Test results might be unreliable or results may be invalid.
- Improper specimen collection, handling, storage or transport may lead to false negative or invalid results and the need for re-testing and loss of the Test specimen.
- Potential mutations within the target regions of the virus genome covered by the Idylla™ Ebola Virus Triage Test may result in failure to detect the presence of the pathogen.
- Due to the difficulty in obtaining clinical specimens, the Test has not been evaluated with blood from infected Ebola patients.
- The Test has not been evaluated with blood from Ebola vaccinated patients. Samples from recently vaccinated individuals may result in false positive results due to potential cross-reactivity with self-replicating vaccines.
- The Idylla™ Ebola Virus Triage Test reacts with Ebola Zaire and Sudan but does not differentiate between Ebola Zaire and Ebola Sudan viruses and an Ebola Virus Detected (EVD) result.
- At clinically relevant levels (10 to 100 cfu/mL) no interference with *Candida glabrata* was observed; at very high concentrations [e.g. 1E+08 cfu/mL] *C. glabrata* could interfere with the detection of Ebola Zaire by the Test.

12 Performance

12.1 Analytical Sensitivity / Limit of Detection

12.1.1 Initial and Refined LOD

Analytical sensitivity (Limit of Detection, LOD) of the Idylla™ Ebola Virus Triage Test was evaluated by testing live Ebola Zaire (strain Makona) virus samples diluted into negative EDTA whole blood. Ebola Zaire samples were prepared from a viral stock of known concentration (3xE+07 pfu/mL) from which the expected concentration was calculated for each prepared dilution. In addition, the actual concentration of the samples was determined using the DoD Ebola Zaire (EZ1) rRT-PCR method on the ABI 7500 Fast Dx Real-Time PCR System (Life Technologies) .

The initial LOD was defined based on two titration experiments (table 1 LOD, initial estimation and table 2 LOD, refinement). After the refinement step, the refined LOD was found to be at 216 pfu/mL corresponding to 178 copies/mL (see table 2).

Table 1. Limit of Detection, Initial Estimation (ND: Not Detected)

Calculated Concentration (PFU/mL)	Calculated Concentration (Copies/mL)	Measured Concentration (Copies/mL)	Idylla™ EVD Rate
10 000	27 167	69 547	3/3
1 000	2 717	3 837	3/3
100	272	135	3/3
10	27	ND	1/3
1	3	ND	0/3

Table 2. Limit of Detection, Refinement

Calculated Concentration (PFU/mL)	Calculated Concentration (Copies/mL)	Measured Concentration (Copies/mL)	Idylla™ EVD Rate
2 150	5 841	4 521	3/
1 000	2 717	1 799	3/3
465	1 264	1 010	3/3
216	588	1	3/
101	273	80	2/3

12.1.2 LOD confirmation

The Confirmed LOD was determined by testing 24 live Ebola Zaire samples at 465 pfu/mL. This LOD experiment used the same virus stock from the initial and refined LOD estimation. The LOD was defined by the lowest concentration at which an EVD result was obtained in ≥ 95% of the samples tested.

For Ebola Zaire the LOD was confirmed at a concentration of 465 pfu/mL (1,010 copies/mL as measured by the EZ1 assay) with 24 out of 24 positive calls.

Table 3. Limit of Detection, Confirmation

Concentration (PFU/mL)	Idylla™ EVD Rate
	24/24

12.2 Analytical Reactivity

Analytical reactivity of the Idylla™ Ebola Virus Triage Test was evaluated by testing the following inactivated Ebola Zaire strains spiked into EDTA whole blood:

- Mayinga
- Kikwit
- GIN/2014/Gueckedou-C05
- GIN/2014/Gueckedou-C07
- GIN/2014/Kissidougou-C15

For all Ebola Zaire strains listed above, spiking was performed at 2x LOD.

All viruses were successfully detected at the lowest tested concentration of 2x LOD.

In addition, in silico analysis was performed to predict reactivity of the Idylla™ Ebola Virus Triage Test with various Ebola Zaire strains. Complete genome sequences from 14 different Ebola Zaire strains available in GenBank were tested. The analysis shows that the Idylla™ Ebola Virus Triage Test oligos and probes detect genomic sequences of the different strains for Ebola Zaire.

