Diagnosis of Ebola Virus Disease: Past, Present, and Future

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SUMMARY

Laboratory diagnosis of Ebola virus disease plays a critical role in outbreak response efforts; however, establishing safe and expeditious testing strategies for this high-biosafety-level pathogen in resource-poor environments remains extremely challenging. Since the discovery of Ebola virus in 1976 via traditional viral culture techniques and electron microscopy, diagnostic methodologies have trended toward faster, more accurate molecular assays. Importantly, technological advances have been paired with increasing efforts to support decentralized diagnostic testing capacity that can be deployed at or near the point of patient care. The unprecedented scope of the 2014-2015 West Africa Ebola epidemic spurred tremendous innovation in this arena, and a variety of new diagnostic platforms that have the potential both to immediately improve ongoing surveillance efforts in West Africa and to transform future outbreak responses have reached the field. In this review, we describe the evolution of Ebola virus disease diagnostic testing and efforts to deploy field diagnostic laboratories in prior outbreaks. We then explore the diagnostic challenges pervading the 2014-2015 epidemic and provide a comprehensive examination of novel diagnostic tests that are likely to address some of these challenges moving forward.

INTRODUCTION

The recent outbreak of Ebola virus disease (EVD) in West Africa has highlighted both the importance of rapid and accurate diagnosis of this disease and the challenges around diagnostic testing. Throughout the 2014-2015 outbreak, diagnosis relied primarily on testing of venipuncture blood samples from symptomatic individuals in a biocontainment laboratory facility, leading to challenges with specimen collection and data management and often a prolonged turnaround time to final results. Consequently, the need for rapid and, particularly, for point-of-care diagnostics generated an unprecedented surge in development of new diagnostic methods for EVD. This review summarizes the evolution of laboratory-based methods for EVD diagnosis, the implementation of these methodologies for field-based testing in outbreak

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FIG 1 Detection of Ebola virus infection in nonfatal versus fatal cases. Solid lines indicate that the analyte of interest is detected in the majority of cases at the corresponding time point (days post-symptom onset); dashed lines indicate that the analyte of interest is detected in the minority of cases at that time point. Data for IgG and IgM detection were compiled from references 9, 10, and 15 to 20. Data for antigen detection were compiled from references 2, 10, 17, and 18. Data for RNA detection were compiled from references 2, 12, 15, 27, 28, and 35. RT-PCR, reverse transcription-PCR; ELISA, enzyme-linked immunosorbent assay.

settings, and recent advances in diagnostic tools that are likely to benefit future clinical and surveillance efforts. As new diagnostic technologies become available, it will be increasingly important for clinicians to understand both the analytic and practical strengths and limitations of each testing platform. Ultimately, the optimal diagnostic approach for a particular setting will depend upon multiple factors, including population characteristics and disease prevalence, the health care setting (e.g., infrastructure and availability of biosafety and infection control measures), training requirements, regional laboratory capacity, regulatory status, and cost.

METHODS FOR DETECTING EBOLA VIRUS INFECTION

Overview

Ebola viruses contain a single-stranded RNA genome that encodes seven viral proteins: nucleoprotein (NP), glycoprotein (GP), polymerase (L), VP24, VP30, VP35, and VP40. Over the past 25 years, several methods for detecting infection and/or disease with Ebola virus have been developed that are amenable for use in clinical laboratory settings (1). These fall into three basic categories: (i) serologic tests that detect host antibodies generated against the virus, (ii) antigen tests that detect viral proteins, and (iii) molecular tests that detect viral RNA sequences (Fig. 1). Specific antiviral antibodies can persist for years following infection; however, the variable onset of antibody responses during acute illness makes serology minimally useful as a diagnostic tool in the acute setting. Conversely, antigen detection and molecular tests have

proven very effective for acute diagnosis, as virus levels in the blood typically rise to high levels within the first few days of symptoms (2). The incubation period following Ebola virus infection typically ranges between 3 and 13 days, but may be as long as 21 days (3, 4); no tests have yet demonstrated the ability to reliably detect Ebola virus prior to the onset of symptoms. Some diagnostic tests have been designed to broadly detect Ebola virus infection, while others distinguish among the five known Ebola virus species (Zaire/Ebola [EBOV], Sudan [SUDV], Tai Forest [TAFV], Reston [RESTV], and Bundibugyo [BDBV]). Major outbreaks of EVD in humans have been attributable to EBOV, SUDV, and BDBV; prior to the 2014-2015 epidemic, the origins of EVD outbreaks were restricted to five African countries: Democratic Republic of Congo (formerly Zaire), Sudan, Gabon, Uganda, and Republic of Congo (Fig. 2).

Cell Culture

The traditional gold standard method to confirm the presence of Ebola virus is viral isolation in cell culture, typically using Vero E6 African Green monkey kidney cells. Propagated virus can be directly visualized by electron microscopy or indirectly visualized by immunofluorescence microscopy within 1 to 5 days of inoculation. While detection of Ebola virus by these methods is definitive, these methods require biosafety level 4 (BSL-4) containment and are typically restricted to research and public health laboratories (5).

FIG 2 Diagnostic testing in Ebola virus outbreaks. The information provided for each outbreak includes the affected country (or countries), the agencies primarily responsible for clinical diagnostic testing during the outbreak, where testing took place (the location is shown in parentheses; field laboratories are also highlighted in red), and the primary testing methods used for clinical diagnosis. The size of the box denotes the relative size of the outbreak, categorized in the following groups: <100 cases, 100 to 200 cases, 200 to 300 cases, 300 to 400 cases, and 400 to 500 cases; the West Africa epidemic exceeded 28,600 cases (132). The color of the box denotes the outbreak's Ebola virus species (EBOV, Zaire/Ebola; SUDV, Sudan; BDBV, Bundibugyo). Abbreviations: DRC, Democratic Republic of Congo; RC, Republic of Congo; MRE, Microbiological Research Establishment; ITMA, Institute of Tropical Medicine, Antwerp; CIRM, Centre International de Recherches Médicales de Franceville; PHAC, Public Health Agency of Canada; KEMRI, Kenya Medical Research Institute; NICD, National Institute of Communicable Diseases; UVRI, Uganda Virus Research Institute; INRB, Institut National de Recherche Biomedicale.

Antibody Detection

Serologic assays for the detection of specific antiviral antibodies in patient serum have been used to demonstrate current or prior infection with Ebola virus since the first outbreak investigations of this virus in 1976 (6, 7). Indeed, an indirect fluorescent antibody detection test (IFAT) was used in 1977 to distinguish the newly discovered Ebola virus from the closely related Marburg virus, based on the viral antigen specificity of antibodies in convalescent-phase serum from individuals who had recovered from infections with these pathogens (6). For this method, cell cultures infected with Ebola virus (or antigen suspensions from these cultures) are irradiated, fixed onto a slide, and incubated with sera

from potentially exposed individuals; bound antibodies are then detected with a fluorescently labeled secondary antibody (e.g., rabbit anti-human IgG) and visualized with immunofluorescence microscopy (8). Although IFAT played a critical role in establishing clinical diagnoses during the first several Ebola outbreaks, it was considered to have suboptimal sensitivity and specificity (9), and the requirement for BSL-4 biocontainment rendered this method unsuitable for large-scale diagnostic efforts.

The development of enzyme-linked immunosorbent assay (ELISA) tests for the detection of Ebola virus-specific IgM and IgG antibodies offered a faster, higher-throughput system for serologic testing. These assays, first developed at the U.S. Army Med-

ical Research Institute of Infectious Diseases (USAMRIID) in 1990 (9) and adopted by the CDC, utilize viral antigens prepared from inoculated cell cultures to bind antibodies present in patient serum (9, 10). ELISAs (for both antibody and antigen detection; see “Protein Antigen Detection,” below) are compatible with gamma irradiation, allowing for inactivation of infectious virus in clinical specimens and subsequent sample processing under BSL-2 conditions; however, viral inactivation via gamma irradiation is available on demand in only a very few specialized institutions. Viral inactivation with heat and detergent treatment prior to serologic testing by ELISA has been described previously (11), but data regarding assay performance under these conditions are not available.

The IgM ELISA in use by the CDC entails an antibody capture platform, utilizing microtiter plates coated with goat antibodies that bind human IgM present in serum samples. After antibodies from a serum sample are captured, Ebola virus-specific IgM is detected by incubating the plate with a preparation of Ebola viral antigens, followed by polyclonal antibodies from Ebola virus-exposed rabbits that bind to captured viral antigens, with final detection mediated by horseradish peroxidase (HRP)-conjugated anti-rabbit antibodies. In contrast, the IgG ELISA in use by the CDC utilizes microtiter plates coated with viral antigens that pull down Ebola virus-specific antibodies present in serum samples; captured IgG is detected with HRP-conjugated mouse antibodies specific for human IgG. A similar IgG ELISA has been developed by the Public Health Agency of Canada and employed in their recent field laboratory efforts (12). Given the challenges inherent in generating authentic antigen preparations from viral cultures in a BSL-4 facility, ELISAs that utilize recombinant viral proteins have been developed (13, 14), but to date they do not appear to have been validated for clinical use. However, a commercially available Ebola Zaire virus IgM and IgG ELISA kit (Alpha Diagnostic International) that utilizes recombinant viral proteins expressed in *Escherichia coli* has been employed in recent clinical research efforts (15). Limited data are available to assess the sensitivity or specificity of these ELISAs. A study evaluating the cross-reactivity of IgM and IgG antibodies in convalescent-phase sera from outbreaks involving four different species of Ebola virus found that IgM antibodies are minimally cross-reactive for Ebola virus antigens from other Ebola virus species, while IgG antibodies readily react with antigens from multiple Ebola virus species (16).

