What Ebola tells us about outbreak diagnostic readiness

Mark D Perkins & Mark Kessel

Healthcare priorities all too often ignore the importance of diagnostics for disease control and case management. The Ebola epidemic illustrates the folly of this attitude when few therapeutic or prophylactic interventions are available.

As the Ebola epidemic flames out in West Africa, both politicians and public health experts are getting a first chance to take stock of what has been learned from how this epidemic was handled, and to engage in a fair amount of finger-pointing. One surprising element in these discussions is how seldom the need for effective diagnostics is mentioned, given their fundamental role in both disease control and case management. This is true not only for Ebola but for other viral infections that spring up on a regular basis. Indeed, without a deployable and effective vaccine, the only Ebola control measure available to stop transmission is the rapid identification of infected patients for isolation and care in treatment centers (or in the case of fatalities, for safe burial). What explains the relative silence around diagnostics for Ebola, and what opportunities are there for innovations that could help control the current epidemic, and speed response mechanisms in future outbreaks?

A turning point?

Not for the first time, the Ebola epidemic that has been ravaging West Africa seems to have reached a turning point. Confirmed case rates have fallen substantially, from over 800 cases a week in early October across the three affected countries, to under 100 cases a week in the first quarter of 2015 (ref. 1). This dramatic decline in incidence started first in Liberia and then was mirrored to a lesser extent in Guinea and Sierra Leone.

This current Ebola outbreak, which began in December 2013, had claimed nearly 60 lives in Guinea when European specialty virology laboratories first confirmed the presence of Ebola virus in shipped samples and the outbreak was officially reported. Movement thereafter to establish laboratories in West Africa to confirm Ebola in suspect individuals, and ideally to trigger contact-tracing activities, remained relatively sluggish early in the outbreak. By August, when the World Health Organization (WHO; Geneva) issued the first of its valuable situation reports, and five months after the epidemic was declared, there were still only two fully functioning laboratories in West Africa capable of confirming cases, despite active transmission evident in two dozen districts across three countries[2]. By October, when the magnitude of the epidemic was headline news, a dozen laboratories had been established, covering over 80% of the affected districts.

Fixed and mobile testing facilities

There are now 26 Ebola laboratories based in the three affected countries (**Fig. 1**)[3,4]. The diagnostic response in this outbreak is now vastly different in scale from that seen in previous epidemics, but the methodologies in use, and by extension the goals and impact of testing, have been similar (**Box 1**).

The so-called mobile laboratories, or, more accurately, deployable laboratories, are difficult places to do good clinical laboratory science. Established in sometimes improvised locations, with limited capacity to keep vermin out or moderate temperatures in, they often serve multiple Ebola treatment centers (ETCs) or other clinical sites, are under intense pressure to release rapid and accurate results, and have constantly rotating expatriate staff and limited local human resources. They are effectively developed-country academic virology laboratories, deployed into highly constrained settings, and serving and maintaining these laboratories to function at a high level has been an enormous task at which many senior virologists have labored. The role of the laboratory has been central in documenting the course of the epidemic, to some degree in determining cause of death in individuals dying outside of treatment centers, and in verifying that clinically improved patients were noninfectious and could be released from care (though in previous epidemics, release into the community was largely based on clinical judgment). One could argue, however, that the impact of the laboratories on disease control has been relatively limited, both because of the mandate of the laboratories and because of the technologies in use.

The reliance on nonautomated molecular approaches in fixed facilities has substantially diminished the capacity of the laboratories to follow the epidemic's shifting epidemiology, to empower active case-finding and to otherwise detect patients early enough to interrupt transmission. There are several indications of inadequate or delayed case detection, including the late presentation of many patients—with a mean of five days of symptoms prolonged before diagnosis—and the turnaround time for results to be transmitted back to patients and caregivers. The most significant overall indicator of inadequate diagnostic impact, though, is the extent of ongoing transmission. Transmission has been effectively blocked in ETCs, but has continued in the community; within homes and villages, through traditional burial practices, and in the pursuit of non-Ebola healthcare. The great majority of healthcare workers contracting Ebola did so not in ETCs, but outside of these centers, often in private clinics or in communities. In this widespread outbreak, or series of outbreaks,

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the chain of transmission was quickly lost, making it impossible to implement full contact tracing. After months of effort and deployment of large numbers of local and international staff, the goal of seeing 100% of all cases arising from among known case contacts had been reached as of late January only in Liberia (7 of 7), whereas in Guinea 54% of cases were on contact lists, and in Sierra Leone only 21%.