Table 4. Analytical Reactivity

Ebola Zaire virus Strains Tested	Specimen Type	Concentration	Idylla™ EVD Rate	*In Silico*
Gabon	Spiked in blood	2x LOD	6/6	No mismatch
Gueckedou-C05	Spiked in blood	2x LOD	6/6	No mismatch
Gueckedou-C07	Spiked in blood	2x LOD	6/6	No mismatch
Kissidougou-C15	Spiked in blood	2x LOD	6/6	No mismatch
Mayinga	Spiked in blood	2x LOD	6/6	No mismatch
Makona-G3707*				No mismatch
Makona-201403007*				No mismatch
Makona-NM042.1*				No mismatch
Makona-G3822*				No mismatch
Makona-GE1*				No mismatch
Makona-Mali-DPR4*				No mismatch
Bonduni*				No mismatch
Luebo*				No mismatch
Lomela-Lokolia19*				No mismatch

**Organisms were tested by in silico analysis only*

12.3 Analytical Specificity

The cross-reactivity of the Idylla™ Ebola Virus Triage Test was evaluated by testing human genomic DNA and a total of 38 organisms, including Ebola viruses. Purified nucleic acids, plasmids and culture-derived materials from various pathogens were used for testing.

The Idylla™ Ebola Virus Triage Test shows no cross-reactivity (no positive EVD result for Ebola) with any of the unrelated tested pathogens that do not belong to the Ebola family (other viruses, bacteria and fungi) (see table 5). However, cross reactivity can be detected with Ebola Sudan because the original device design contains an Ebola Sudan probe that specifically detects Ebola Sudan. Performance characteristics of the Idylla™ System have not been sufficiently evaluated for the detection of Ebola Sudan.

Table 5. Cross-Reactivity Testing

Organism	Specimen	Concentration	Unit/200µL loaded in Cartridge	N	Cross-Reactivity Results
Acinetobacter baumannii	Nucleic Acids	4.76E+09	cfu	3	0
Aspergillus fumigatus	Nucleic Acids	2.00E+05	copies	3	0
Bundibugyo Ebolavirus virus	Nucleic Acids	90	copies	3	0
Candida albicans	Nucleic Acids	3.63 - 4.27	µg	3	0
Candida glabrata	Nucleic Acids	2.59E+08	cfu	3	0
Candida krusei	Nucleic Acids	2.00E+05	copies	3	0
Crimean-Congo hemorrhagic virus	Nucleic Acids	5.00E+04	copies	3	0
Dengue virus subtype1	Nucleic Acids	1.86E+05	CCID50	3	0
Dengue virus subtype2	Nucleic Acids	1.72E+06	CCID50	3	0

Cross-reactivity testing (continued)

Organism	Specimen	Concentration	Unit/200µL loaded in Cartridge	N	Cross-Reactivity Results
Dengue virus subtype3	Nucleic Acids	1.86E+05	CCID50	3	0
Dengue virus subtype4	Nucleic Acids	1.00E+06	CCID50	3	0
Ebola Sudan virus (Gulu Strain)*	Live Virus stock	1641	copies	23	22
Ebola Sudan virus (Gulu Strain)*	Inactivated Virus stock	4.00E+03 (2x LOD)	copies	6	6
Enterecoccus faecium	Nucleic Acids	1.10E+09	cfu	3	0
Enterococcus faecalis	Nucleic Acids	3.22E+09	cfu	3	0
Escherichia coli	Nucleic Acids	2.00E+05	copies	3	0
Hepatitis B	Nucleic Acids	4.20E+06	copies	3	0
Hepatitis C	Replicon cell stock	2.80E+03	cells	3	0
Human DNA	Whole blood	N/A	µg	73	0
Human immunodeficiency virus-1	Culture stock	3.05E+05	CCID50	3	0
Influenza A	Culture stock	6.74E+05	copies	3	0
Influenza B	Culture stock	1.26E+07	copies	3	0
Klebsiella oxytoca	Nucleic Acids	2.00E+05	copies	3	0
Klebsiella peumoniae	Nucleic Acids	2.00E+05	copies	3	0
Lassa virus	Nucleic Acids	2.20E+05	copies	3	0
Marburg virus Musoko	Plasmid	6	ng	3	0
Marburg virus Ravn	Nucleic Acids	1.00E+07	copies	3	0
Marburg virus Voege	Nucleic Acids	1,46E+09	copies	3	0
Plasmodium falciparum	Nucleic Acids	3.14E+06	copies	3	0
Plasmodium vivax	Nucleic Acids	1581	ng	3	0
Pseudomonas aeruginosa	Nucleic Acids	2.00E+05	copies	3	0
Reston Ebolavirus	Nucleic Acids	1.84E+04	copies	3	0
Rift Valley fever virus	Nucleic Acids	9.00E+05	copies	3	0
Salmonella enterica Typhimurium	Nucleic Acids	16.4	µg	3	0
Schistosoma mansoni	Nucleic Acids	0.1	µg	3	0
Staphylococcus aureus	Nucleic Acids	2.00E+05	copies	3	0
Staphylococcus epidermis	Nucleic Acids	2.00E+05	copies	3	0
Streptococcus pneumoniae	Nucleic Acids	2.00E+05	copies	3	0
Tai Forest Ebola virus	Nucleic Acids	2.60E+04	copies	3	0
Trypanosoma brucei gambiense	Nucleic Acids	603	ng	3	0

