## Annex 1. Predictive values of Ebola RDTs and implications for decision-makers

Based on the currently available evidence rapid antigen detection tests have variable sensitivity and therefore a negative test result, in certain circumstances, cannot exclude Ebola infection. Furthermore, test specificity also varies and therefore a positive test result may be a false positive result. Both false negative and false positive results for Ebola have serious implications for individuals/patients and public health.

However, sensitivity and specificity of a test are not the only parameters of importance in evaluating its appropriateness for use. The prevalence (% of true positives) of the disease is important in assessing its positive and negative predictive value (PPV and NPV, respectively) .The predictive value of the same test can differ between different populations in the same country or within a district.

**Table A1. PPV and NPV of an antigen-based RDT with sensitivity=92%, specificity=85%**

| Prevalence | PPV | NPV | False Negatives | False Positives |
|---|---|---|---|---|
| 100.0% | 100.0% | 0.0% | 8.0% | 0.0% |
| 90.0% | 98.2% | 54.1% | 7.2% | 1.5% |
| 80.0% | 96.1% | 72.6% | 6.4% | 3.0% |
| 70.0% | 93.5% | 82.0% | 5.6% | 4.5% |
| 60.0% | 90.2% | 87.6% | 4.8% | 6.0% |
| 50.0% | 86.0% | 91.4% | 4.0% | 7.5% |
| 40.0% | 80.3% | 94.1% | 3.2% | 9.0% |
| 30.0% | 72.4% | 96.1% | 2.4% | 10.5% |
| 20.0% | 60.5% | 97.7% | 1.6% | 12.0% |
| 10.0% | 40.5% | 99.0% | 0.8% | 13.5% |
| 5.0% | 24.4% | 99.5% | 0.4% | 14.3% |
| 2.5% | 13.6% | 99.8% | 0.2% | 14.6% |
| 1.0% | 5.8% | 99.9% | 0.1% | 14.9% |
| 0.5% | 3.0% | 100.0% | 0.0% | 14.9% |
| 0.2% | 1.2% | 100.0% | 0.0% | 15.0% |
| 0.1% | 0.6% | 100.0% | 0.0% | 15.0% |

### Causes and operational implications of negative and positive RDT results

**False negative** results are most likely linked to the inability of the assay to detect a low viral copy number (assuming test is well performed; other less likely causes could be interfering substances); the false negative status is likely to be resolved by retesting the patient at a later stage (at 24/48/72 hours, if symptoms persist; and preferably refer samples from symptomatic suspect patients with negative RDT for PCR testing).

**False positive** results are most likely linked to cross-reactivity or other non-specific binding of reagents in the test to an non-Ebola antigen in the sample; false positive results are less likely to be resolved over time, as the cause of false positivity will remain.

### Advice concerning use of RDTs in a low prevalence epidemiological context

For an assay with performance characteristics similar to the example given above the number of false positive results will become higher than the number of true positives as the prevalence of infection (% of true positives) falls. There is a risk that use of such a test in this epidemiological context will undermine trust in the testing procedures and in the broader public health response. Therefore widespread use of RDTs that do not meet the WHO Target Product Profile is not recommended. Special settings where use of such RDTs for Ebola may be beneficial are given in other WHO guidance[1].

[1] 'Interim guidance on the use of rapid Ebola antigen detection tests', Geneva, World Health Organization, March 2015. Available online at http://www.who.int/csr/resources/publications/ebola/ebola-antigen-detection/en/

## Annex 2. Considerations for the selection of PCRs for the diagnosis of Ebola virus disease

### Table 1a: IVDs with WHO Emergency Use and Assessment Listing (EUAL)
### Version: 09 June 2015

The WHO EUAL assessment comprises WHO assessment of manufacturing capacity and quality management systems, documentary evidence of safety and performance, independent verification of analytical sensitivity (LOD)

