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United States FDA's emergency use authorization of Ebola virus diagnostics: current impact and lessons for the future

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The Ebola outbreak that took hold in West Africa in 2014 outran the epidemic response capacity of many organizations. Five months after the epidemic was first declared, there were still only two laboratories in West Africa with the capacity to confirm Ebola virus infection. In the summer of 2014, before the first case of imported Ebola occurred in the USA, the US FDA announced it would issue Emergency Use Authorizations for Ebola virus *in vitro* diagnostics to speed their availability. Between October 2014 and March 2015, the FDA issued Emergency Use Authorizations for nine diagnostic products. The actions of the FDA not only allowed nationwide deployment of Ebola virus testing capacity in the USA but also established an attractive regulatory goalpost for companies developing assays for use in West Africa. Here, we comment on the diagnostic assays for which the FDA has issued emergency authorizations and their fitness for purpose.

Ebola virus causes a hemorrhagic fever disease (Ebola virus disease) in humans associated with significant mortality. Since its discovery in 1976, only sporadic outbreaks of disease have been reported in a geographically limited area of Central Africa, resulting in slightly more than 2300 cases in the 39-year period [1]. Sadly, the past year has witnessed the largest Ebola virus disease outbreak in the recorded history of West Africa [2]. In early March 2014, the Ministry of Health of Guinea was notified of several cases of patients with fever, severe diarrhea and vomiting with a high fatality rate that turned out to be among the first Ebola cases of the epidemic, although it is now known that the index case was actually an 18-month-old boy from Guinea who contracted the illness and died in December 2013 [3]. The disease ultimately spread to other West African countries, including Liberia, Sierra Leone, Mali, Nigeria and Senegal. In addition, a small number of cases were imported into the USA, UK and Spain. According to the WHO, as of 12 April 2015, there have been a total of 25,826 cases with 10,704 deaths in a total of nine countries [4].

The use of rapid and reliable laboratory diagnostic tests is critical to slow the proliferation of this epidemic or future epidemics. Physicians and public health personnel rely on the results of diagnostic assays to make decisions regarding quarantine, assess the clinical efficacy of various treatment regimens, aid in contact tracing and mapping the geographic spread of disease. A range of diagnostic methods is available for Ebola virus. These include virus isolation, ELISAs to detect antigen or antibodies, reverse transcriptase-PCR (RT-PCR) and electron microscopy, all of which have played major roles in the diagnosis of Ebola virus infections and have been summarized elsewhere [5–8]. However, many of these methods are cumbersome, slow and complex to perform. In addition, some of them require a high level

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of laboratory biocontainment (i.e., BSL-4) and highly trained technical staff. Recently, the WHO published a target product profile for Ebola virus diagnostics that outlined 'desired' and 'acceptable' characteristics [9]. The ideal test would require no laboratory infrastructure, involve no more than three steps, produce results in less than 30 min and have no biosafety requirements beyond the use of standard personal protective equipment. Furthermore, their ideal assay would be portable, need no power supply and require no maintenance. Needless to say, we are not there yet.

When the Ebola epidemic was first recognized in Guinea in March 2014, no US FDA-authorized diagnostic assays for the virus were available, and the assays used in deployable laboratories in West Africa and elsewhere early in the epidemic (e.g., assays from CDC, Department of Defense [DoD] and several companies) were from a US regulatory perspective; research use-only assays implemented on an emergency basis. Due to the threat of imported Ebola, US laboratories sought assays cleared by the FDA. In the summer of 2014, the Secretary of the US Health and Human Services declared that circumstances existed to justify the Emergency Use Authorization (EUA) of *in vitro* diagnostics for the detection of Ebola virus. EUA authority allows the FDA to strengthen the nation's public health protections against biological, chemical and radiological threats by facilitating the availability and use of medical countermeasures during public health emergencies [10]. Since that time, the FDA has granted EUAs to nine different assays for the presumptive detection of Ebola virus in specimens from individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors. All except two of these assays are designed to be specific for Ebola Zaire.