IgM and IgG ELISAs were first clinically employed by the CDC during the 1995 outbreak of Ebola Zaire virus in Kikwit, Democratic Republic of Congo, and have since been a cornerstone of EVD outbreak investigations. However, these tests have limited utility in diagnosing acute EVD due to the variable onset of humoral responses. In a study of 29 EVD survivors from the 1995 Kikwit outbreak, IgM and IgG antibodies appeared between days 2 and 10 and days 6 and 19 after symptom onset, respectively (17). In this survivor cohort, IgM was detectable in all patients between days 10 and 29 of illness and persisted at least through day 30 and up to day 168 (the latest time point tested). IgG was detectable in nearly all patients by day 19 of illness and persisted at least through day 661 and up to day 749 (the latest time point tested). Similarly, a retrospective analysis of convalescent-phase serum samples collected during three outbreaks with different Ebola virus species (1995, Kikwit, Democratic Republic of Congo; 2000, Gulu, Uganda; 2007, Bundibugyo, Uganda) showed a loss of IgM in most patients by day 80 of illness, with persistence of IgG through the

final time point tested (day 117) (16). Importantly, evaluation of acutely ill patients from the 1995 Kikwit outbreak as well as the 1996 Gabon outbreaks demonstrated that antibody responses are often not detected during EVD infections with fatal outcomes (10, 18, 19). A recent study of acutely ill EVD patients infected during the 2014–2015 epidemic and treated in U.S. or European facilities showed the onset of IgM and IgG responses between 6 and 11 days and 9 and 11 days after symptom onset, respectively (15); differences in antibody responses between fatal and nonfatal infections in this cohort were not reported. In survivors, IgG has been shown to persist for years following exposure (9, 10, 20). In summary, the current literature suggests that IgM antibody responses during EVD infection are variable, with the onset of detection ranging from 2 to 11 days following symptom onset and persisting through at least day 30 but typically not beyond day 80 in nonfatal infections (Fig. 1). IgG responses typically become detectable in the second week of illness in EVD survivors and can persist for years (Fig. 1), providing a useful tool for population-level seroprevalence studies.

Protein Antigen Detection

The detection of viral protein antigens circulating in blood provides a reliable method for diagnosing acute EVD in symptomatic patients, as viral proteins typically accumulate to detectable levels within a few days of disease onset. An ELISA for the detection of Ebola virus antigens, first developed at USAMRIID in 1989, utilizes a pool of 8 monoclonal mouse antibodies reactive against EBOV and SUDV for antigen capture and polyclonal antibodies from hyperimmune rabbit serum (reactive against EBOV, SUDV, and RESTV) for antigen detection (21). This assay was first evaluated by the CDC for clinical use during the 1995 outbreak in Kikwit, Democratic Republic of Congo (10), and it performed well for clinical diagnosis of acute EVD in a field laboratory deployed by the CDC during the 2000 outbreak in Gulu, Uganda (2), offering the fastest method of virus detection available at the time (~5 h). By this method, viral antigen can be detected in serum as early as the first day of symptoms, and detectable antigen is present in nearly all EVD patients by day 3 of illness (2, 10, 17, 18) (Fig. 1). Antigen levels rise throughout the course of disease in fatal cases. During nonfatal infections, antigen levels are comparable to those in fatal cases during the first 7 to 10 days of illness, after which they typically decline to undetectable levels by day 16 (2, 10, 17, 18) (Fig. 1).

The CDC antigen detection ELISA became part of the standard diagnostic testing suite used by the CDC in subsequent outbreaks; however, limited access to the antibody reagents may have limited its use by other agencies. ELISA antigen detection tests utilizing monoclonal antibodies against NP (22), VP40 (23), or GP (24) proteins (generated from mice immunized with purified or recombinant Ebola virus proteins) have been developed and are in place at some national reference laboratories (25), but the use of these assays for clinical diagnosis has not been reported, as real-time reverse transcription-PCR (RT-PCR) techniques have now replaced these tests (discussed in the “Real-Time RT-PCR” section below). During the recent outbreak, lateral flow immunoassays (LFIs) emerged as powerful tools for rapid antibody-mediated antigen capture that can be performed at the point of care. Novel LFIs for EVD diagnosis are discussed in the “Rapid antigen detection tests” section, below.

Conventional RT-PCR

Diagnostic RT-PCR tests for Ebola virus, developed by the CDC, were first evaluated on serum samples collected from acutely ill patients during the 1995 Kikwit outbreak (26). These assays used PCR to amplify the L, GP, and NP genes, followed by size-based amplicon detection via gel electrophoresis. An important advantage of this method was the simple, chemical inactivation of infectious virus during the initial steps of RNA extraction by using a chaotropic agent such as guanidine thiocyanate, allowing subsequent sample processing to be carried out on the benchtop. (Of note, the efficacy of viral inactivation under such conditions has recently been questioned; viral inactivation methods are discussed in more detail in the “Specimen Management and Biosafety for Diagnostic Testing” section). Conventional RT-PCR was found to be more sensitive than antibody and antigen detection ELISAs when evaluated over the complete course of symptomatic infection (see below), and in 1999 the CDC recommended its use in conjunction with antigen detection ELISA testing for diagnosis of acute EVD (26).

Conventional RT-PCR testing for EVD was first evaluated by a regional laboratory during the 1996 outbreaks in Gabon, at the Centre International de Recherches Medicales de Franceville (CIRMF). In this setting, an RT-PCR test detecting the L gene of Ebola virus in peripheral blood mononuclear cells was more sensitive than serum IgM or antigen detection by the CDC ELISAs (antigen was detected in 83% of samples that tested positive by RT-PCR, and IgM was positive in 67%) (27). Furthermore, RT-PCR detected viral RNA in two specimens collected on the day of symptom onset that were negative for antigen at this time point, suggesting that RT-PCR may detect infection earlier in disease (27). The CDC first used RT-PCR testing of serum samples for clinical diagnosis of acute EVD during the 2000 outbreak in Gulu, Uganda, and similarly found that a nested RT-PCR assay for NP gene detection was able to detect viral RNA up to 72 h before antigen became detectable by ELISA and up to 72 h following the loss of antigen detection in convalescent patients (2). Of note, this study also showed that an RT-PCR assay for L gene detection showed inferior sensitivity to the nested RT-PCR assay for NP gene detection, warranting caution in RT-PCR assay selection (2).

Importantly, early application of conventional RT-PCR demonstrated that it also performed well for detection of virus in other body fluids, such as saliva and seminal fluid. Indeed, RT-PCR for the NP gene was able to detect persistent viral RNA in multiple body fluids from convalescent patients for several weeks beyond the cessation of symptoms (28), and in a small study the yield of RT-PCR for the L and NP genes in saliva samples from acutely ill patients was consistent with that of serum RT-PCR testing (29). (Testing of body fluids is discussed in more detail in the “Detection of Viral Persistence in Nonblood Body Fluids” section.) Furthermore, experience soon demonstrated that RT-PCR tests could be rapidly developed and adapted to newly identified viral strains, as exemplified during the first known outbreak of BDBV in 2007 (30).

Real-Time RT-PCR

Real-time RT-PCR assays utilizing fluorogenic probes designed for the detection of Ebola virus were first developed (31) and tested in EVD patient serum samples (32) in the early 2000s. In a retrospective evaluation of patient samples collected during the

2000 Gulu outbreak, the CDC found that estimates of viral RNA copy number based on real-time RT-PCR correlated well with quantification of viral loads by plaque assay, and that lower cycle threshold (C_T) values (and thus higher viral RNA copy numbers) were associated with higher mortality (2). Several reports from the 2014–2015 epidemic have confirmed that high viral loads are associated with a poor prognosis, with most studies demonstrating a significantly higher mortality in patients with $>10^7$ RNA copies/ml blood at the time of diagnosis (typically seen as C_T values of <25) (4, 12, 33–36). Recent evidence from a longitudinal study of four EVD survivors suggests that C_T values greater than ~ 35 in the setting of convalescence are not associated with infectious virus (37); however, a C_T value of ≤ 40 is typically used as the cutoff to designate a positive sample (37). Because existing data indicate that detection of RNA by real-time RT-PCR is variable in the first 72 h of illness, current guidelines recommend that suspected EVD patients who test negative in this period should be retested after 72 h of symptoms (38, 39), or earlier if their condition deteriorates. The reported durations of persistence of detectable RNA in the serum or plasma of EVD survivors appear to depend on the RT-PCR assay used, although differences in study populations between testing centers render comparisons between assays difficult. Field laboratories in the 2014–2015 epidemic (employing a variety of commercial and laboratory-developed assays) reported variable times to clearance of detectable RNA in their survivor populations, ranging from day 13 to 45 of illness (12, 15, 35) (Fig. 1). The duration of RNA detection by RT-PCR during convalescence has important implications for the reentry of EVD survivors into the community, and the variable performance of RT-PCR assays during this period must be carefully considered when establishing discharge criteria. The clinical significance of very low levels of virus RNA in convalescent patients who are clinically well is unknown.

Compared to conventional RT-PCR, real-time amplicon detection using sequence-specific probes offers greater specificity and more rapid results (typically 2 to 3 h); however, limited data are available regarding the sensitivity and specificity of the various laboratory-developed and commercial Ebola virus real-time RT-PCR assays now employed by public health reference laboratories (25). Only one study to date has compared the analytic characteristics of commercially available Ebola virus real-time RT-PCR assays, and it demonstrated up to 100-fold variations in the limits of detection and 1,000-fold variations in the lower limits of quantitation (40). Many well-validated, portable thermocyclers with real-time fluorescence detectors are commercially available (e.g., models manufactured by Applied Biosystems, Roche Diagnostics, Cepheid, and Bio-Rad) and have been successfully employed in field laboratories in recent outbreak settings.