| Assay name | RealStar® Filovirus RT-PCR Kit 1.0 (CE-IVD) | RealStar® Ebolavirus RT-PCR Kit 1.0 (FDA EUA)* | Liferiver™ Ebola virus (EBOV) real time RT-PCR kit | Xpert® Ebola Assay (Also FDA EUA)** |
|---|---|---|---|---|
| **Manufacturer** | altona Diagnostics GmbH | altona Diagnostics GmbH | Shanghai ZJ Bio-Tech Co., Ltd. | Cepheid |
| **Kit format** | One step RT PCR assay | One step RT PCR assay | One step RT PCR assay | Integrated cartridge |
| **Kit size** | 96 reactions | 96 reactions | 25 reactions | 10 reactions |
| **Kit components** | Master A and B mixes<br>Internal control<br>EBOV Positive control<br>MARV Positive control<br>Molecular grade water | Master A and B mixes<br>Internal control<br>EBOV Positive control<br>Molecular grade water | EBOV super mix<br>RT PCR enzyme mix<br>Internal control<br>Positive control<br>Molecular grade water | Cartridge with all reagents on board. Inactivation solution. Swab.<br>Transfer pipettes<br>Ebola sample reagent box |
| **Viruses detected** | Zaire ebolavirus (ZEBOV)<br>Bundibugyo ebolavirus (BEBOV)<br>Reston ebolavirus (RESTV)<br>Sudan ebolavirus (SEBOV)<br>Tai Forest ebolavirus (TAFV)<br>Marburg virus (MARV) | Zaire ebolavirus (ZEBOV)<br>Bundibugyo ebolavirus (BEBOV)<br>Reston ebolavirus (RESTV)<br>Sudan ebolavirus (SEBOV)<br>Tai Forest ebolavirus (TAFV) | Zaire ebolavirus (ZEBOV),<br>Sudan ebolavirus (SUDV),<br>Taï Forest ebolavirus (TAFV),<br>Bundibugyo ebolavirus (BDBV) | Zaire ebolavirus (ZEBOV) |
| **Gene Target** | L | L | NP | NP, GP |
| **Laboratory instruments required but not provided with kit/assay/** | Real time PCR platform<br>Specify instruments:<br>- Mx 3005P™ QPCR System (Stratagene)<br>- VERSANT™ kPCR Molecular System AD (Siemens)<br>- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied | Real time PCR instrument<br>- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)<br>- LightCycler® 480 Instrument II (Roche)<br>- CFX96 system/Dx real-time | Real time PCR platform:<br>Bio-Rad CFX 96; SLAN®-96; ABI Prism®7500; LightCycler®480 Instruments<br>Desktop centrifuge<br>Plate centrifuge<br>Vortex mixer<br>Micropipettes | GeneXpert Dx System or GeneXpert Infinity systems<br>Vortex mixer<br>Micropipettes<br>Powder free gloves<br>Biosafety Level 3 laboratory |

| Assay name | RealStar® Filovirus RT-PCR Kit 1.0 (CE-IVD) | RealStar® Ebolavirus RT-PCR Kit 1.0 (FDA EUA)* | Liferiver™ Ebola virus (EBOV) real time RT-PCR kit | Xpert® Ebola Assay (Also FDA EUA)** |
|---|---|---|---|---|
| | Biosystems)<br>- LightCycler® 480 Instrument II (Roche)<br>- Rotor-Gene™ 3000/6000 (Corbett Research)<br>- Rotor-Gene Q5/6 plex Platform (QIAGEN)<br>- CFX96 system/Dx real-time system (BIORAD)<br>Desktop centrifuge<br>Plate centrifuge<br>Vortex mixer<br>Micropipettes<br>Barrier pipette tips<br>Powder free gloves<br>96 well reaction plates<br>Nuclease-Free Water<br>Biosafety Level 3 laboratory | system (BIORAD)<br>Desktop centrifuge<br>Plate centrifuge<br>Vortex mixer<br>Micropipettes<br>Barrier pipette tips<br>Powder free gloves<br>96 well reaction plates<br>Nuclease-Free Water<br>Biosafety Level 3 laboratory | Barrier pipette tips<br>Powder free gloves<br>Biosafety Level 3 laboratory | |
| **Other materials required but not provided** | QIAamp viral RNA mini Kit (Qiagen)<br>96 well plates or reaction tubes and suitable optical seal | QIAamp viral RNA mini Kit (Qiagen)<br>96 well plates or reaction tubes and suitable optical seal | RNA Isolation kit (ZJ Biotech), or<br>QIAamp viral RNA mini Kit (Qiagen)<br>96 well plates or reaction tubes and suitable optical seal | Chlorine |
| **Laboratory space** | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification | Specimen inactivation should be performed under enhanced biosafety conditions at the site of collection |
| **Electricity only** | Yes | Yes | Yes | Yes |
| **Equipment maintenance** | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment | Annual calibration self-check |
| **Technical support by manufacturer** | Required | Required | Required | Required |