** Patients infected with Ebola Sudan virus may lead to EVD call when tested with Idylla™.*

12.3.1 In Silico Cross-Reactivity

Cross-reactivity was evaluated in silico by aligning the primer and probe sequences with the complete human genome sequence and for the organisms listed below (see table 6). The potential to generate positive amplification (amplicon) was assessed to identify potential cross-reactivity. No amplicon was detected for any of the species assessed in the in silico analysis, suggesting that cross-reactivity is unlikely to occur. Microbial interference for Candida glabrata and other organisms was further tested (please see section Microbial Interference below).

Table 6. In silico cross-reactivity: tested organism

ORGANISMS			
Acinetobacter baumannii	*Adenovirus*	*Aspergillus fumigatus**	*Borellarecurrentis*
Bundibugyo Ebola virus	*Candida albicans*	*Candida glabrata**	*Candida krusei*
Chikungunya virus	*Coxiella burnetti*	*Crimean-Congo hemorrhagic virus*	*Dengue virus Subtype*
Dengue virus Subtype 2	*Dengue virus Subtype 3*	*Dengue virus Subtype 4*	*Enterococcus faecalis*
Enterococcus faecium	*Enterovirus*	*Escherichia coli**	*Hemophilus influenza*
Hepatitis A	*Hepatitis B*	*Hepatitis C*	*Human genome*
Human immunodeficiency virus-1	*Influenza virus A*	*Influenza virus B*	*Klebsiella oxytoca**
Klebsiella pneumoniae*	*Lassa virus*	*Leptospira genus*	*Marburg virus Ravn*
Marburg-Ci67	*Marburg-Musoke*	*Neisseria meningitidis*	*Pichia kudriavzevii*
Plasmodium falciparum*	*Plasmodium malariae*	*Plasmodium vivax**	*Pseudomonas aeruginosa**
Respiratory syncytial virus	*Reston Ebola virus*	*Rickettsia africae*	*Rickettsia conorii*
Rickettsia prowazekii	*Rickettsia typhi*	*Rift Valley Fever virus*	*Rotavirus*
Salmonella enterica typhimurium*	*Schistosoma mansoni*	*Shigella dysenteriae*	*Stahpylococcus aureus*
Staphylococcus epidermidis	*Staphylococcus saprophyticus*	*Streptococcus pneumonia*	*Streptococcus pyogenes*
Tai Forest Ebola virus	*Trypanosoma brucei brucei treu927*	*Trypanosoma brucei gambiense*	*Vibrio cholera*
Yersinia enterocolitica	*Yersinia pestis*		

** Organism had some degree of homology with either primers or probes. While the organisms DNA sequence theoretically does not give rise to an amplicon, interference was tested in additional studies.*

12.3.2 Microbial Interference

The microbial interference of the Idylla™ EBOV Test was evaluated by spiking nucleic acids, from non-Ebola pathogens for which oligo binding has been observed in silico, at highest concentration possible in a sample containing Ebola Zaire virus target RNA at a concentration of 3x LOD.