The first such authorization (5 August 2014) was for the US. DoD EZ1 real-time RT-PCR assay for the presumptive detection of Ebola Zaire virus. This authorization was limited to the use of the assay on specified instruments by laboratories designated by the DoD. In addition, it limited the use of the assay to Trizol-inactivated whole blood or Trizol-inactivated plasma specimens [10]. Soon to follow were two real-time RT-PCR assays from the US CDC targeting the Ebola Zaire genes encoding viral nucleoprotein (NP) and viral protein 40 (VP40). Both CDC assays were authorized to run on the BioRad CFX96 Touch Real-Time PCR instrument or the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument using whole blood, serum or plasma. The CDC assays were also authorized for use on urine specimens when tested in conjunction with patient-matched whole blood, serum or plasma specimen. The DoD EZ1 real-time RT-PCR assay mentioned above is currently established in over 50 States and Local Public Health Laboratory Response Network reference laboratories in the USA for the presumptive detection of Ebola Zaire virus, followed by confirmation using the CDC-developed assays and other confirmatory methods.

In October 2014, the FDA issued an EUA to BioFire Defense for the FilmArray Biothreat-E test and the FilmArray Biothreat NGDS BT-E assay for the presumptive detection of Ebola Zaire. The FilmArray is a multiplex PCR system that integrates sample preparation, amplification, detection and analysis. The assay requires a limited amount of hands-on-time and has a turn-around time of 75 min. The menu of tests available on this BioFire platform includes three different FilmArray multiplex panels – a Respiratory Panel, a Blood Culture Identification Panel and a Gastrointestinal Panel – that are currently FDA cleared for *in vitro* diagnostic use. The FilmArray Biothreat-E Test is authorized for use in moderate and high-complexity laboratories and is marketed commercially by BioMerieux, which recently acquired BioFire. The FilmArray Biothreat NGDS BT-E assay, which is similar but developed through a different funding mechanism, is not commercially available.

In November 2014, the FDA issued an EUA for the RealStar® Ebolavirus RT-PCR Kit 1.0 from altona Diagnostics GmbH of Hamburg, Germany. This assay was authorized for use on specified instruments in Clinical Laboratory Improvement Amendments (CLIA)-certified high-complexity laboratories. The RealStar assay, which targets conserved regions of the L gene, detects, but does not distinguish between, different Ebola virus species. Soon after the assay was approved for emergency use, Qiagen announced that it would offer global distribution. Another assay, LightMix® Ebola Zaire rRT-PCR by Roche, is a probe-based one-step real-time RT-PCR assay authorized for use on specified instruments in CLIA-certified high complexity laboratories or similarly qualified non-US laboratories.

In March 2015, the FDA issued an EUA for the ReEBOV™ Antigen Rapid Test from Corgenix for the presumptive detection of Ebola virus in capillary (fingerstick) or venous whole blood or plasma. This assay, currently in dipstick format, detects VP40 proteins from Ebola Zaire, Sudan and Bundibugyo, but does not distinguish between them. The EUA labeling states that test results must be confirmed with additional testing, such as with a molecular assay, and that the test is not intended for general Ebola virus infection screening, such as airport screening or contact tracing. The limit of detection of the ReEBOV™ test was 1×10^6 plaque forming units (PFU)/ml (625 ng/ml recombinant VP) using live virus spiked into whole blood. A WHO study found 78.3% sensitivity and 90.7% specificity in 147 fresh whole-blood samples among suspected cases, and a sensitivity of 91.8% and specificity of 84.6% in a similar collection of 146 frozen plasma samples (altona comparator). A Corgenix study found sensitivity 62.1% and specificity 96.7% in 176 frozen plasma samples using the Trombley PCR assay as a comparator. For a number of reasons, including the necessity to confirm both positive and negative results with PCR, and the low positive predictive value even in West Africa at this stage in the epidemic, WHO published a guidance document in March declaring that where PCR testing is available, rapid tests for the detection of Ebola antigens should not be used in the routine management of Ebola in this outbreak [11].