| Assay name | RealStar® Filovirus RT-PCR Kit 1.0 (CE-IVD) | RealStar® Ebolavirus RT-PCR Kit 1.0 (FDA EUA)* | Liferiver™ Ebola virus (EBOV) real time RT-PCR kit | Xpert® Ebola Assay (Also FDA EUA)** |
|---|---|---|---|---|
| **Specimen inactivation using enhanced biosafety required by user prior to performing the test** | Specimen inactivated by addition of lysis buffer. | Specimen inactivated by addition of lysis buffer. | Yes | Specimen added to Sample Reagent bottle that contains lysis buffer. |
| **Nucleic acid extraction required by user** | Yes | Yes | Yes | No |
| **Suitable specimen for testing** | Plasma collected in EDTA, cell free-body fluids, swab washes | Plasma collected in EDTA | Venous whole blood or plasma collected in EDTA, serum | Venous whole blood collected in EDTA |
| **Storage conditions for reagents** | -20°C (requires cold chain) | -20°C (requires cold chain) | -20°C (requires cold chain) | 2-8°C (requires cold chain) |
| **Recommended time to process the specimen after collection** | Plasma or serum stored at 2-8°C for up to 6 hours<br>Freezing at -20°C or -80°C in aliquots recommended for long-term storage | Plasma stored at 2-8°C for up to 6 hours<br>Freezing at -20°C or -80°C in aliquots recommended for long-term storage | Plasma or serum stored at 2-8°C for up to 6 hours<br>Freezing at -20°C or -80°C in aliquots recommended for long-term storage | Fresh blood specimen should be processed immediately<br>The Reagent-treated blood specimens may be stored for 72hrs at 2-8C, for 48hrs at 8-30C or 24hrs at 28-35C |
| **WHO evaluation: Limit of Detection (LOD)** | "The LoD was not assessed by WHO during EUAL assessment of the product. However the manufacturer claim is 1.39 copies/µl specimen eluate as determined by probit analysis using *in-vitro* transcribed RNA without taking into account the RNA extraction procedure. FDA EUA data shows a sensitivity of 1 PFU Zaire ebolavirus/ml plasma (1 PFU = approx. 3.400 copies)." | "The LoD was not assessed by WHO during EUAL assessment of the product. However the manufacturer claim is 1 PFU Zaire ebolavirus/ml plasma (1 PFU = approx. 3.400 copies) as determined during the FDA Emergency Use Authorization process." | 4.23 $10^4$ copies /ml blood | (1.34 – 4.23) $10^3$ copies /ml blood |
| **Throughput: Time to results** | 4-6 hours for a negative, less for a positive | 4-6 hours for a negative, less for a positive | 4-6 hours for a negative, less for a positive | 90 minutes |
| **Maximum number of specimens per day/instrument** | Approximately 50 specimens | Approximately 50 specimens | Approximately 180 specimens | Depending on version of the instrument |

| Assay name | RealStar® Filovirus RT-PCR Kit 1.0 (CE-IVD) | RealStar® Ebolavirus RT-PCR Kit 1.0 (FDA EUA)* | Liferiver™ Ebola virus (EBOV) real time RT-PCR kit | Xpert® Ebola Assay (Also FDA EUA)** |
|---|---|---|---|---|
| | | | | 24 - 96 |
| **Reagents shelf life** | 9 months | 9 months | 12 months | 12-24 months (expected from accelerated stability testing) |
| **Minimum specimen volume** | 140 µL plasma for nucleic acid extraction | 140 µL plasma for nucleic acid extraction | 140-200 µL blood, plasma or serum for nucleic acid extraction | 100 µL whole blood |
| **Limitations of the assay** | Presence of RT-PCR inhibitors may cause invalid results.<br>Potential for false positives due to amplicon carry-over | Presence of RT-PCR inhibitors may cause invalid results.<br>Potential for false positives due to amplicon carry-over | Presence of RT-PCR inhibitors may cause invalid results. Potential for false positives due to amplicon carry-over | Presence of RT-PCR inhibitors may cause invalid results. |
| **Trained manpower** | Laboratory technicians<br>Biosafety training<br>Donning and doffing PPE | Laboratory technicians<br>Biosafety training<br>Donning and doffing PPE | Laboratory technicians<br>Biosafety training<br>Donning and doffing PPE | Trained health workers<br>Biosafety training<br>Donning and doffing PPE |
| **Biosafety requirements** | Specimen inactivation should be performed under BSL3 conditions | Specimen inactivation should be performed under BSL3 conditions | Specimen inactivation should be performed under BSL3 conditions | Specimen inactivation should be performed under BSL3 conditions |
| **Waste management** | Follow national guidelines | Follow national guidelines | Follow national guidelines | Follow national guidelines |