Table 7. Microbial Interference Testing

Sample Name	Concentration of Pathogen	Interference
Aspergillus fumigatus	5,00E+05 copies/mL	No
Candida glabrata*	6,45E+08 cfu/mL	Yes
Candida glabrata*	100 cfu/mL	No
Candida glabrata*	10 cfu/mL	No
Escherichia coli	5,00E+05 copies/mL	No
Haemophilus influenza	98 µg/mL	No
Klebsiella oxytoca	5,00E+05 copies/mL	No
Klebsiella pneumonia	5,00E+05 copies/mL	No
Plasmodium falciparum	7,85E+06 copies/mL	No
Plasmodium vivax	3,95 µg/mL	No
Pseudomonas aeruginosa	5,00E+05 copies/mL	No
Salmonella typhimurium	41,1 µg/mL	No
Schistosoma mansoni	0,25 µg/mL	No

**At clinically relevant levels (10 to 100 cfu/mL) no interference with C. glabrata was observed; at very high concentrations [e.g., 6,45E+08 cfu/mL] C. glabrata may interfere with the device.*

12.4 Mock Clinical

In the absence of access to prospective clinical samples, a clinical evaluation of positive percent agreement (PPA), negative percent agreement (NPA) was performed using contrived Ebola Zaire samples. The samples were prepared by spiking known concentrations of a live Ebola Zaire virus stock, Makona strain (3.00E+07 PFU/mL or 8.15E+07 copies/mL) in EDTA whole blood from individual healthy subjects (see tables 8 and 9). Idylla™ Ebola Virus Triage Test results were determined as described in the section Interpretation of Results above. Invalid samples were excluded from analysis. Per the mock clinical results (table 9), the Idylla™ EBOV Test demonstrated a PPA of 97.3% and an NPA of 100%.

Table 8. Summary of the mock clinical performance study with Ebola Zaire samples.

		Concentration (PFU/mL)	EVD	NEVD	Invalid
Negative	(n=49)	ND	0	46	3
Positive	10x LOD (n=22)	46500	22	0	0
	3x LOD (n=21)	1395	20	1	1
	1.5x LOD (n=32)	698	29	1	2
Total			71	48	6

Table 9. Agreement Analysis

Idylla™ Result	Positive	Negative	Agreement	
			Point Estimate	95% CI
EVD	71	0	PPA 97.3% (71/73)	90.6% - 99.3%
NEVD	2	46	NPA 100% (46/46)	92.3% - 100%
Total	73	46		

13 References

Ref n°	Detail
1	Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Waste Management;Approved Guideline – Third Edition. CLSI document GP05-A3 [ISBN 1-56238- 744-8]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA (2011)
2	Clinical and Laboratory Standards Institute (CLSI). Protection of lab workers from occupational acquired infections; Approved Guideline – Fourth Edition. CLSI document M29-A4 [ISBN 1-56238-961-0]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA (2005)
3	Clinical and Laboratory Standards Institute (CLSI). MM13-A -- Collection Transport Pr ration an rage of Specimens for Molecular Methods; Approved Guideline. CLSI document MM13 [ISBN 1-56238-5 7]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wa Pennsylvania 19087-1898 USA (2005)
4	WHO. Laboratory diagnosis of Ebola virus disease, WHO/EVD/GUIDA /LAB/14.1 http://apps.who.int/iris/bitstream/10665/134009/1/WHO_EVD_G DANCE_LA 14.1_eng.p
5	CDC Guidance for U.S. Laboratories for Managing and Testing outine Specimens hen There is a Concern About Ebola Virus Disease, http://www.cdc.gov/vhf/ bola/healt e-us/lab tories/safe-specimen-management.htmL.
6	CDC Guidance for Collection, Transport and Submiss of Sp ens for Ebola Virus Testing, http://www.cdc.gov/vhf/ebola/healthcare-us/laborato /s cimens.ht
7	Universal Precautions, Infection Control f rrhag vers in the African Health Care Setting and with Information for Healthcare W ker in the U State (http://www.cdc.gov/vhf/ebola/healt are-us/index.htm depending upon their location of testing

14 Commonly Used Symbols

SYMBOL	USED FOR
	Catalog Number
	Manufacturer
	Temperature limit
	Use by Date
	Batch Code
	Consult Instructions for Use
	Contains sufficient for <n> Tests
	Do not reuse
	Do not use if package is damaged
	Patient number (indicates location on Cartridge where sample ID can be added)
	CE mark
	*In vitro* diagnostic medical device
GTIN	Global Trade Item Identifier (Global Trade Identification Number)
	Key code icon (keycode). Use the code printed next to this icon when you want to obtain user documentation
	Emergency Use Authorization. For use under Emergency Use Authorization (EUA) only.

