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By Madeleine Johnson

NEW YORK (GenomeWeb) — At least five companies have indicated they will pursue or are considering pursuing Emergency Use Authorization from the US Food and Drug Administration to make nucleic acid tests for Ebola available, while three RT-PCR assays have already been granted EUA since an emergency was declared in early August.

The three real-time RT-PCR tests that have already been granted EUA — one from the US Department of Defense, and two from the US Centers of Disease Control — can now be legally used to diagnose patients.

Roche and BioMérieux's BioFire Diagnostics subsidiary recently disclosed they will also pursue EUA for lab-based assays on existing platforms, and Cepheid said that it is considering a similar move. Meantime, UK-based Primerdesign is ready to ship its lyophilized qPCR test kits, while Madison, Wisc.-based Lucigen is building a point-of-care test for use in decentralized settings. Additional Ebola diagnostics are also known to exist, but it's unclear whether any of these will also seek EUA.

The majority of these assays use standard RT-PCR, but a few have novel methodologies that may help control the current outbreak in Africa, prevent an outbreak in the US, and possibly advance the field of molecular diagnostics.

The FDA has rarely granted EUA in the past, but has a new mandate under the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 to prepare for potential public health emergencies, rather than simply respond to them. This includes waiving of good manufacturing practices and a product's risk evaluation and mitigation requirements if EUA is deemed

necessary.

When the current Ebola outbreak met requirements for an emergency in early August, the first EUA was granted to the Department of Defense test. This was based on the Secretary of Homeland Security's determination that "the Ebola virus presents a material threat against the United States population sufficient to affect national security," according to a notice from the Health and Human Services Department.

The FDA "works extremely rapidly to make a determination on an Emergency Use Authorization once the information is submitted to the agency for review,"an agency spokesperson told *GWDN* in an email.

Authorization requires reviewing data about a test's performance in detecting Ebola virus in human specimens, and determining that the standards for authorization are met. "It is important to note that the FDA has been reaching out to commercial companies to encourage them to work with us on the development of rapid diagnostic tests," the agency spokesperson said.

RT-qPCR

The FDA initiated the emergency use application process by granting EUA to the DoD's EZ1 test on August 6 of this year, as covered in *GWDN*.

The DoD test uses real-time RT-PCR for qualitative detection of Ebola Zaire virus, the strain implicated in the current West Africa outbreak.

The complete DoD EZ1 assay consists of two primer/probe sets, EZ1 and RNase P, along with the assay master mix and positive template controls for each primer/probe set.

The test works on whole blood (with or without EDTA as an anticoagulant), plasma, or Trizol-inactivated whole blood or plasma specimens.

Nucleic acid extraction uses the Qiagen QIAamp viral RNA mini kit and centrifugation. This is followed by real-time RT-PCR, authorized on the Applied Biosystems 7500 Fast Dx system from Thermo Fisher Scientific, the JBAIDS instrument from BioFire Defense, or the Roche LightCycler, according to the FDA letter of authorization.

In the meantime, two CDC real-time RT-PCR assays were granted EUA on October 10 of this year.

The CDC's NP assay consists of two primer/probe sets, NP and Rnase P, and targets nucleic acids coding for Ebola nucleoprotein, or NP, which plays a central role in virus replication.

Nucleic acid extraction is authorized with the MagMax Pathogen RNA/DNA kit and the Dynal Bead Retriever, both from Life Technologies. Samples can be whole blood collected with EDTA as the anticoagulant, plasma, serum, or urine. Sample prep is followed by RT-qPCR on the ABI 7500 Fast Dx system, according to the FDA letter of authorization.

The second CDC test consists of two primer/probe sets, VP40 and Rnase P, and targets viral matrix protein 40, an important coordinator of the viral life cycle of Ebola.

It uses the same sample types, extraction, and amplification methods as the NP test, according to the FDA letter of authorization.

In its third quarter earnings call last week, Cepheid CEO John Bishop responded to an analyst's query about the firms Ebola strategy.

Cepheid has some experience with pathogens like Ebola as it was part of a Canadian initiative funded in 2006 to develop viral hemorrhagic fever assays, Bishop noted.

In addition, the company's GeneXpert platform already has a presence in the developing world through its high-burden developing country program for tuberculosis diagnostics, and it is a "totally closed" system, so there is no interaction of fluids with the hardware, he said.