Despite its potential diagnostic advantages, RT-PCR methodology (both conventional and real-time approaches) requires significant laboratory infrastructure, electrical power, multiple temperature-sensitive reagents, the operation and maintenance of specialized equipment, and technical expertise in molecular biology, potentially complicating deployment in resource-limited settings. The performance of these RT-PCR-based assays has been found to vary even among national reference laboratories (41), and reliable results are contingent upon both appropriate sample handling prior to analysis (to avoid RNA degradation) and avoidance of cross-contamination; thus, careful oversight and quality assurance measures are necessary to ensure

adequate sample integrity and assay performance in field laboratory settings. Furthermore, assay design must take into account the potential for false-negative results due to PCR inhibitors present across specimen types, as well as sequence variation in novel virus strains/species.

FIELD DIAGNOSTIC LABORATORIES IN EBOLA VIRUS OUTBREAKS

Importance of Field Diagnostic Capacity

Diagnostic testing during Ebola virus outbreaks can take place in a spectrum of settings that include international reference laboratories (i.e., samples are shipped out of the country), regional reference laboratories (often requiring the ground transport of samples over long distances), field laboratories situated at or near patient care units (which, depending on location, may still require substantial ground transport of samples), and potentially at the point of care (see the “Rapid antigen detection tests” section, below). Historically, diagnostic testing has been carried out in international reference laboratories (Fig. 2), and nine sites are currently listed as WHO Collaborating Centres for the diagnosis of Viral Hemorrhagic Fevers (VHF): Public Health Agency of Canada (PHAC), Institut Pasteur de Lyon (IPL) in France, Institut Pasteur de Dakar (IPD) in Senegal, CIRMF in Gabon, Bernhard-Nocht Institute for Tropical Medicine (BNITM) in Germany, Kenya Medical Research Institute (KEMRI), National Institute of Communicable Diseases (NICD) in South Africa, Uganda Virus Research Institute (UVRI), and the U.S. CDC (38). (Note that other national laboratories with the broad designation of being “WHO collaborating centers,” such as the Public Health England VHF laboratory, also offer validated tests for Ebola virus). In addition, some countries have established national reference VHF laboratories, and these have greatly improved regional diagnostic capacities. While these laboratories provide outstanding technical capacity and rigorous biocontainment, it has become clear that delays associated with remote diagnostic testing hinder outbreak responses (42). Reducing the time to diagnosis has a significant impact in several aspects of a response effort, as follows.

1. Clinical management of suspected EVD patients. Due to the nonspecific clinical presentation of EVD, many patients who do not have the disease are admitted to isolation wards, where they may be exposed to those who have EVD (43). Earlier confirmation of EVD (and, in some cases, establishing an alternative diagnosis) allows for more effective infection control measures and allocation of limited clinical resources.
2. Discharge and community reintegration of EVD survivors and non-EVD patients. Negative test results are often necessary for patients to be accepted back into their communities and to receive health care at non-Ebola facilities.
3. Postmortem testing. Timely testing can allow families to proceed with burial practices as appropriate and can assist in surveillance and contact tracing efforts.
4. Contact tracing. Earlier confirmation of true EVD cases greatly increases the efficiency of contact tracing efforts.

Although the advantages of expeditious testing are evident, the logistical and safety challenges of operating a field laboratory during an outbreak of a pathogen requiring high biosafety level ca-

capacity are daunting. To maintain adequate biosafety and containment, laboratory technicians must either have access to a negative-pressure glove box or comprehensive personal protective equipment. Furthermore, infrastructure for a consistent power supply, water access, waste disposal, transport and storage of temperature-sensitive reagents, and equipment maintenance are essential. The logistical challenges involved in operating a field molecular diagnostic laboratory under resource-constrained conditions have recently been described (44).

Field Diagnostic Laboratory Efforts in Prior Outbreaks

Despite the challenges, successful field diagnostic laboratory operations have been carried out in several past Ebola virus outbreaks (Fig. 2). Notably, a diagnostic laboratory run by the CDC was set up during the first known Ebola outbreak in 1976 at a local hospital in Zaire, at which immunofluorescence microscopy was utilized for acute diagnosis. It was not until the 2000 Gulu outbreak that another major field diagnostic laboratory operation was attempted (45). The CDC again set up a lab in a local hospital, which was equipped with antigen-capture ELISA and conventional RT-PCR testing. PHAC operated field diagnostic laboratories with real-time RT-PCR testing in the 2003 (11), 2007 (46), and 2012 (47) Ebola outbreaks in DRC. Field experience has also been gained from mobile laboratories with molecular testing capacity deployed in outbreaks with other high-biosafety-level pathogens (e.g., Lassa and Marburg viruses) (48), including those operated by partners of the European Mobile Laboratory Consortium, the VHF Consortium, and other members of the WHO Global Outbreak Alert and Response Network.

LABORATORY DIAGNOSIS OF EVD IN THE 2014-2015 EPIDEMIC

Overview

The unprecedented scope of the West African Ebola epidemic necessitated a major influx of laboratory resources from the international community. Indeed, following the initial confirmation of Ebola virus infection in specimens from Guinea tested by WHO Collaborating Centers for VHF in Europe (49) and Africa, agencies from across the globe ultimately deployed nearly 40 field laboratories to West Africa, with many operating as part of the WHO Emerging and Dangerous Pathogens Laboratory Network (EDPLN). While several of the participating agencies had experience with field laboratory deployment in outbreak scenarios, few had been directly involved with prior Ebola virus outbreaks. Furthermore, although laboratory-developed Ebola virus RT-PCR assays were in routine use at multiple national reference laboratories, no EVD diagnostic tests had regulatory approval for clinical use at the beginning of the outbreak. To address these issues, the WHO set forth guidelines for laboratory diagnosis of EVD to promote standardization of biosafety and quality control measures (38, 50) and initiated an Emergency Use Assessment and Listing (EUAL) process for EVD diagnostic tests (51). The FDA also evaluated EVD diagnostic tests for issuance of Emergency Use Authorization (EUA) status.

Real-time RT-PCR performed on blood specimens has become the standard methodology for diagnosis of acute EVD in an outbreak setting, while real-time RT-PCR performed on oral fluid specimens has become the standard for postmortem testing (38, 50). Thus, all field laboratories deployed to West Africa during the

outbreak were equipped for inactivation of infectious virus under adequate biocontainment, RNA extraction, and real-time RT-PCR (with strict measures to prevent cross-contamination between samples), and they were staffed with laboratory technicians proficient in molecular biology techniques. Despite the critical contribution of these laboratories to the response effort, persistent barriers around RT-PCR diagnosis (largely attributable to the challenges in collecting and transporting samples [see “Specimen Management and Biosafety for Diagnostic Testing,” below]) have resulted in prolonged turnaround times that compromise clinical management and infection control efforts (42). Furthermore, it is unclear how these expatriate-managed resources will be integrated into regional laboratory capacity as the epidemic wanes.

In response to these challenges, and in preparation for establishing sustainable surveillance and rapid response capacity in affected countries, the WHO issued a Target Product Profile (TPP) for the development of safe, rapid, and cost-effective EVD diagnostic tests that can be used at or near the point of care by local laboratory technicians and health care workers (52). Test features prioritized in the TPP include the intended use in decentralized health care facilities with no or minimal laboratory infrastructure, excellent performance characteristics (clinical sensitivity of >95% and specificity of >99%), use of minimally invasive diagnostic specimens (e.g., capillary blood, oral fluid), and simple test procedures (e.g., no or minimal preanalytic sample processing, minimal timed and overall procedural steps, preferably no precise volume transfers, and an integrated internal control). In addition, the TPP describes desirable operational test characteristics, including long-term reagent stability under tropical conditions with no cold chain requirements, small and portable equipment with minimal or no power requirements or need for maintenance, and minimal training needs. Novel Ebola virus diagnostic tests have now reached the field, and include automated nucleic acid amplification tests (NAATs) as well as rapid antigen detection tests (53). Those that have received WHO and/or FDA EUA status at the time of this article are described in Table 1. No ELISA-based tests for antigen or antibody detection are currently approved for acute EVD diagnosis.

Of note, all tests with FDA EUA status are approved for “presumptive” testing only; any positive presumptive Ebola virus test in the United States must be confirmed at the CDC (when performed at the CDC, the CDC Ebola virus NP and VP40 RT-PCR assays may currently be used for confirmatory testing, although a combination of testing modalities may be employed) (54). Ebola virus is listed as a Tier 1 Select Agent by the U.S. Department of Health and Human Services (55); once a patient specimen has been confirmed to contain infectious Ebola virus by viral culture, all clinical specimens from that patient are subject to Select Agent regulations and must be appropriately destroyed, decontaminated, or transferred to a Select Agent facility until the patient is shown to have cleared the infection (56). In the United Kingdom, confirmation of infection by any recognized Ebola virus detection method is sufficient to trigger restrictions on sample handling and disposal (57). Other Western countries also have variants of these procedures to maintain the safety of health care workers and for biosecurity. The WHO does not systematically designate “presumptive” versus “confirmatory” tests in their EUAL; however, WHO guidance documents state that nucleic acid amplification tests are preferred when feasible and that rapid antigen detection tests should serve as “presumptive” or “screening” tests in remote

settings without access to immediate molecular testing or to assist in triaging high-risk patients when case loads are high (50, 58). When necessary, confirmatory testing may be performed by a recognized national reference viral hemorrhagic fever (VHF) laboratory; alternatively, specimens can be sent to one of the nine WHO Collaborating Centres for VHF (38). Therefore, country-specific strategies for presumptive and confirmatory testing must be established that take into account the epidemiologic setting and available laboratory resources.