*FDA EUA restricts the use of this assay to CLIA High Complexity Laboratories and Similarly Qualified Non-U.S. Laboratories
**FDA EUA restricts the use of this assay to CLIA Moderate and High Complexity Laboratories or in similarly qualified non-U.S. laboratories
Information on certain characteristics listed in the Table will be updated by WHO as more data become available

**Table 1b: IVDs with US FDA Emergency Use Authorization (FDA EUA)**
**Version: 09 June 2015**

The FDA EUA assessment comprises FDA assessment of documentary evidence of safety and performance, requirements for reporting of performance issues, and control of end users.

| Assay name | FilmArray Biothreat-E test | FilmArray NGDS BT-E Assay | LightMix® Ebola Zaire rRT-PCR Test | EZ1 Real-time RT PCR Assay | CDC Ebola Virus NP Real-time RT-PCR Assay | CDC Ebola Virus VP40 Real-time RT-PCR Assay |
|---|---|---|---|---|---|---|
| **Manufacturer** | BioFire, Biomerieux | BioFire Defense, LLC for US Dept. of Defense | Roche | US Dept. of Defense<br>(in house assay) | US CDC<br>(in house assay) | US CDC<br>(in house assay) |
| **Kit format** | Integrated film pouch<br>Multiplex assay | Integrated film pouch<br>Multiplex assay | One step RT PCR assay | One step RT PCR assay | One step RT PCR assay | One step RT PCR assay |
| **Kit size** | 6 reactions | 30 reactions | 96 reactions | 40 reactions | 500 reactions | 500 reactions |
| **Kit components** | Test pouches<br>Single-use sample buffer ampoules<br>Single-use freeze-dried protease vials<br>Single-use pre-filled hydration injection vials<br>Single-use sample injection vials<br>Transfer pipettes | Test pouches<br>Single-use sample buffer ampoules<br>Single-use freeze-dried protease vials<br>Single-use pre-filled hydration injection vials<br>Single-use sample injection vials<br>Transfer Pipettes | EBOV primers and probes<br>Amplification control and primers/probes<br>EBOV positive template control | EZ1 master mix<br>RNAseP master mix<br>RT-Taq polymerase<br>EZ1 Positive Template Control<br>RNAseP (RP) control<br>RP Positive Template Control | Ebola Virus NP Real-time RT-PCR Primer and Probe Set (includes 2 sets of primers and probes: the NP2 set and the RNase P set) | Ebola Virus VP40 Real-time RT-PCR Primer and Probe Set (includes 2 sets of primers and probes: the VP40 set and the RNase P set) |
| **Viruses Detected** | ZEBOV | ZEBOV | ZEBOV | ZEBOV | ZEBOV | ZEBOV |
| **Gene Target** | L | NP | L | GP | NP | VP40 |
| **Laboratory instruments required but not provided with kit/assay** | FilmArray instrument with laptop computer<br>FilmArray Pouch loading station compatible with use of FilmArray injection vials | FilmArray instrument with laptop computer<br>FilmArray Pouch loading station compatible with use of FilmArray injection vials | PCR platform - LightCycler® 480 II Instrument or cobas z 480 Analyzer<br>LightCycler® Software (Version 1.5 or higher) or cobas z 480 Software (Version 1.5 or higher with UDF Version 1.0 or | Real time PCR platform - Applied Biosystems 7500 Fast Dx Real-time PCR Systems, Roche LightCycler, or BioFire Defense Joint Biological Agent Identification and Diagnostic System (JBAIDS) | Real-time PCR instrument - Applied Biosystems 7500 Fast Dx Real-time PCR Systems or Bio-Rad CFX96 Touch Real-time PCR Detection<br>Extraction instrument - Life Technologies Dynal | Real-time PCR instrument - Applied Biosystems 7500 Fast Dx Real-time PCR Systems or Bio-Rad CFX96 Touch Real-time PCR Detection<br>Extraction instrument - Life Technologies Dynal |