15 Contact Information

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Documentation	All of the available user documentation can be obtained from the Biocartis website or the freephone numbers included on the Safety Sheet. www.biocartis.com/support • Subscribe as a customer to get access to the Biocartis Secure Area and log in • Find all documentation and help on the Secure Area site (IFU, other information) www.biocartis.com/ifu • Repository of all Idylla IFU and Operator Manual • Use the keycode on the product labeling

1 05/2016 First version for FDA

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Quick Reference Guide

Idylla™ Ebola Virus Triage Test

SPECIMEN REQUIREMENTS

- EDTA venous whole blood
- 200µL

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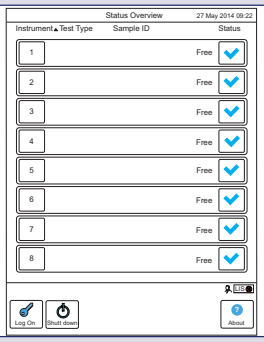

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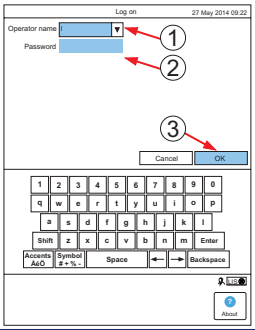

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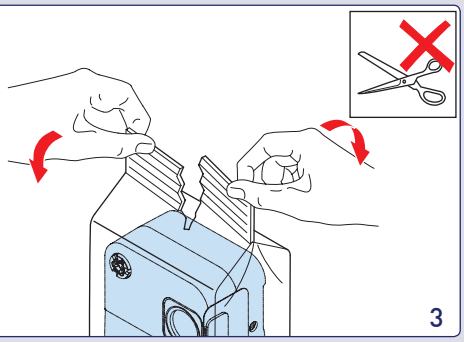

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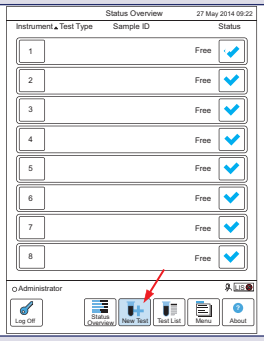

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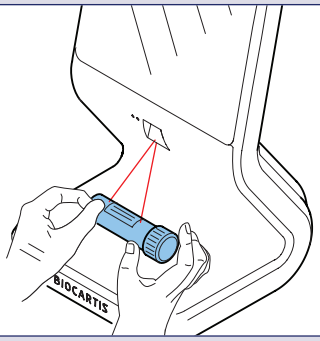

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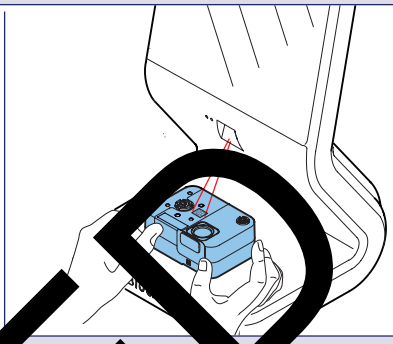

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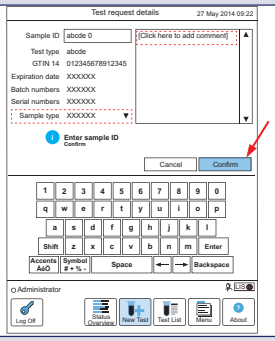

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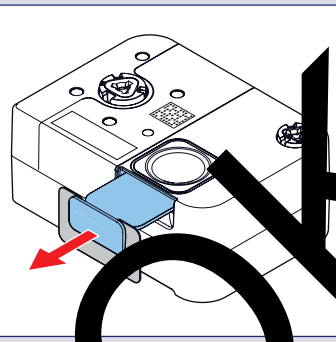

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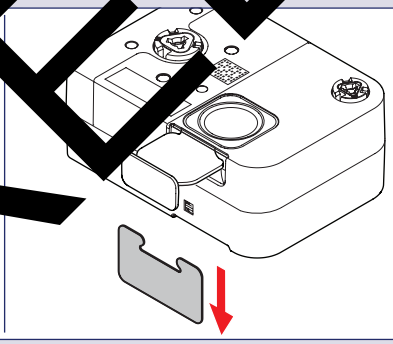