EVD Diagnostic Tests with Emergency Use Authorization

Standard (nonautomated) real-time RT-PCR tests. The RealStar Filovirus Screen RT-PCR kit 1.0 (Altona Diagnostics GmbH; a two-target multiplex assay for simultaneous detection of Ebola virus [all species] and Marburg virus L gene targets; approved for use in plasma specimens) was the first real-time RT-PCR test to receive EUA status from the WHO (59) and was widely used by field laboratories in the most recent epidemic. A laboratory evaluation of this assay was carried out at the BNITM (this study is briefly described in the WHO EUAL report [59]; however, details of the study have not been published). The analytic sensitivity of the RealStar Filovirus assay compared well with two laboratory-developed RT-PCR assays for RNA detection in plasma samples spiked with RNA extracted from infected cell culture supernatants, and no cross-reactivity to other viral hemorrhagic fever viruses was observed. Of note, recent studies have raised concern that the RealStar Filovirus assay, as deployed in the field, is not adequately sensitive (60, 61) (discussed below). A similar assay from the same manufacturer, RealStar Ebolavirus RT-PCR kit 1.0 (Altona Diagnostics GmbH; L gene detection for all Ebola virus species; approved for use in plasma specimens) received FDA EUA status (62). Data regarding the clinical performance of this assay have not been published. The second RT-PCR test to be added to the WHO EUAL was the Liferiver-Ebola virus (EBOV) real-time RT-PCR kit (Shanghai ZJ BioTech; NP gene detection for EBOV, SUDV, TAFV, and BDBV; approved for use in whole blood [source not specified], plasma, and serum specimens). As described in the WHO EUAL report (63), the analytic sensitivity of this assay was evaluated at the BNITM using whole blood inoculated with infectious cell culture supernatants; data regarding clinical performance are not available.

Four other standard real-time RT-PCR tests have been granted EUA status by the FDA: CDC Ebola virus NP and VP40 real-time RT-PCR assays (U.S. CDC; EBOV NP and VP40 gene detection, respectively; approved for use with venous whole blood, plasma, serum, and urine) (64, 65), DoD EZ1 real-time RT-PCR assay (U.S. Department of Defense; EBOV GP gene detection [50]; approved for use with venous whole blood and plasma) (66), and the LightMix Ebola virus Zaire test (Roche; EBOV L gene detection; approved for use with whole blood [source not specified]) (67). Per the FDA authorizations (64–66), use of the U.S. CDC and DoD assays is restricted to facilities designated by these agencies; thus, these assays are not commercially available to clinical laboratories. Deployment of the CDC NP and VP40 assays in a field laboratory in Sierra Leone was recently described (68), although an evaluation of assay performance in this setting has not been provided. Data regarding clinical performance of the EZ1 and LightMix assays are not available.

Ancillary requirements for each of the standard real-time RT-PCR tests discussed above include capacity for cold chain main-

TABLE 1 Ebola virus diagnostic tests with WHO and/or FDA EUA status^a

Assay name (manufacturer)	FDA/WHO EUA	Assay format	Detection target (virus species)	Approved specimen type(s) under EUA	Preanalytic processing (approved kits/reagents)	Approved instrument platform(s) under EUA	Facility restrictions for FDA EUA	Infrastructure requirements ^b	Time to results ^c
RealStar Filovirus Screen RT-PCR kit 1.0 (Altona Diagnostics GmbH)	WHO	Real-time RT-PCR	L gene RNA (EBOV, BDBV, RESTV, SUDV, TAFV)	Plasma	Sample lysis, RNA extraction (QIAamp viral RNA minikit, Qiagen)	ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), CFX96 system/Dx (Bio-Rad), Mx 3005P QPCR (Stratagene), Rotor-Gene 3000/6000 (Corbett Research), Rotor-Gene Q 5/6 plex (Qiagen), Versant kPCR Molecular System AD (Siemens)	NA	-20°C storage, UPS	4-6 h
RealStar Ebolavirus RT-PCR kit 1.0 (Altona Diagnostics GmbH)	FDA	Real-time RT-PCR	L gene RNA (EBOV, BDBV, RESTV, SUDV, TAFV)	Plasma	Sample lysis, RNA extraction (QIAamp viral RNA minikit, Qiagen)	ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), CFX96 system/Dx (Bio-Rad)	CLIA high complexity or similar qualification	-20°C storage, UPS	4-6 h
EZI Real-time RT-PCR Assay (U.S. DoD, LDT)	FDA	Real-time RT-PCR	GP gene RNA (EBOV)	Venous WB, plasma	Sample lysis, RNA extraction (QIAamp viral RNA minikit, Qiagen)	ABI Prism 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), JBAIDS instrument (Biofire Defense)	Laboratories designated by U.S. DoD	-20°C storage, * UPS	4-6 h
CDC Ebola Virus NP real-time RT-PCR assay (CDC, LDT)	FDA	Real-time RT-PCR	NP gene RNA (EBOV)	Venous WB, plasma, serum, urine (if paired with blood)	Sample lysis, RNA extraction (MagMax Pathogen RNA-DNA kit, Applied Biosystems; Dynal bead retriever; Invitrogen)	ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), CFX96 system/Dx (Bio-Rad)	Laboratories designated by CDC	-20°C storage, * UPS	4-6 h
CDC Ebola Virus VP40 real-time RT-PCR assay (CDC, LDT)	FDA	Real-time RT-PCR	VP40 gene RNA (EBOV)	Venous WB, plasma, serum, urine (if paired with blood)	Sample lysis, RNA extraction (MagMax Pathogen RNA-DNA kit, Applied Biosystems; Dynal bead retriever, Invitrogen)	ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), CFX96 system/Dx (Bio-Rad)	Laboratories designated by CDC	-20°C storage, * UPS	4-6 h
LightMix Ebola Zaire rRT-PCR test (TIB MOLBIOL Syntheselabor GmbH)	FDA	Real-time RT-PCR	L gene RNA (EBOV)	WB	Sample lysis (TriPure, Roche), RNA extraction (MagNA Pure 96 DNA and Viral Nucleic Acid Kit, Roche; High Pure Viral Nucleic Acid Kit, Roche)	LightCycler 480 (Roche), cobas z 480 Analyzer (Roche)	CLIA high complexity or similar qualification	2-24°C storage, UPS	4-6 h
Lifiver Ebola virus (EBOV) real-time RT-PCR kit (Shanghai ZI BioTech)	WHO	Real-time RT-PCR	NP gene RNA (EBOV, SUDV, TAFV, BDBV)	WB, plasma, serum	Sample lysis, RNA extraction (QIAamp Viral RNA minikit, Qiagen; QIAamp DSP virus Spin kit, Qiagen; Liferiver RNA isolation kit, Liferiver)	ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), CFX96 system/Dx (Bio-Rad), SLAN-96 (Hongshi)	NA	-20°C storage, * UPS	4-6 h

FilmArray NGDS BT-E assay (BioFire Defense)	FDA	Automated, cartridge- based RT-PCR	L gene RNA (EBOV)	WB, plasma, serum	Inoculation of sample injection vial containing viral inactivation buffer	FilmArray instrument (Biofire)	Laboratories designated by U.S. DoD	15–25°C storage, * UPS	75 min
FilmArray Biothreat- E test (BioFire Defense)	WHO, FDA	Automated, cartridge- based RT-PCR	L gene RNA (EBOV)	Per FDA and WHO: WB, urine (if paired with blood)	Inoculation of sample injection vial containing viral inactivation buffer	FilmArray instrument (Biofire)	CLIA moderate or high complexity or similar qualification	15–25°C storage, UPS	75 min
Xpert Ebola assay (Cepheid)	WHO, FDA	Automated, cartridge- based RT-PCR	NP, GP gene RNA (EBOV)	Per FDA; venous WB ^a ; per WHO: venous WB	Sample inactivation	GeneXpert platform (Cepheid)	CLIA moderate or high complexity or similar qualification	2–28°C storage, UPS	100 min
ReBOV Antigen Rapid Test (Corgenix)	WHO, FDA	Chromatographic lateral flow immunoassay	VP40 protein antigen (EBOV, SUDV, BDBV)	Per FDA; fingerstick WB, venous WB, plasma; per WHO: plasma, serum	None	NA	Adequately equipped facilities, including treatment centers and public health clinics	2–8°C storage	15–25 min
OraSure Ebola Rapid Antigen test (OraSure Technologies)	WHO, FDA	Chromatographic lateral flow immunoassay	VP40 protein antigen (EBOV, SUDV, BDBV)	Per FDA; fingerstick WB, venous WB, cadaveric oral fluid; per WHO: WB, cadaveric oral fluid	None	NA	Adequately equipped facilities, including treatment centers and public health clinics	2–30°C storage	30 min
SD Q Line Ebola Zaire Ag (SD Biosensor)	WHO	Chromatographic lateral flow immunoassay	NP, GP, VP40 protein antigens (EBOV)	WB, plasma, serum	None	NA	NA	1–40°C storage	20–30 min

^a Abbreviations: EUA, emergency use authorization; FDA, U.S. Food and Drug Administration; WHO, World Health Organization; RT-PCR, reverse transcription-PCR; WB, whole blood; EBOV, Zaire/Ebola virus; SUDV, Sudan virus; BDBV, Bundibugyo virus; RESTV, Reston virus; TAFV, Tai Forest virus; CDC, Centers for Disease Control and Prevention; CLIA, Clinical Laboratory Improvement Amendments; UPS, uninterrupted power supply; LDT, laboratory-developed test; NP, nucleoprotein; GP, glycoprotein; NA, not applicable or not available.

^b Storage temperature requirements are taken from the following package inserts, except where otherwise indicated: RealStar Filovirus Screen RT-PCR kit 1.0 IVD package insert, August 2014; RealStar Ebola virus RT-PCR kit 1.0 EUA package insert, November 2014; LightMix Ebola Zaire rRT-PCR test EUA package insert MDx 40-0666-96; FilmArray BioThreat-E EUA package insert REIT-0302-01, October 2014; Xpert Ebola assay IVD package insert 301-4826, revision A, June 2015; ReBOV Antigen Rapid test EUA package insert 14005-01, March 2015; OraQuick Ebola Rapid Antigen test EUA package insert, March 2016; SD Q Line Ebola Zaire Antigen Test package insert R1-20150901.indd, September, 2015. For assays without accessible package inserts (marked with an *), storage requirements were taken from the WHO guidance document on Ebola *in vitro* diagnostic assays (51).