Also in March 2015, an EUA was issued for the Xpert® Ebola Assay manufactured by Cepheid. This automated RT-PCR assay

is to be used with EDTA venous whole-blood specimens on the GeneXpert Instrument System in CLIA moderate-to-high complexity laboratories (or similarly qualified non-US labs). The assay fully integrates sample processing, amplification and detection and begins with an inactivation step (placing blood-soaked swab from venous or fingerprick blood into a chaotropic solution) to minimize biosafety risks. Although users of many tests may start their sample processing work with an inactivation step, this is the only assay that includes inactivation materials in the kit and integrates inactivation into sample collection. There are two molecular targets (NP and glycoprotein [GP]) in the Xpert Ebola assay that can mitigate the risk of mutational drift affecting sensitivity and help discriminate true infection from circulating GP sequences that might occur following inoculation with a recombinant viral vaccine carrying the Ebola GP gene [12].

The FDA EUA process was developed to strengthen the national capacity to respond to threats to public health in the USA. The process has served a wider function, both in this Ebola epidemic and in previous settings when the EUA mechanism was invoked for influenza or coronavirus outbreaks: it has become a rapid and publicly transparent mechanism for garnering initial data on assay quality and performance that can help drive decision making in countries facing local epidemics. Given the weakness of regulatory mechanisms in many developing countries, the EUA serves as an important gating process, and in emergency settings can act as a useful surrogate for full local regulatory approval. The speed of action on submissions to the FDA EUA was appropriate to the urgency of the situation and was significantly accelerated by the FDA decision to accept mock clinical trial data.

The compelling nature of the epidemic, and the high degree of attention paid to it by the press, has prompted many diagnostic groups or companies to work in this area, despite obvious uncertainty about the size or longevity of the commercial opportunity. In a detailed analysis of the Ebola diagnostic development landscape, the Foundation for Innovative New Diagnostics identified over 75 companies with assays in some stage of development as early as October 2014. Obviously, many or most of these assays will never be commercially launched, but the prospect of public funding to assist product development and procurement; the newsworthiness of Ebola-associated activities; and the availability of a streamlined regulatory process through the FDA, all served to attract many diagnostic companies that otherwise would not have engaged.

Emergency authorization makes the US. procurement and use of assays possible and describes legal limits on their implementation. It does not, however, act as a guidance process for procurement toward meeting local needs. The Ebola assays authorized by the FDA vary widely in terms of gene targets, performance, speed, and most notable, ease of use. The stated limits of detection of the Ebola assays listed by the FDA vary hugely, from 0.13 PFU/ml to 6×10^5 PFU/ml (see Table 1) although methodologic differences, both in preparation of reference material and manner of testing, make these numbers difficult to assess. More important for developing world settings, such as the provinces of West Africa where this epidemic has played out, is ease of use and capacity for being integrated into the health system for sustained use for detection of other diseases and Ebola surveillance. Most of the assays used in the current outbreak are quite complex to use and their implementation for Ebola has minimally engaged local scientists and technicians. Moreover, supply chain management for many of these assays can be daunting. As seen in the table, integrated systems such as from BioFire and Cepheid can require little or no additional procurement of reagents and disposables. On the other hand, many of the assays require many additional materials that must be procured from a range of vendors.

There will certainly be future Ebola outbreaks, and it cannot be assumed that assays developed around the current strains of Ebola Zaire will maintain unchanging performance. Ebola virus, similar to other RNA viruses, may rapidly evolve: EBOV is estimated to evolve at about 7×10^{-4} substitutions per site per year [13]. One study examined the amount of genetic drift that has occurred in the virus over time from the first outbreak in Yambuku, Zaire, in 1976 or a large outbreak that occurred in Kikwit, Zaire, in 1995 and the current West African outbreak [14]. In this study, the authors identified more than 600 SNPs between the genome sequences of viruses from these outbreaks. Clearly, this level of genetic diversity could significantly affect the sensitivity and specificity of Ebola virus molecular diagnostic assays, especially if they were originally designed using the Yambuku-1976 or Kikwit-1995 strains. In fact, another recent study compared the sequences of the primer/probe sets from 11 published assays with the corresponding sequences of viruses from Sierra Leone and found a total of nine nucleotide discrepancies in either the forward or reverse primer or probe sequences [15]. Further laboratory validation is needed, however, to know whether these discrepancies affect the sensitivity and/or specificity of the assays. Clearly, this is an issue that needs to be addressed when designing diagnostic assays for rapidly evolving viruses. As such, future efforts could include designing broad range diagnostics using primers with degenerate bases covering all known strains of the virus or the use of multiplex assays. In addition, sequencing efforts should continue to be on the lookout for mutations that could impact the effectiveness of existing and emerging diagnostic assays.