| Assay name | FilmArray Biothreat-E test | FilmArray NGDS BT-E Assay | LightMix® Ebola Zaire rRT-PCR Test | EZ1 Real-time RT PCR Assay | CDC Ebola Virus NP Real-time RT-PCR Assay | CDC Ebola Virus VP40 Real-time RT-PCR Assay |
|---|---|---|---|---|---|---|
| | | | higher)<br>Extraction Instrument - MagNA Pure 96 Instrument<br>LightCycler® 480 Multiwell Plate 96 white, with seals<br>Barrier pipette tips<br>RNase, DNase free-disposable plasticware<br>Plate centrifuge<br>Vortex mixer<br>Microfuge<br>TriPure Isolation Reagent<br>Microfuge tubes, 1.5-ml | Desktop centrifuge<br>Plate centrifuge<br>Vortex mixer<br>Micropipettes<br>Barrier pipette tips<br>Powder free gloves<br>Heat block or water bath<br>Biosafety cabinet<br>Freezer, -20°C<br>Refrigerator, 2-8°C | BeadRetriever System or MagMAX Express-96 Deep Well Magnetic Particle Processor<br>Powder-free gloves and surgical gowns<br>Aerosol barrier pipette tips<br>Microcentrifuge tubes<br>Vortex mixer<br>Microcentrifuge<br>Micropipettes<br>Multichannel micropipettes<br>Racks for 1.5 mL microcentrifuge tubes<br>2 x 96-well -20 °C cold blocks<br>PCR reaction Optical Adhesive Film Kit | BeadRetriever System or MagMAX Express-96 Deep Well Magnetic Particle Processor<br>Powder-free gloves and surgical gowns<br>Aerosol barrier pipette tips<br>Microcentrifuge tubes<br>Vortex mixer<br>Microcentrifuge<br>Micropipettes<br>Multichannel micropipettes<br>Racks for 1.5 mL microcentrifuge tubes<br>2 x 96-well -20 °C cold blocks<br>PCR reaction Optical Adhesive Film Kit |
| **Other materials required but not supplied** | Bleach<br>De-ionized water | Bleach<br>De-ionized water | LightCycler® Multiplex RNA Virus Master<br>MagNA Pure 96 DNA and Viral NA Small Volume Kit<br>MagNA Pure 96 Tips<br>MagNA Pure 96 Processing Cartridges<br>MagNA Pure 96 Output Plate<br>MagNA Pure 96 Sealing Foil<br>MagNA Pure 96 System Fluid | QIAamp viral RNA mini Kit-(Qiagen)<br>96 well plates or reaction tubes and suitable optical seal<br>TRIzol LS reagent or TRI Reagent LS | EBOV NP rRT-PCR Assay Positive Control<br>Human Specimen Control SuperScriptTM III Platinum® One-Step qRT-PCR Kit<br>Molecular grade water, nuclease-free<br>Extraction reagents<br>MagMax Pathogen RNA/DNA kit<br>Isopropanol<br>Ethanol<br>DNA Away™ | EBOV VP40 rRT-PCR Assay Positive Control<br>Human Specimen Control SuperScriptTM III Platinum® One-Step qRT-PCR Kit<br>Molecular grade water, nuclease-free<br>Extraction reagents<br>MagMax Pathogen RNA/DNA kit<br>Isopropanol<br>Ethanol<br>DNA Away™ |