^c Including preanalytic processing, if applicable.

^d Venipuncture whole blood, swab of fingerstick blood, and swab of oral fluid are all listed in the CE-IVD-approved package insert (June 2015), while only venipuncture whole blood is included in the FDA and WHO EUAs.

tenance, collection and transport of venipuncture blood, sample lysis/inactivation, manual RNA extraction, operation of a thermocycler with fluorescence detection, and manual recording and reporting of results. The estimated time to results including sample preparation and analytic procedures is 4 to 6 h. Where applicable, data regarding the clinical performance of these tests in comparison to novel assay platforms are described below.

Automated real-time RT-PCR tests. Several fully automated PCR platforms have been developed in recent years that integrate nucleic acid extraction, PCR amplification, and detection of reaction products, typically yielding results in 90 min or less. These platforms are designed for use in more decentralized health care settings and minimize manual processing, thereby improving safety and facilitating use by technicians with minimal training. However, these tests still require specialized instruments and accessory laptop computers, which in turn require electrical power and equipment maintenance.

The Xpert Ebola assay (Cepheid), which has EUA status from both the WHO and FDA (69, 70), is an automated, cartridge-based system for RNA extraction and real-time RT-PCR detection of EBOV NP and GP genes. Patient samples (of note, venipuncture whole blood, swabs of fingerstick blood, and swabs of oral fluid are all listed in the package insert with CE-IVD approval [Xpert Ebola IVD package insert 301-4826, revision A, June 2015], while only venipuncture whole blood is included in the FDA and WHO EUAs) are placed directly into a prefilled sample reagent vial; an aliquot is then loaded into a cartridge and the test is run on a module-format GeneXpert instrument. Kit components for this test require storage at 2 to 28°C. According to the most recent package insert (June 2015), 20 min in the sample reagent completely inactivates up to 4.6×10^6 PFU of EBOV. Ideally, this would allow for subsequent sample processing to be carried out on a benchtop, facilitating use at or near the point of care; however, since blood viral loads in acutely ill patients can exceed 10^8 RNA copies/ml (4), more information about the efficiency of viral inactivation in such specimens may be needed to consider use of the Xpert platform outside a biocontainment laboratory. Notably, the GeneXpert system has been widely used in developing regions for molecular detection of *M. tuberculosis* complex, though deployment has been limited primarily to district-level labs due in part to the requirement for a stable and continuous power supply.

An evaluation of the analytical performance of the Xpert Ebola assay showed a limit of detection of 73 viral genome copies/ml for inactivated virus, 1 PFU/ml for infectious virus, and 232 RNA copies/ml (71). The analytic and clinical performance characteristics of the Xpert Ebola assay were further evaluated at the NICD in South Africa (72). This study presented a comparison of results obtained from the Xpert Ebola assay and a laboratory-developed real-time RT-PCR assay (L gene target) performed on 281 frozen serum and plasma samples collected from EVD suspect patients in Sierra Leone. Agreement between the Xpert Ebola assay and the L gene RT-PCR assay (as performed on two different thermocyclers) was 100% in specimens yielding C_T values of ≤ 35 by the L gene RT-PCR assay; discrepancies between tests were seen at C_T values of 35 to 45. Viral isolation in cell culture was successful for 91/125 specimens that tested positive by either of the molecular tests; importantly, both the Xpert Ebola assay and the L gene RT-PCR assay detected 100% of specimens from which virus could be recovered in culture.

The field performance of the Xpert Ebola assay was recently

evaluated on whole blood and buccal swab samples submitted for routine clinical RT-PCR testing in a field biocontainment laboratory in Sierra Leone (73). Compared to the benchmark Trombley RT-PCR assay (NP gene target) (74), the Xpert assay showed 100% sensitivity with 95.8% specificity in fresh venipuncture whole-blood specimens ($n = 211$; 22 Ebola virus-positive specimens), and 100% sensitivity with 100% specificity in buccal swab specimens (mixture of fresh and frozen specimens; $n = 64$; 20 Ebola virus-positive specimens). All but one of 8 discordant whole-blood specimens (Trombley negative/Xpert positive) had been collected from known EVD patients (who had previously tested positive by the Trombley assay and were under monitoring for viral clearance), suggesting a revised Xpert assay specificity of 99.5%. Of note, a small fraction of specimens failed Xpert testing due to endogenous/exogenous internal control failure or system failure, highlighting both the need for adequate sample and reagent integrity and the potential requirement for technical expertise for troubleshooting and equipment maintenance. The Xpert Ebola assay performed similarly well in a study carried out at an Ebola treatment center near Conakry, Guinea, showing 100% sensitivity with 96.0% specificity in fresh venipuncture whole-blood specimens ($n = 218$; 26 Ebola virus-positive specimens) compared to an in-house RT-PCR assay (NP gene target) used for routine clinical diagnostic testing at the national reference laboratory at Gamal Abdel Nasser University of Conakry, located near the treatment center (75). All discordant specimens (benchmark RT-PCR negative/Xpert positive) were obtained from known EVD patients undergoing monitoring for viral clearance, again suggesting a higher actual specificity for the Xpert assay and highlighting the performance of this assay in the convalescent stage of EVD. Importantly, this study demonstrated the feasibility of operating the Xpert platform at the site of patient care; however, biosafety and logistical concerns were noted.

The BioFire Defense FilmArray assays (FilmArray Biothreat-E test and FilmArray NGDS BT-E assay) are automated real-time RT-PCR tests for detection of the EBOV L gene. The FilmArray Biothreat-E test has EUA status from both the FDA (76) and WHO (77), with approved use of whole blood (source not specified) and paired urine specimens, while the FilmArray NGDS BT-E test has FDA EUA status (78) for use with whole blood (source not specified), plasma, and serum specimens. For both FilmArray assays, a pouch preloaded with lyophilized reagents for RNA extraction and real-time RT-PCR is rehydrated, followed by preparation of the patient sample in a sample injection vial containing sample lysis reagents. The contents of the sample injection vial are then loaded into the pouch and run on a single-assay FilmArray instrument. Per the package insert (BioThreat-E EUA IFU RFIT-PRT-0302-01, October 2014), sample preparation and loading of the pouch are to be performed in a biosafety cabinet. Kit components require storage at 15 to 25°C. Use of the FilmArray NGDS BT-E test is restricted to laboratories designated by the U.S. DoD (78).

Several studies have evaluated the Filmarray Biothreat-E assay in clinical specimens. The largest of these studies, carried out in Public Health England laboratories in both Sierra Leone and the United Kingdom, compared the results from venipuncture whole blood tested by the FilmArray Biothreat-E test with paired plasma tested by routine clinical RT-PCR (Trombley assay) (79). The Biothreat-E test demonstrated 84% sensitivity with 89% specificity in specimens collected in Sierra Leone ($n = 60$; 25 Ebola virus-pos-

itive specimens), and 75% sensitivity with 100% specificity in specimens collected in the United Kingdom ($n = 108$; 4 Ebola virus-positive specimens), compared to the Trombley RT-PCR. In a smaller study, the Filmarray Biothreat-E assay was tested in clinical specimens collected from six EVD patients who were treated in U.S. hospitals (Emory University and University of Nebraska Medical Center) and compared to clinical RT-PCR results (CDC NP2 and VP40 assays performed by the CDC Viral Special Pathogens Branch) (80). The Biothreat-E assay showed 86% sensitivity with 80% specificity compared to benchmark RT-PCR in whole-blood specimens tested by both assays ($n = 27$) and 89% sensitivity with 75% specificity in urine specimens ($n = 13$). Twenty additional whole-blood specimens were tested against paired plasma assayed by benchmark RT-PCR and showed 100% sensitivity with 71% specificity. In a study conducted in a hospital-associated research laboratory in Sierra Leone (81), results obtained from on-site testing of whole-blood specimens from EVD suspect patients with the Filmarray Biothreat-E assay were fully concordant with clinical RT-PCR results (CDC NP and VP40 assays) obtained from plasma specimens collected at a later time and tested in CDC field laboratories (5 EVD-positive patients and 57 EVD-negative patients were tested by both assays). Finally, the Filmarray Biothreat-E assay was utilized at an Ebola treatment center in Guinea on urine ($n = 7$) and saliva ($n = 18$) specimens collected from EVD patients (confirmed by routine RT-PCR testing of venous blood; QuantiTect Probe RT-PCR [Qiagen] and RealStar Filovirus Type RT-PCR kit 1.0 [altona Diagnostics]) (82). All urine and saliva specimens tested positive by the Biothreat-E test, demonstrating the utility of this platform in testing noninvasive specimen types near the point of patient care.

Rapid antigen detection tests. Three EVD rapid diagnostic tests (RDTs) have received WHO and/or FDA EUA status, all of which are lateral flow immunoassays (LFIs) (Table 1). LFIs are designed for use at the point of care and have been successfully used to diagnose other infectious diseases (e.g., HIV, malaria) in resource-poor settings. The ReEBOV Antigen Rapid Test kit (Corgenix, Inc.) was the first LFI for EVD to receive EUA status (both WHO and FDA) (83, 84). This test is a chromatographic dipstick immunoassay for detection of the Ebola virus VP40 matrix protein (EBOV, SUDV, BDBV). The FDA EUA allows for the testing of whole blood (collected by either fingerstick or venipuncture) or plasma (83), while the WHO EUA lists whole blood (source not specified), plasma, and serum (84) as acceptable specimen types. When used at the bedside, a drop of fingerstick blood is applied directly to the nitrocellulose test strip, and the strip is then placed into a tube with buffer to initiate flow of the sample along the test strip. If present in the sample, VP40 is captured by gold-labeled anti-VP40 antibodies, forming immune complexes that are subsequently deposited along a stripe of anti-VP40 antibodies printed onto the dipstick at a specific location. The gold nanoparticles produce a pink-red line that can be visually interpreted in 15 to 25 min. No electronic equipment is needed to operate the test, though reagents do require refrigeration for storage.