In conclusion, the FDA EUA process has provided a feasible and useful regulatory target and an excellent source of information on the performance of individual assays. Although rapidly responsive, the FDA EUA process does not erase the primary lesson learned repeatedly during this outbreak – that it is almost impossible to work quickly enough *during* an outbreak to meet disease control needs. This is true for provision of treatment centers, recruitment of health workers and establishing effective supply chains – but it is also true for the development and delivery of diagnostics. Assay development needs to be supported *between* epidemics, so that the types of tests that are needed (notably simple to use, automated and near-patient systems) can be available for deployment at the early days of the outbreak, before transmission has taken hold in hard-to-reach communities. This will require, among other things,

Table 1. Available assays issued emergency use authorization by the US FDA.

Name of assay	Manufacturer	Sample types	Species detected	Gene target	LOD	Reagent storage	Reagents & disposables not included[†]	Sample prep
EZ1 Real-time RT-PCR assay	US Department of Defense	Whole blood, plasma, Trizol-inactivated whole blood, or Trizol-inactivated plasma	Ebola Zaire	?	5000 PFU/ml with Trizol inactivated whole blood or plasma. 100 PFU/ml with live virus	−20°C	8	Exogenous
CDC Ebola virus NP real-time RT-PCR assay	US CDC	Whole blood, serum, plasma, and urine	Ebola Zaire	NP	30 TCID50/ reaction with live virus in whole blood. 1–2 logs higher with inactivated	−20°C	14	Exogenous
CDC Ebola Virus VP40 real-time RT-PCR assay	US CDC	Whole blood, serum, plasma, and urine	Ebola Zaire	VP40	3 TCID50/ reaction with live virus in whole blood. 1–2 logs higher with inactivated	−20°C	14	Exogenous
FilmArray Biothreat-E test	BioFire	Whole blood, urine	Ebola Zaire	?	$6x10^5$ PFU/ml with irradiated virus spiked into whole blood	15–25°C	1	Integrated
RealStar® Ebolavirus RT-PCR Kit 1.0	altona	EDTA plasma	Ebola Zaire, Sudan, Tai Forest, Bundibugyo	L	1 PFU/ml with RNA spiked into plasma	−20°C	5	Exogenous
LightMix® Ebola Zaire rRT-PCR Test	Roche	EDTA whole blood or TriPure-inactivated EDTA whole blood	Ebola Zaire	L	4781 PFU/ml with irradiated virus spiked into whole blood	4–24°C	13	Exogenous
ReEBOV™ Antigen Rapid Test	Corgenix	Capillary or venous whole blood, and plasma	Zaire Ebola, Sudan and Bundibugyo	VP40	1×10^6 PFU/ml with live virus spiked into whole blood	2–8°C	2	N/A
Xpert® Ebola Assay	Cepheid	EDTA whole blood	Zaire Ebola	GP, NP	232 copies/ml RNA in whole blood, or 0.13–1 PFU/ml live virus in whole blood	2–28°C	0	Integrated, immediate inactivation

[†]Number of items necessary to procure that are not included in the test kit (excluding bleach, gloves and blood drawing materials).
GP: Glycoprotein; LOD: Limit of detection; NP: Nucleoprotein; PFU: Plaque forming units; RT-PCR: Reverse transcriptase-PCR; TCID50: Median tissue culture infective dose; VP: Viral protein.

development funding, a flexible regulatory approach as represented by the FDA EUA, and access to reference materials.

Disclaimer

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