| Assay name | FilmArray Biothreat-E test | FilmArray NGDS BT-E Assay | LightMix® Ebola Zaire rRT-PCR Test | EZ1 Real-time RT PCR Assay | CDC Ebola Virus NP Real-time RT-PCR Assay | CDC Ebola Virus VP40 Real-time RT-PCR Assay |
|---|---|---|---|---|---|---|
| | | | High Pure Viral Nucleic acid extraction kit<br>TriPure Isolation reagent<br>Absolute ethanol<br>Isopropanol | | RNase Away™<br>10% bleach | RNase Away™<br>10% bleach |
| **Laboratory space** | No requirement for multiple rooms.<br>Testing should be performed under the appropriate biosafety conditions in accordance with CDC and WHO guidelines. | No requirement for multiple rooms.<br>Testing should be performed under the appropriate biosafety conditions in accordance with CDC and WHO guidelines. | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification. | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification. | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification.<br>Specimen extraction must be performed inside biosafety cabinet level 3 if specimens have not been inactivated.<br>Addition of extracted specimens into RT-PCR reactions may not need to be performed inside biosafety cabinet level 3. | Unidirectional workflow; laboratory area must contain dedicated workspace and equipment for specimen extraction, preparation of mastermix and amplification.<br>Specimen extraction must be performed inside biosafety cabinet level 3 if specimens have not been inactivated.<br>Addition of extracted specimens into RT-PCR reactions may not need to be performed inside biosafety cabinet level 3. |
| **Electricity only** | Yes | Yes | Yes | Yes | Yes | Yes |
| **Equipment maintenance** | Per Instrument manual | Per Instrument manual | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment | Recommended maintenance and calibration for real time PCR platforms, micropipettes and standard laboratory equipment |
| **Technical support** | Required | Required | Required | Required | Required | Required |

| Assay name | FilmArray Biothreat-E test | FilmArray NGDS BT-E Assay | LightMix® Ebola Zaire rRT-PCR Test | EZ1 Real-time RT PCR Assay | CDC Ebola Virus NP Real-time RT-PCR Assay | CDC Ebola Virus VP40 Real-time RT-PCR Assay |
|---|---|---|---|---|---|---|
| **by manufacturer** | | | | | | |
| **Specimen inactivation using enhanced biosafety required by user prior to performing the test?** | No | No | Optional | Optional | Optional | Optional |
| **Nucleic acid extraction required by user** | No | No | Yes | Yes | Yes | Yes |
| **Suitable specimen for testing** | Venous whole blood, undiluted urine<br>*urine must be tested in conjunction with blood specimen | Venous whole blood, plasma and serum | Whole blood collected in EDTA or TriPure-inactivated EDTA whole blood collected in EDTA | Venous whole blood, plasma, Trizol-inactivated whole blood, Trizol-inactivated plasma | Venous whole blood, serum, plasma and urine with matched blood specimen | Venous whole blood, serum, plasma and urine with matched blood specimen |
| **Storage conditions for reagents** | 15-25°C | 15-25°C | 4-24°C primers.<br>-15 to -25°C LightCycler® Multiplex RNA Virus Master (requires cold chain) | -20°C (requires cold chain) | -20°C (requires cold chain) | -20°C (requires cold chain) |
| **Recommended time to process the specimen after collection** | No specific specimen stability claims. General specimen storage recommendations for NAT tests are applicable. | No specific specimen stability claims. General specimen storage recommendations for NAT tests are applicable. | Following extraction the specimen extracts can be stored at 2-8$^{o}$C until PCR amplification. If not used immediately freezing at -80°C in aliquots recommended for long-term storage | Freezing at -20°C in aliquots recommended for long-term storage. Freeze-thaw cycles not to exceed 3 | Whole blood can be stored up to 7 days at 2-8°C prior to extraction. Maintain at -70°C if processing delayed | Whole blood can be stored up to 7 days at 2-8°C prior to extraction. Maintain at -70°C if processing delayed |
| **Limit of detection (LOD) as <u>claimed by manufacturer</u>, not verified by independent assessment** | 6x10$^{5}$ PFU/mL with irradiated virus spiked into whole blood | 1x10$^{4}$ PFU/mL with irradiated virus spiked into whole blood, plasma or serum | 4,781 PFU/mL with irradiated virus spiked into EDTA-whole blood | 5,000 PFU/mL with Trizol inactivated whole blood or plasma. 1,000 PFU/mL with live virus spiked in Trizol-inactivated whole blood | 30 $TCID_{50}$/reaction with inactivated virus in whole blood or urine | 30 $TCID_{50}$/reaction with inactivated virus in whole blood or urine. |