In a recent field validation study in Sierra Leone, the ReEBOV RDT was performed at the bedside on fingerstick blood samples from suspected EVD patients presenting at Ebola care centers, in parallel with collection of venipuncture blood for clinical diagnostic testing (RealStar Filovirus Screen RT-PCR kit 1.0 [altona Diagnostics]) performed in a field reference laboratory; separately, the RDT was also performed on venipuncture blood in the field

reference laboratory (61). This study demonstrated that the RDT was feasible to perform in restricted patient care areas (red zones) by operators wearing full personal protective equipment and that interoperator agreement (for reading the RDT) was high. In both point-of-care settings ($n = 105$; 28 Ebola virus-positive specimens) and reference laboratory settings ($n = 277$; 45 Ebola virus-positive specimens), the RDT demonstrated 100% sensitivity and 92% specificity compared to clinical results obtained with the altona RealStar assay. Importantly, most of the EVD patients tested in this study had relatively high viral loads in blood, and the RealStar assay as performed in the field laboratory during this study was observed to have imperfect sensitivity (as mentioned above, others have made similar observations regarding the altona assay [60]). Comparison of altona to an alternative RT-PCR benchmark assay (Trombley) indicated that the altona assay was falsely negative in some samples with a C_T of >30 according to the Trombley assay, leading those authors to recognize that comparison of the ReEBOV RDT to an imperfect reference standard (altona) had led to overestimation of true RDT sensitivity and underestimation of true RDT specificity. Therefore, while these results suggest that the ReEBOV RDT could be very useful as a rapid point-of-care test for EVD in high-risk populations, its performance in patients with low viral loads (e.g., those presenting very early or very late in their disease course) remains to be ascertained.

The OraQuick Ebola Rapid Antigen Test (OraSure Technologies, Inc.) is a similar chromatographic LFI (detection of VP40 matrix protein [EBOV, SUDV, and BDBV]) that recently received FDA (85, 86) and WHO (87) EUA status. In addition to whole blood (venipuncture or fingerstick specimens listed in the FDA EUA [85]; source not specified in the WHO EUA [87]), the OraQuick RDT is the first EVD diagnostic test to receive approval for use with cadaveric oral fluid (approved in EUAs from both the FDA [86] and the WHO [87]). For testing whole blood (either fingerstick or venous), the specimen is collected into a plastic micropipette provided in the kit and applied to a sample port in an assay device containing the nitrocellulose test strip. Cadaveric oral fluid can be sampled directly by swabbing the oral mucosa with the flat pad at the end of the collection device. Alternatively, fluid from an oral swab stored in viral transport medium can be collected into a provided micropipette and applied to the sample port. The assay device is then placed into a prefilled vial of buffered developer solution. The presence of Ebola virus antigens is visually detected by the deposition of gold-labeled antibodies bound to viral proteins along the test line, as described above for the ReEBOV RDT. The test is interpreted as positive or negative after 30 min (OraQuick Ebola EUA package insert, publication 3001-2812, March 2016); per the package insert, the intensity of the test line is not proportional to the amount of virus in the blood. Kit components require storage at 2 to 30°C and can be used at 15 to 40°C.

Limited published data are available to evaluate the clinical performance of the OraQuick RDT. The WHO EUA report describes a retrospective analysis of frozen venipuncture whole blood specimens collected from Ebola suspect patients in Sierra Leone (87). In this study, the OraQuick RDT demonstrated 84% sensitivity ($n = 25$ Ebola virus-positive specimens) and 98% specificity ($n = 50$ Ebola virus-negative specimens) compared to clinical real-time RT-PCR testing (RT-PCR assay not specified). Of note, the sensitivity of the assay was higher in samples with C_T values <25 . The WHO EUA also describes a retrospective analysis

of frozen cadaveric oral fluid specimens collected in Sierra Leone (87). Compared to the results obtained by retesting with the Xpert Ebola assay, the OraQuick RDT demonstrated 94% sensitivity ($n = 51$ Ebola virus-positive specimens) and 100% specificity ($n = 193$ Ebola virus-negative specimens). Additionally, the package insert (OraQuick Ebola EUA package insert, 3001-2812, March 2016) describes three studies conducted in West Africa evaluating RDT results obtained from direct sampling of cadaveric oral fluid compared to routine RT-PCR performed on fluid collected by oral swab and stored in viral transport medium. All specimens from these studies tested negative by RT-PCR (total $n = 539$), and the RDT showed 99 to 100% specificity across studies. The CDC recently evaluated the implementation of the OraQuick RDT for testing febrile patients ($n = 1,000$) presenting to primary care facilities in Guinea who were considered to be at low risk for EVD (88). No patients tested positive by the RDT (RT-PCR testing was not performed in parallel for comparison). No information is yet available on the accuracy, operational feasibility, or interreader reliability of this assay when conducted at the point of care in Ebola care centers.

The test most recently added to the WHO EUAL (89) is the SD Q Line Ebola Zaire Ag test (SD Biosensor, Inc.), a chromatographic LFI that simultaneously detects NP, GP, and VP40 antigens of EBOV in whole blood (source not specified), plasma, or serum. Gold-labeled mouse monoclonal antibodies form complexes with antigens present in the specimen and deposit along three antigen-specific test lines for visual detection. To perform the test, three drops of specimen are added to a sample port on the assay device, using a provided disposable dropper; alternatively, 100 μ l of specimen can be added using a precision pipette. The test is visually read at 20 to 30 min, with the appearance of any of the three test lines interpreted as positive (SD Q Line Ebola Zaire Ag test package insert R1-20150901.indd, September 2015). Kit components can be stored between 1 and 40°C.

The SD Q Line Ebola Zaire Ag test was evaluated as part of a laboratory-based study in Sierra Leone comparing the performance of several antigen detection tests for EVD (a brief description of the study is provided in the WHO EUAL report for the SD Q Line test [89]; a full account of the study has not been published). In this study, a total of 446 specimens (100 fresh venous whole blood, 346 frozen plasma; 126 total Ebola virus-positive specimens) were tested by rapid antigen detection tests in four field laboratories (the other tests included in the study were not specified). Sensitivity and specificity were evaluated using the RealStar Filovirus Screen RT-PCR kit 1.0 (Altona Diagnostics) as a benchmark assay. The SD Q Line Ebola Zaire Ag test demonstrated 84.9% sensitivity with 99.7% specificity. No studies have yet evaluated the performance of this test at the point of care.

Specimen Management and Biosafety for Diagnostic Testing

Specimen collection and tracking. The current standard for real-time RT-PCR testing requires the collection of venipuncture blood into EDTA-coated blood tubes or, when blood collection is not possible or in the setting of postmortem testing, the collection of an oral swab into viral transport medium. Guidelines for safe specimen collection, packaging, and transport procedures along with lists of necessary materials are provided by the WHO (38, 90, 91) and CDC (39). During the 2014-2015 epidemic, chronic resource limitations and inadequate training often prevented adher-

ence to the WHO guidelines for sample collection and transport. A steady supply chain of the recommended venipuncture and packaging materials was lacking, requiring phlebotomists to improvise for these procedures. This compromised the safety of individuals drawing and handling blood samples as well as the integrity of the samples (e.g., hemolysis, cracked tubes, inadequate labeling), hindering sample analysis and reporting of results. Furthermore, as EDTA tubes were often unavailable, testing was not infrequently performed on serum using assays for which this specimen type was not approved. In the future, adequate support for specimen management systems must be paired with analytic resources to enable safe and expeditious EVD diagnostic testing. The ability to use clinical specimens that entail less invasive sampling, such as fingerstick blood or oral fluid swabs, would alleviate some of these challenges. Moving forward, guidelines for the safe collection of alternative diagnostic specimens, such as urine and semen, will be needed (see "Detection of Viral Persistence in Non-blood Body Fluids," below).

In addition to good practices for sample collection, packaging, and transport, reliable specimen tracking and modes of communication are imperative for accurate and efficient reporting of results to clinicians. Early in the 2014-2015 epidemic, the use of patient name, age, and home town to identify specimens frequently led to confusion, as this information often did not provide unique patient identification. To address this problem in Sierra Leone, suspected Ebola patients and dead bodies undergoing postmortem screening were identified by local field surveillance teams and allocated a case number, including a district identification code. This code was used by diagnostic laboratories as well as local and central epidemiology and case control networks to identify each person and their associated samples when reporting results externally. Within each laboratory, samples were identified by an internal lab number so that multiple samples from each patient could be recorded. Patients who were subsequently admitted to a treatment center would receive a hospital identification number, but the district- and country-level data collection systems for case surveillance and geographical distribution relied on the original case identifier assigned by the surveillance team. Daily data were collected centrally by the Ministry of Health and Sanitation, assisted by staff from the CDC, WHO, the United Kingdom and Sierra Leone Armed Forces, and staff from other national and international agencies. Several efforts were made to simplify this system, ranging from preprinted labels for use by local staff (with the idea that a set of labels would follow the patient to allow consistent identification of future records and samples) to electronic medical record systems. In practice, traditional paper forms and identification numbers handwritten onto specimen containers remained the norm across the network of laboratories and treatment centers. The implementation of a systematic case identification scheme dramatically improved specimen tracking and reporting; however, unacceptable delays in blood collection could occur when surveillance officers were not present to assign case ID numbers. In these situations, it proved very useful to implement an alternative patient/specimen identification system specific for a clinical site (and thus managed by the clinical team). Finally, reliable internet connectivity is needed to facilitate the timely reporting of results via email when treatment centers are situated remotely from diagnostic laboratories. When such connectivity was not available, cell phones were used to convey results by text message. In a postoutbreak setting, strategies for efficient

results communication that are both reliable and maintain patient privacy must be considered.