Diagnostic priorities where infection originates

If available, rapid testing done at or near where patients live or first present for care (near-patient testing) would answer a number of both clinical and disease control needs for Ebola. On the ground in West Africa, unmet diagnostic needs come easily to the lips of healthcare workers assisting in the Ebola response. The most pressing of these is alleviating the danger and inconvenience put on patients and healthcare workers due to delays in obtaining testing results, and the need to transport either patient or blood sample to a laboratory facility. Symptomatic individuals encountered passively by the health system, or actively through case-contact investigations, must be either transported overland—often with several family members and/or other patients—for sometimes several hours to an ETC where blood will be sampled and sent to a laboratory facility, or placed in holding centers to wait while blood is sent out for testing (**Box 1**). Both situations present obvious transmission risk, both for uninfected Ebola suspects, and for healthcare workers and drivers. Given the logistic complexities involved, the batch processing in the laboratories and the manual delivery of results, the turnaround time for decision making is often measured in days rather than in hours. Communities are aware of these delays and risks, which does not encourage patients to seek early testing.

The need for more mobile and accelerated testing capability extends across the spectrum of Ebola risk situations. Without near-patient testing, symptomatic individuals arriving by air from an affected country may be held in costly isolation for hours to days while awaiting testing results. When faced with the death of a loved one, families have emotional burial proceedings interrupted for extended periods while buccal swab samples are sent to the laboratory. The fear of Ebola has had a huge impact on the availability of non-Ebola care, especially wet procedures. Many horror stories exist of patients without Ebola symptoms, who required surgical or obstetric care, suffering or dying in the absence of laboratory proof of their Ebola-free status. One study found a 70% fall in the availability of inpatient health services in Sierra Leone with the advent of the Ebola epidemic[3]. Almost all the testing priorities in epidemic countries could be facilitated by near-patient methodologies (**Box 2**). Early diagnosis will also be critical to the success of antiviral treatments as they are developed[4].

Global diagnostic priorities

Priorities for testing are notably different in developed countries, where there has been extensive discussion of airport screening and presymptomatic detection. The question of Ebola screening at airports is complex. Noncontact thermography using thermal scanners or infrared thermometers makes airport scanning for fever feasible, but it remains a relatively rare symptom among international travelers. The prevalence of fever among international airport arrivals was 0.002% in Korea and 0.06% in Australia[5,6]. Prospective studies have found that, at least for influenza viruses and dengue fever, that infrared thermography had relatively low sensitivity and predictive value[7–9]. Airport screening is complicated by the large number of travelers with a very low probability of disease (leading to high false-discovery rates), and the cost and disruption of screening large numbers of negatives with a corresponding need for very high predictive values. The vast majority of cross-border transmission in Africa has been with road and foot traffic, not on airplanes.

Media coverage of imported Ebola in the United States and Europe sparked discussion of the potential to extend testing to presymptomatic individuals with an exposure risk. There is a limited amount of scientific data on asymptomatic infection, though surveys of the prevalence of anti-Ebola antibodies after epidemics suggests that asymptomatic infection may be relatively common. One post-outbreak serosurvey[10,11] showed that 71% of people with Ebola-specific IgG antibodies had not suffered clinical disease. Leroy *et al.*[12] have followed 24 contacts of symptomatic Ebola patients and repeatedly sampled blood evidence of Ebola infection, using Ebola-specific antibody responses, viral culture, antigen-detection enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase (RT)-PCR during a one-month period following exposure. IgM and IgG antibody responses developed in 11 of the 24 contacts, all of whom remained asymptomatic. None had circulating virus detectable by viral culture or reverse transcription