| Assay name | FilmArray Biothreat-E test | FilmArray NGDS BT-E Assay | LightMix® Ebola Zaire rRT-PCR Test | EZ1 Real-time RT PCR Assay | CDC Ebola Virus NP Real-time RT-PCR Assay | CDC Ebola Virus VP40 Real-time RT-PCR Assay |
|---|---|---|---|---|---|---|
| | | | | or Trizol-inactivated plasma. | | |
| **Throughput: Time to results** | 75 minutes | 75 minutes | 4-6 hours | 4-6 hours | 4-6 hours | 4-6 hours |
| **Maximum number of specimens per day/instrument** | 8 specimens<br>(1 pouch processed at a time) | 8 specimens<br>(1 pouch processed at a time) | Approximately 50 specimens | Approximately 40 specimens | Approximately 40 specimens | Approximately 40 specimens |
| **Reagents shelf life** | Not known | Not known | Not known | Not known | Rehydrated primers and probes may be stored frozen for up to 12 months | Rehydrated primers and probes may be stored frozen for up to 12 months |
| **Minimum specimen volume** | 200µL blood or urine | 200µL blood, plasma, or serum | 200µL for nucleic acid extraction | 140-200µL whole blood or plasma for nucleic acid extraction (70µL whole blood or plasma for each QIAamp spin column. | 100µL whole blood, serum, plasma or urine for nucleic acid extraction | 100µL whole blood, serum, plasma or urine for nucleic acid extraction |
| **Limitations of the assay** | Presence of RT-PCR inhibitors may cause invalid results.<br>Specimens from patients who have received therapeutics or vaccines may exhibit false positive test results | Presence of RT-PCR inhibitors may cause invalid results.<br>Specimens from patients who have received therapeutics or vaccines may exhibit false positive test results | Presence of RT-PCR inhibitors may cause invalid results.<br>Potential for false positives due to amplicon carry-over<br>Performance of assay only validated on EDTA Whole blood and triPure inactive EDTA whole blood<br>This test should not be used to test specimens from asymptomatic individuals | Presence of RT-PCR inhibitors may cause invalid results.<br>Potential for false positives due to amplicon carry-over<br>Specimens from patients who have received therapeutics or vaccines may exhibit false positive test results | Presence of RT-PCR inhibitors may cause invalid results.<br>Potential for false positives due to amplicon carry-over<br>Specimens from patients who have received therapeutics or vaccines may exhibit false positive test results | Presence of RT-PCR inhibitors may cause invalid results. Potential for false positives due to amplicon carry-over<br>Specimens from patients who have received therapeutics or vaccines may exhibit false positive test results |
| **Trained manpower** | Laboratory technicians | Laboratory technicians | Laboratory technicians | Laboratory technicians | Laboratory technicians | Laboratory technicians |

| Assay name | FilmArray Biothreat-E test | FilmArray NGDS BT-E Assay | LightMix® Ebola Zaire rRT-PCR Test | EZ1 Real-time RT PCR Assay | CDC Ebola Virus NP Real-time RT-PCR Assay | CDC Ebola Virus VP40 Real-time RT-PCR Assay |
|---|---|---|---|---|---|---|
| | Biosafety training<br>Donning and doffing PPE | Biosafety training<br>Donning and doffing PPE | Biosafety training<br>Donning and doffing PPE | Biosafety training<br>Donning and doffing PPE | Biosafety training<br>Donning and doffing PPE | Biosafety training<br>Donning and doffing PPE |
| **Biosafety requirements** | Testing under BSL3 conditions | Testing under BSL3 conditions | Specimen inactivation should be performed under BSL3 conditions | Specimen inactivation should be performed under BSL3 conditions | Specimen inactivation or extraction should be performed under BSL3 conditions | Specimen inactivation or extraction should be performed under BSL3 conditions |
| **Waste management** | Follow national guidelines | Follow national guidelines | Follow national guidelines | Follow national guidelines | Follow national guidelines | Follow national guidelines |
| **FDA EUA restrictions regarding sites for use** | CLIA moderate to high complexity labs and similar non-US facilities | US Department of Defense-specified laboratories that currently perform testing with the FilmArray System | CLIA high complexity labs and similar non-US facilities | Qualified laboratories designated by the US Dept. of Defense | Qualified laboratories designated by the US CDC | Qualified laboratories designated by the US CDC |

Information on certain characteristics listed in the Table will be updated by WHO as more data become available