Viral inactivation in diagnostic specimens. When utilizing diagnostic methods that involve manual processing, viral inactivation is critical for the safety of laboratory workers. In addition, rendering specimens noninfectious for high-biocontainment pathogens allows for subsequent sample processing under BSL-2 conditions, which greatly improves laboratory workflow and throughput. Traditional viral inactivation methods for specimens suspected to contain hemorrhagic fever viruses include chemical inactivation, gamma irradiation, and heat treatment (92–94). However, gamma irradiation (typically using a ^{60}Co gamma cell) is usually restricted to public health and research laboratories, and some forms of chemical inactivation are considered unsafe for laboratory workers (e.g., beta-propiolactone is potentially carcinogenic). Regarding viral inactivation in specimens for EVD diagnosis, careful consideration must be given to whether the method is compatible with downstream diagnostic tests, with particular concern for maintaining the sensitivity of viral RNA detection by RT-PCR. The feasibility of any given method will vary according to the laboratory setting; however, methods involving chemical inactivation and heat treatment are usually practical for hospital-associated clinical laboratories (54) and field laboratories deployed during an Ebola outbreak (95).

Several studies have evaluated the efficiency of viral inactivation (determined by viral culture of treated specimens) mediated by common reagents used in the initial processing of specimens for RT-PCR (commonly referred to as “sample lysis”). The first of these studies, carried out by USAMRIID (96), found that TRIzol LS reagent (Invitrogen; contains phenol and the chaotropic salt guanidine isothiocyanate) and buffer AVL (Qiagen; contains guanidine isothiocyanate) were each capable of inactivating Ebola Zaire virus when the virus stock was diluted with the inactivating reagent (4 parts reagent:1 part sample; 10^6 PFU/ml after dilution) and incubated for 10 min at room temperature. Of note, this study did not test for the inactivation of virus present in whole blood or blood components; thus, the findings may not be generalizable to clinical specimens commonly used for EVD diagnosis. A later study by the CDC (97) found that NC lysis buffer (Applied Biosystems; contains guanidine hydrochloride) provided complete inactivation of Ebola Zaire virus (1.5×10^5 PFU/ml before dilution) when used at a 3 part reagent:1 part sample dilution for 10 min at room temperature, but not when used in the standard dilution for RNA extraction (1:1). By comparison, in the same study, Tripure reagent (Roche; contains phenol and a guanidine salt) provided complete viral inactivation under the same conditions at a 1:1 dilution. Again, viral inactivation was not tested in whole blood or blood component specimens. Most recently, an important study from the Defense Science and Technology Laboratory (95) showed that buffer AVL, when used at a 4 parts reagent:1 part sample dilution for 10 min at room temperature, did not reliably inactivate Ebola Zaire virus (10^8 50% tissue culture infective doses [TCID₅₀]/ml in marmoset serum or 10^6 TCID₅₀/ml in murine blood before dilution). In addition, neither ethanol (4:1 dilution, 10 min at room temperature) nor heat (60°C for 15 min) yielded complete inactivation of the murine blood samples. However, the murine blood samples were completely inactivated following treatment with a combination of buffer AVL and ethanol or buffer AVL and heat treatment, with no loss of efficiency in downstream RNA extraction and RT-PCR.

Based on these findings, protocols were adopted in a Public Health England field laboratory in Sierra Leone in which diagnostic serum and whole blood specimens were treated with a mixture of buffer AVL plus ethanol (11 parts reagents:1 part sample for 10 min) or with buffer AVL (8:1 dilution for 10 min) followed by heat (60°C for 15 min) prior to downstream processing on the benchtop (95).

Ideally, viral inactivation methods compatible with ancillary tests that support the clinical management of suspected or confirmed EVD patients should be defined and selected, including tests for malaria infection, blood chemistry, complete blood count, and coagulation status. In 2005, the CDC published guidelines recommending the treatment of serum specimens from suspected viral hemorrhagic fever patients with a combination of heat (56°C) and chemical (Triton X-100; Sigma-Aldrich) inactivation methods prior to routine handling (treatment length not specified) (98). This protocol was shown to be compatible with the detection of *Plasmodium falciparum* by RT-PCR and with a commonly used LFI (HRP-2 antigen detection; BinaxNOW; Alere) (99). However, heat treatment was shown to significantly diminish the performance of other *Plasmodium* antigen detection tests (100). Of note, thin blood smear preparations for morphological diagnosis of malaria are considered noninfectious for hemorrhagic fever viruses following 15 min of methanol fixation (54, 98); this method was recently shown to fully inactivate blood containing EBOV at 10^8 TCID₅₀/ml (101). Heat treatment alone (60°C for 60 min) is compatible with the measurement of thermally stable blood components, including electrolytes, glucose, and blood urea nitrogen (94, 102). Data are lacking regarding methods for the inactivation of Ebola virus that are compatible with routine hemocytometry, measurement of liver enzymes, or evaluation of coagulation status. There is a need for updated formal guidelines in this arena that account for recent evidence and experience with viral inactivation methods and that consider novel diagnostic platforms (both for EVD diagnosis as well as ancillary testing) and alternative specimen types. When adequate viral inactivation methods are not available, testing may be performed under BSL-3 biocontainment with the use of a biosafety cabinet and/or appropriate personal protective equipment. Modern closed-path analyzers also provide a high degree of safety if used appropriately, since they are designed to protect operators from blood-borne viruses and have suitable cleaning protocols. Smaller platforms can be adapted for use in a simple cabinet in high-risk situations, and such analyzers were used in several laboratories in the later stages of the outbreak to measure basic blood chemistry and hematology parameters (54, 103).

Detection of Viral Persistence in Nonblood Body Fluids

As discussed above, the demonstration of viral clearance from blood is recommended for the discharge of EVD survivors back into the community. However, viral persistence in other body fluids after clearance of viremia may have important implications for disease transmission (104, 105), and laboratory testing in this context is needed to support infection control efforts and to provide appropriate counseling for survivors and their communities.

Viral persistence in seminal fluid. What appears to be the first evidence that Ebola virus is secreted in seminal fluid was described in a case report of a laboratory-acquired infection with SUDV that occurred during investigations of the 1976 Sudan outbreak (106); infectious virus was detected in semen as late as day 61 following the onset of symptoms. Studies of EVD survivors from later out-

breaks (Democratic Republic of Congo 1995, Gabon 1996, and Uganda 2000) demonstrated that Ebola virus is frequently detected in seminal fluid after viremia has cleared, with infectious virus detected by viral culture in semen specimens collected as late as day 82 following the onset of symptoms and viral RNA detected by RT-PCR as late as day 101 (17, 28, 107, 108). These findings were extended in a recent study of male EVD survivors in Sierra Leone, in which semen collected from 46/93 (49%) survivors (ranging from 2 to 10 months following the onset of symptoms) tested positive by RT-PCR (CDC NP and VP40 RT-PCR assays) in a field diagnostic laboratory (109). This study demonstrated a decrease in the prevalence of viral RNA in seminal fluid specimens over time (9/9 [100%] of specimens collected 2 to 3 months after symptom onset, 24/40 [65%] of specimens collected 4 to 6 months after symptom onset, and 11/43 [26%] of specimens collected 7 to 9 months after symptom onset) as well as increasing C_T values over time (suggesting dropping viral loads). In this study, viral RNA was detected in semen as late as 284 days (9 months) after symptom onset, and the earliest time point at which a semen specimen tested negative by RT-PCR was 128 days (4 months) after symptom onset. In line with these findings, a recent study investigating viral persistence in a variety of body fluids collected from EVD survivors in Sierra Leone (described in more detail under “Viral persistence in other body fluids,” below) reported the detection of viral RNA by RT-PCR (Trombley assay) in a semen specimen collected 114 days following discharge from an Ebola treatment unit (110). The presence of infectious virus in specimens collected from the survivor cohorts described in these recent studies (determined by viral culture) has not yet been reported. Surrogate measures of infectivity based on detecting whole or intact virus genome are used for nonculturable viruses such as norovirus and could be applied to semen studies to obtain an indication of likely infectious potential.

Despite the apparently high frequency of viral persistence in seminal fluid, the overall rate of sexual transmission of EVD is thought to be very low (109, 111). However, a case of male-to-female sexual transmission in Liberia 6 months after the onset of symptoms in the male patient was verified by viral sequencing (112), raising concern that sexual transmission events may occur even after an outbreak is declared to be over (defined by the WHO as 42 days following the resolution of the final case, as determined by death or two negative RT-PCR results) (113). Accordingly, the WHO currently recommends that male survivors be offered the opportunity to test their semen for Ebola virus by RT-PCR starting at 3 months after the onset of illness (testing is not recommended in the first 3 months because all semen specimens should be assumed to be positive in this time frame), with repeat testing every month until two negative RT-PCR results are obtained (114). However, there are currently no guidelines addressing collection methods (attention to both biosafety and sample integrity issues is needed) or optimal testing strategies (e.g., viral inactivation, sample processing, assay selection) of seminal fluid specimens from survivors. A recent study demonstrated that the DoD EZ1 RT-PCR assay performs similarly for whole blood and semen specimens spiked with EBOV (115), but none of the EVD diagnostic tests with EUA status are currently approved for use with seminal fluid specimens.