Cepheid is now speaking with potential investment partners. With funding, a fieldable prototype Ebola assay might be available in eight weeks, with a preproduction test ready in 16 weeks. The company has also already lined up collaborations with Biosafety Level-4 labs, Bishop said. Additional information about Cepheid's potential foray into Ebola diagnostics can be found in a recap of the company's Q3 earnings in *PCR Insider this week.*

Also in a Q3 earnings call last week, Roche revealed it would seek EUA for a research-use-only Ebola test.

Under a worldwide exclusive agreement with Tib Mobiol, Roche distributes the LightMix Modular Ebola Virus Zaire Assay. That assay is optimized to run on Roche's LightCycler systems, the company told *GWDN* in an email. "It is being prepared for filing for Emergency Use Authorization in the US, CE-mark approval, and WHO prequalification," a Roche spokesperson said in an email to *GWDN*.

Finally, on the RT-qPCR front, BioMérieux affiliate BioFire has an existing Ebola assay that runs on the FilmArray system, "but this test was developed solely for use in environmental surveillance," a spokesperson said in an email.

The FilmArray system is currently primarily used to run clinical infectious disease panels through a combination of real-time PCR and high-resolution melt curve analysis. It is also a fully automated and closed platform.

"As this test was developed for non-clinical testing, it is necessary to provide FDA with evidence that it enables healthcare professionals for testing patient samples with all the necessary reliability and precision," she said.

BioFire is now working with the FDA to determine if the FilmArray Ebola assay might qualify for EUA, a process in which the FDA has been "extremely responsive and constructive," the representative noted.

Alternative molecular approaches

Southampton, UK-based Primerdesign is pursuing EUA, and is currently testing its assays in Sierra Leone, said Rob Powell, director of research and development.

The firm's qPCR-based kit is unique in that all reagents are dried. "There's no cold transport

chain required, and in this kind of field testing that's a real advantage for getting kits into far flung places," Powell said.

The test still requires a simple laboratory environment, he said, but the method avoids centrifugation so has less likelihood of generating aerosols or small droplets. It runs on whole blood samples, using magnetic bead-based RNA extraction. The company has also tried to make this test very "de-skilled," so it can be used with less technical expertise.

The firm currently has the capacity to ship 50,000 lyophilized tests within a week, Powell said.

"If people are not going to get tests into the field by November, it is going to be too late," Powell asserted. "I think that's the key part of the story that people are missing; this will be totally out of control by Christmas … if a major impact is not made."

While all the aforementioned tests use some form of RT-PCR to make Ebola's RNA-based genome detectable, Lucigen's Ebola assay uses the firm's OmniAmp nucleic acid detection and the AmpliFire platform from Douglas Scientific for amplification and detection. As covered in a separate article in *PCR Insider* this week, this method makes the Lucigen test uniquely suited to decentralized settings.

Other assays

MoBiTec and Bioneer both have RT-PCR kits for Ebola detection, as does Germany's Altona Diagnostics. BioInnovation Solutions has collaborated with the US Army Medical Research Institute of Infectious Diseases to develop a sequencing-based viral panel which includes Ebola.

Affiliated with the US Department of Energy, Oak Ridge National Laboratory in Tennessee has also developed and patented an RNA virus rapid test that can detect Ebola and is now seeking licensing partners, according to a statement. The diagnostic detects active viral replication in infected cells and is "highly fieldable, and nearly reagentless."

A path to commercialization?

In non-emergency situations, *in vitro* molecular diagnostic kits or devices need to be tested with live virus, then put through the rigors of clinical trials.

For rare, but dangerous, pathogens like Ebola, both of these steps can be hurdles. There are currently only seven BSL-4 labs operating in the United States, according to the Federation of American Scientists, and Ebola can only be safely handled in these highly regulated spaces. Scarce patient samples are hard to come by just for prototype analysis.

An alternative route for testing live virus on prototypes, then, might be running samples from potentially-infected patients during an emergency.

Data from EUAs during the H1N1 influenza outbreak of 2009-2010 was not simply thrown away when the threat died down, after all. And at least a handful of the companies authorized to supply *in vitro* diagnostics for influenza during that outbreak now have commercially available, FDA-approved influenza tests.

In a sort of post-mortem of the H1N1 EUA experience, participants at a US Institute of Medicine

workshop agreed that "the use of unapproved medications and devices under an EUA presents a potential opportunity to collect data on their use and results in clinical settings," according to a published report.

They also noted important caveats. Collecting and analyzing data in an emergency is challenging, and "nothing like the randomized, controlled clinical trials that are necessary to evaluate safety and efficacy."

Additional reporting by Ben Butkus.

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