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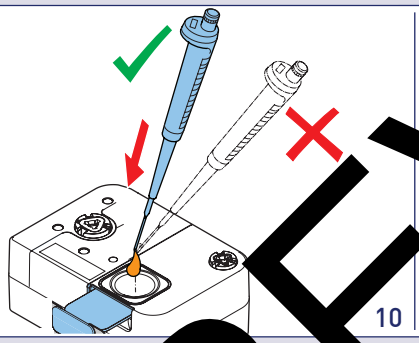

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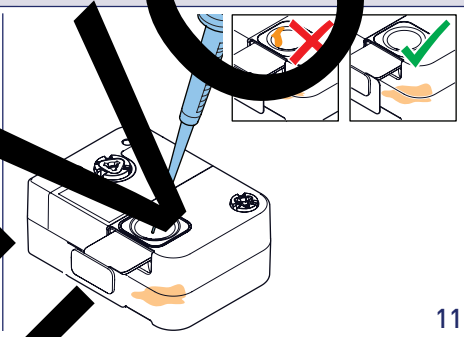

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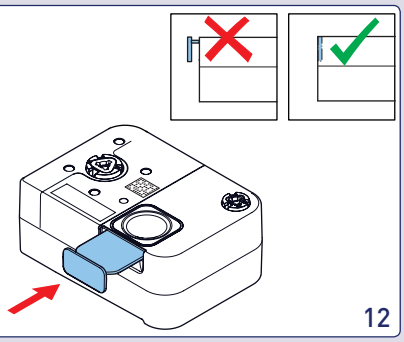

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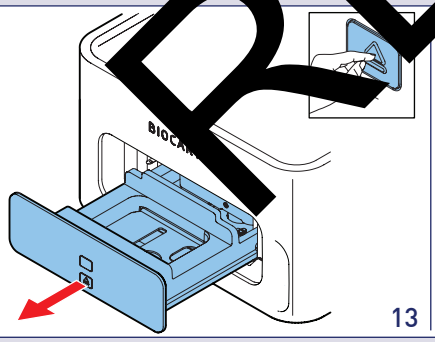

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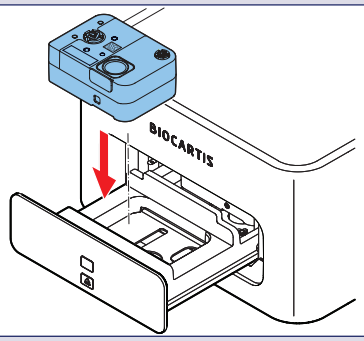

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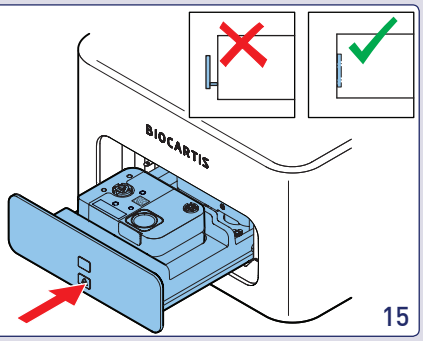

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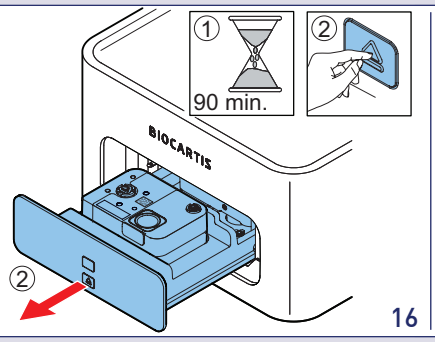

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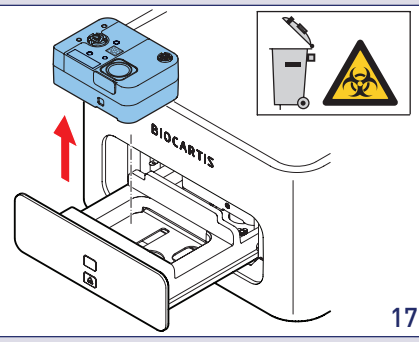

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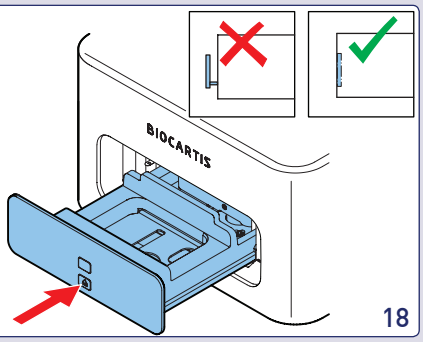

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