## Annex 3: Considerations for the selection of rapid antigen detection tests for the diagnosis of Ebola virus disease

### IVDs with WHO Emergency Use and Assessment Listing (EUAL)
### Version: 09 June 2015

The WHO EUAL assessment comprises WHO assessment of manufacturing capacity and quality management systems, documentary evidence of safety and performance, independent verification of analytical sensitivity (LOD) and clinical performance (sensitivity and specificity)

| **Assay name** | **ReEBOV™ Antigen Rapid Test** (also US FDA EUA authorized) |
|---|---|
| **Manufacturer** | Corgenix, Inc. |
| **Kit Components** | |
| **Kit format** | Dipstick - Lateral flow rapid chromatographic immunoassay |
| **Kit size** | 50 reactions |
| **Kit components** | Test dipsticks<br>Specimen buffer dropper bottles<br>Lyophilized negative control (negative human serum)<br>Lyophilized positive control (recombinant VP40 antigen spiked in negative human serum)<br>Test tubes with caps<br>Disposable test tube rack<br>Visual aid - ReEBOV™ Antigen Rapid Test Results Card |
| **Virus Detected** | Zaire ebolavirus (ZEBOV) |
| **Target** | VP40 antigen |
| **Other materials required but not supplied** | ReEBOV™ Antigen Accessory Kit<br>• 200 Disposable Lancets<br>• 200 Cotton Balls<br>• 200 Alcohol Wipes<br>Precision pipettors capable of delivering between 10 µL and 250 µL, with appropriate tips<br>Deionized water |
| **Infrastructure** | |
| **Laboratory space** | No requirement except for biosafety requirements |

| Assay name | **ReEBOV™ Antigen Rapid Test** (also US FDA EUA authorized) |
|---|---|
| **Electricity only** | No |
| **Equipment maintenance** | N/A |
| **Technical support by manufacturer** | Not required |
| **Performance characteristics** | |
| **Specimen inactivation using enhanced biosafety required by user** | No |
| **Suitable specimen for testing** | Fingerstick (capillary) whole blood,<br>venous whole blood collected in EDTA, or<br>plasma collected in EDTA |
| **Storage conditions for reagents** | Store at 2–8°C. Do Not Freeze (requires cold chain) |
| **Recommended time to process the specimen after collection** | Fresh whole blood specimens should be obtained immediately prior to application to the test dipstick.<br>Plasma can be stored at 2–8°C for up to 1 week, or otherwise store at -20°C |
| **Accurate pipetting of blood critical** | Yes when using plasma. |
| **WHO Performance evaluation** | Sensitivity (95% CI) ; 91.8% (84.5, 96.8)<br>Specificity (95% CI); 84.6% (78.8, 89.4)<br>Limit of Detection: 2.11E+08 RNA copies/ml |
| **Throughput: Time to results** | 15-25 minutes |
| **Maximum number of specimens per day** | If one operator, ~19 per day if run consecutively one at a time (assuming 8 hour day/ 25 min per test) but can stagger testing therefore could probably test 60-100 per day |
| **Reagents shelf life** | 1 year at 2–8°C |
| **Min specimen volume** | 1 full drop of blood from Fingerstick or 30µL venous whole blood or plasma |

| Assay name | **ReEBOV™ Antigen Rapid Test** (also US FDA EUA authorized) |
|---|---|
| **Limitations of the assay** | Circulating EBOV VP40 antigen may be absent or undetectable if the patient has progressed to their humoral immune response and anti-EBOV VP40 antibody titers may have developed.<br>Negative results do not preclude Ebola virus infection, particularly within the first 72 hours after appearance of symptoms, and should not be used as the sole basis for patient management decisions.<br>Test is not intended for use for general Ebola infection screening, such as airport screening or contact tracing and should not be used on asymptomatic individuals.<br>Testing patient specimens containing excess hemoglobin may result in false negative readings.<br>Testing patient specimens containing rheumatoid factor may result in false positive readings.<br>Specimens from patients who have received therapeutics or vaccines may exhibit false positive test results or other confounding test results. |
| **Trained manpower** | Trained personnel<br>Biosafety training<br>Donning and doffing PPE |
| **Biosafety requirements** | Where advanced biocontainment facilities (BSL-4) are not available, the use of all possible universal precautions is highly recommended including safety goggles and/or face shields, masks or respiratory equipment, disposable gowning, boots and gloves. It is highly recommended that health care workers are appropriately trained in the donning and doffing of personal protective equipment. |
| **Waste management** | Follow national guidelines |
| **FDA EUA restrictions regarding sites for use** | Use in laboratories or facilities adequately equipped, trained, and capable of such testing (including treatment centres and public health clinics) |