Viral persistence in other body fluids. Several small studies and case reports of EVD survivors have documented the persistence of infectious virions and/or viral RNA in a variety of body

fluids after clearance of viremia. Persistence of culture-confirmed infectious virus has been reported in breast milk at day 15 of illness (108), urine at day 26 of illness (116), and ocular fluid at week 14 of illness (117); in each case, the finding of persistence was described for a single patient. Unpublished reports have linked two cases of Ebola in neonates to breast milk from an asymptomatic mother confirmed by PCR to harbor viral RNA (T. Brooks, personal communication). More extensive evidence has accumulated for the persistence of viral RNA in body fluids, with detection by RT-PCR in sweat or skin swabs as late as day 40 of illness (15, 116), in vaginal secretions as late as day 33 of illness (15, 28, 118), in urine as late as day 30 of illness (116, 118), in rectal swabs or stool as late as day 29 of illness (15, 28, 118), in conjunctival fluid as late as day 28 of illness (28, 118), in saliva as late as day 24 of illness (15, 118), and in amniotic fluid (timing unspecified) (119). Sampling methods and testing strategies have not been consistent across studies, and little is known about the correlation of positive RT-PCR results with risk of viral transmission. Regardless, the existing evidence strongly suggests that virus may persist in multiple body fluids for several weeks following the clearance of viremia. Importantly, a recent study conducted at an EVD survivor clinic in Sierra Leone showed that 555 body fluid specimens (105 oral swabs, 103 axillary swabs, 92 conjunctival swabs, 69 urine specimens, 54 forehead swabs, 21 vaginal swabs, and 17 rectal swabs) collected from 112 EVD survivors (median of 142 days postdischarge from an Ebola treatment unit; earliest specimens collected ~40 days following discharge) all tested negative by RT-PCR (110). The findings from this large survivor study are consistent with the data from prior outbreaks described above and suggest that body fluids from non-immune-privileged sites are very unlikely to harbor persistent virus after 6 weeks from clearance of viremia. Of note, we emphasize that exposure to body fluids from immune-privileged sites (e.g., semen, breast milk, ocular fluid, and cerebrospinal fluid) may continue to confer a risk of transmission for an extended period of time. WHO guidelines currently state that breast milk from lactating EVD survivors may be tested by RT-PCR if desired by the patient, with retesting every 48 h for those who test positive until two consecutive negative results are obtained (114). The WHO recommends against testing vaginal fluid (114); recommendations regarding the testing of other body fluids have not been provided. Validation of EVD diagnostic tests on a wider variety of specimen types should be a priority, and guidelines are needed to address specimen collection and biosafety issues for testing of alternative body fluid specimens.

Viral Sequencing in the 2014-2015 Epidemic

Viral sequencing tools hold the potential to benefit EVD diagnostic efforts on multiple fronts, including (i) *de novo* diagnosis in the setting of an emerging outbreak, (ii) identification of viral strains responsible for new transmission chains in an ongoing outbreak, (iii) estimation of viral mutation rate, and (iv) analysis of the impact of viral mutations on the performance of molecular diagnostic tests. During the 2014-2015 epidemic, next-generation sequencing (NGS) platforms enabled the characterization of large numbers of viral genomes in a relatively short time frame (days to weeks), and advances in portable sequencing tools have made it possible to acquire sequencing data acutely in field facilities. Initial sample-processing steps for current sequencing strategies parallel those required for standard RT-PCR testing (i.e., viral inactivation, nucleic acid extraction, reverse transcription, and amplification);

subsequent steps include the preparation of a sequencing library, generation of raw sequence data, and data analysis. Effective field deployment of portable sequencing devices has been achieved (120) and will likely play an important role in surveillance and outbreak response efforts moving forward; however, these efforts remain challenged by the procedural complexity and the relatively low sensitivity for viral detection of current sequencing methods.

The emergence of a new EBOV strain in Guinea was determined by conventional Sanger sequencing of patient specimens collected in early 2014, performed by WHO Collaborating Centres for VHF in France and Germany (49). As part of this initial effort, whole-genome sequences were characterized from three specimens. Several large studies using NGS techniques were conducted over the course of the 2014–2015 epidemic, representing an international effort that ultimately provided a rich database of hundreds of EBOV whole-genome sequences. The first of these studies, carried out in a U.S. laboratory (Illumina platform), sequenced 99 EBOV genomes in specimens collected from EVD patients in Sierra Leone from May to June 2014, revealing evidence for a single transmission event from a viral reservoir followed by human-to-human transmission (121). In a subsequent study carried out in China (BGISEQ-100 platform; Ion Proton), EBOV whole-genome sequences were obtained from 175 EVD patient specimens collected in Sierra Leone from September to November 2014, revealing seven novel EBOV sublineages (122). A total of 232 additional EBOV genomes were sequenced in the United States (Illumina platform) from patient specimens collected from June 2014 to January 2015 in Sierra Leone (123); importantly, the study demonstrated that no significant mutations had accumulated in known primer sites for RT-PCR diagnostic tests. The most recent large sequencing study, performed in Europe (Illumina platform), provided 179 EBOV genomes from patient specimens collected in Guinea from March 2014 to January 2015 (124). The broad time range captured in this data set allowed the authors to illustrate the emergence of two distinct EBOV lineages, one of which was restricted to Guinea in the early months of the epidemic and a second lineage which spread from Guinea to Liberia and Sierra Leone. In an important effort to increase sequencing capacity within West Africa, an Illumina Miseq platform was established at the Liberian Institute for Biomedical Research in February 2015 with support from USAMRIID (125). This new genomic capacity has allowed for ongoing monitoring of EBOV sequences within Liberia (125) and provided key evidence for a case of sexually transmitted EVD (112). Near-real-time sequencing was provided in Sierra Leone by Cambridge University (using an Ion Torrent sequencer) in the closing stages of the outbreak, and this facilitated rapid contact tracing by identifying the probable location of exposure in patients who had traveled home when sick (A. Arias, submitted for publication).

Nanopore sequencing is a relatively new technology that offers advantages compared to standard NGS methods, including longer sequencing reads and the ability to perform real-time sequence analysis concurrently with data acquisition. The MinION device (Oxford Nanopore Technologies), a portable nanopore sequencer, has been evaluated as a diagnostic tool for outbreak responses. Via an unbiased, metagenomic approach for pathogen identification on the MinION platform, EBOV was correctly identified in RNA extracted from whole-blood specimens collected during the 2014 Democratic Republic of Congo Ebola outbreak in under 6 h (126). The potential for deployment of the MinION

device in a resource-limited setting was demonstrated in a field diagnostic laboratory in Liberia, where it was utilized to sequence EBOV genomes from eight clinical specimens (120). These studies lay an important foundation for the further development of field-ready sequencing tools with the capacity for rapid pathogen identification and in-depth characterization.

SUMMARY AND FUTURE DIRECTIONS

Real-time RT-PCR testing is an accurate and high-throughput modality and has become the standard for EVD diagnosis. Several standard (nonautomated) real-time RT-PCR tests are approved for emergency use by the WHO and FDA, and four of these (RealStar Filovirus, RealStar Ebolavirus, Liferiver, and LightMix assays) are commercially available as kits. Diagnosis by standard real-time RT-PCR in an outbreak setting requires field laboratories with substantial infrastructure, operation and maintenance of complex equipment, and expertise in molecular techniques. While such resources were eventually deployed in the 2014–2015 epidemic, their integration into sustainable regional laboratory capacities for ongoing surveillance and response to future outbreaks will be a great challenge. Furthermore, the requirement for collection and transport of venipuncture blood will continue to confer additional safety and logistical hurdles. In order to face these challenges, it is imperative that international partners work together with national health ministries to strengthen laboratory capacity in regions where Ebola is endemic, including the development of practical improvements to pre- and postanalytic processes and the training of local laboratory technicians in molecular diagnostic techniques, biosafety practices, and quality control.

Novel diagnostic platforms, such as automated NAATs and rapid antigen detection tests, that can be used in decentralized health care settings with minimal laboratory infrastructure will likely play a major role moving forward. More field data are needed to establish the appropriate use of these novel tests. Existing evidence suggests that RDTs, if thoughtfully integrated into testing algorithms, could have an immediate impact as point-of-care tests in high-risk populations. Given the persistent challenges in obtaining expeditious RT-PCR results, a point-of-care RDT with imperfect sensitivity and specificity (especially if used in combination with RT-PCR testing) still stands to confer substantial benefits to case management and infection control efforts and should improve the utilization of limited clinical and public health resources (127). Accordingly, current WHO guidelines recommend initial testing with an RDT when RT-PCR testing is not immediately available and to assist in triage and case management when clinical and laboratory resources are overwhelmed (58). Three commercially available RDTs (ReEBOV, OraSure, and SD Q Line tests; all are chromatographic LFIs) have received WHO and/or FDA EUA status. In addition, an LFI developed by the Defense Science and Technology Laboratory in the United Kingdom performed well (100% sensitivity with 92% specificity compared to laboratory testing by RT-PCR) when performed by local staff at patient bedsides in Sierra Leone (128), further supporting the utility and feasibility of performing RDTs at the point of care in Ebola treatment centers. Several innovative RDT platforms are in development (53, 127, 129, 130, 131) and have shown promising results in laboratory-based evaluations. Automated NAATs, such as the GeneXpert and Filmarray systems, that provide rapid, sample-to-answer results with minimal operator dependence or potential for cross-contamination have great potential impacts;

however, their implementation in decentralized health care settings will require careful consideration of biosafety and operational challenges (e.g., access to uninterrupted electricity, temperature control, and expertise for test implementation/quality control and equipment maintenance). As more clinical validation data become available and practical experience is compiled, local regulatory agencies, in collaboration with the WHO, will be responsible for developing updated EVD testing algorithms specific for different health care settings in both high-prevalence outbreak and low-prevalence surveillance scenarios. In addition to assay selection for acute diagnostic testing, further guidance is needed to optimize biosafety practices (e.g., viral inactivation methods) and testing of alternative specimen types for viral persistence in EVD survivors. As testing algorithms and guidelines evolve, it remains a critical responsibility of health care agencies to ensure means for safe and efficient specimen management, tracking, and reporting.

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