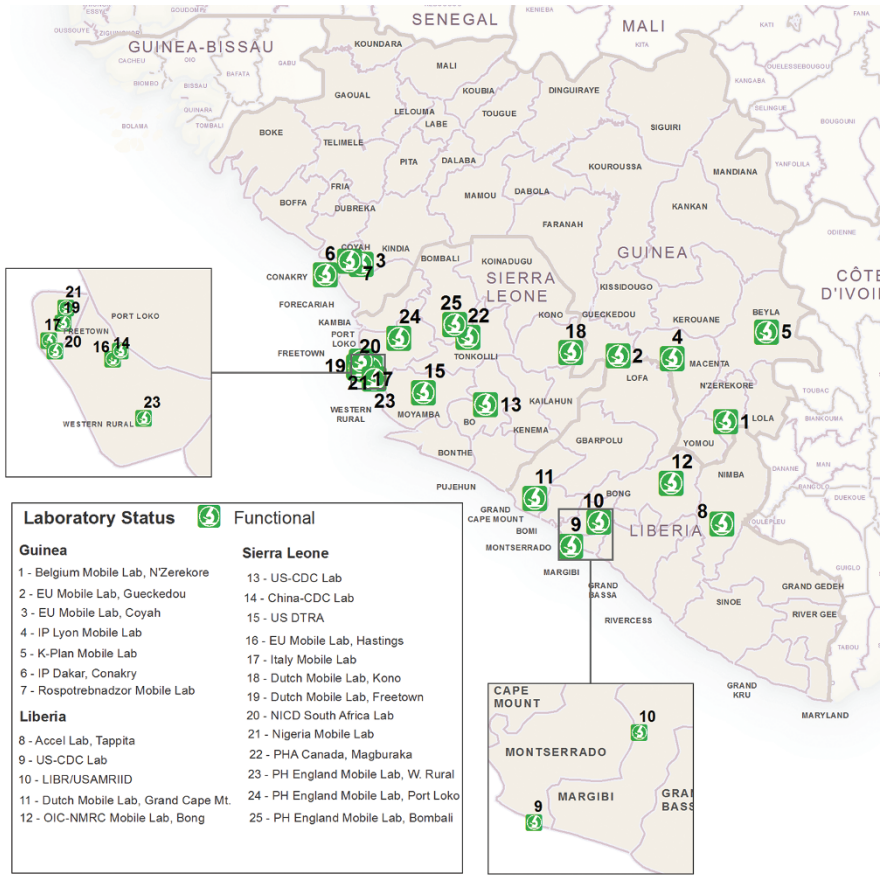

Figure 1 Location of Ebola diagnostic laboratories in affected countries[5].

Box 1 Current tests

The reference assays in use in the Ebola laboratory network are all based on manually performed, reverse-transcription, real-time PCR detecting viral RNA. Reverse transcription (RT)-PCR was first used during an Ebola outbreak in Gabon 15 years ago[23], and quickly thereafter real-time versions of the assay became the principal diagnostic tools in outbreak management as reflected in the practices of the laboratories in the WHO Emerging and Dangerous Pathogens Laboratory Network (EDPLN).

The above assays were all designed originally for research use only, and they share many features in common that limit their use as clinical tools for rapid response—temperature-sensitive reagents that must be kept cold, manual RNA extraction, multiple additional reagents and auxiliary equipment, batch testing, manual readout and reporting, as well as multiple precision steps requiring substantial training. Added to these complexities are the high biosafety demands of working with a dangerous pathogen, complicating not only the handling of specimens but also the transfer of hard-copy information from the clinic and potential contamination of durable instrumentation.

There are multiple different PCR assays in use in the deployed laboratories, including both home-brew and commercial assays targeting one or more genes (late (*L*), nucleoprotein (*NP*), minor matrix protein (*VP40*) or glycoprotein (*GP*)). Though the PCR testing itself may take less than an hour, the RNA extraction protocols and other preparatory steps result in a four- to six-hour turnaround time for results, and samples arriving in the afternoon are often processed the next day. The laboratory methods currently in use require not only highly trained technical staff but also substantial logistical support to ensure the continuous availability of a host of reagents (e.g., inactivation or lysis buffers, extraction reagents, proteinases, amplification mixes) and supplies. Despite good intentions of the international community to strengthen human resources in the health sector of the affected countries in the wake of the epidemic, it cannot readily be imagined that the maintenance and staffing of multiple laboratories working on the current model would be feasible.

PCR. Complementary DNA sequences were detected in 7 of the 11 asymptomatic individuals after 80 cycles of nested PCR.

Despite these findings, there is no evidence from this or any of the previous outbreaks that asymptomatic individuals have been responsible for any disease transmission. Moreover, detecting presymptomatic infection would be both technical and socially difficult. Any circulating virus in the presymptomatic period would likely be at very low concentrations, below the limits of detection of commonly deployed RT-PCR assays. Even among symptomatic patients, a fraction of patients arriving early for care are negative for Ebola on initial PCR testing. In developing countries, testing asymptomatic individuals would pose not only huge financial and logistical burdens, but would be nearly impossible to implement on ethical and social grounds. Introducing a needle for phlebotomy into a healthy individual who later develops symptoms would result in important confusion about cause and effect—confusion that could seriously hamper the already strained relations between Ebola health workers and the affected communities.

Near-patient testing opportunities

Near-patient testing for Ebola or other hemorrhagic fever viruses is technically feasible, and there is a strong push to evaluate and implement such methods in this epidemic, hasten disease-elimination efforts and prepare for a more flexible response to future epidemics. Given the tardiness and unpredictability of antibody responses and the impracticality of viral culture, the only possible technical approaches for near-patient diagnosis are detection of viral RNA or detection of virus-encoded proteins. The former is possible because of the remarkable evolution of simplified molecular-testing systems, and the latter because of the high concentrations of circulating virus in many patients.

Viral RNA detection. Viral load rises steeply to very high levels in most Ebola patients within a few days of infection. Papers from Towner and others[13,14] show that patients with fatal infections tend to have 10^5 to 10^9 copies of viral RNA in their blood from within a day or two of first reporting symptoms, but that those with nonfatal infections—who may nonetheless transmit disease—may have viral loads several logs lower, especially early and late in the course of infection.

Antigen detection. Viral antigen is found systemically early in Ebola virus infection, and though most abundant in spleen and liver, it is readily detected in blood and to a lesser extent in saliva. A polyclonal ELISA was first developed in 1992 in the US Army Medical Research Institute of Infectious Diseases laboratories and has been used in several outbreaks, including in the Democratic Republic of Congo in 1995, Gabon in 1996 and Uganda in 2000 (refs. 13,15–17).

Several antigen-detection tests have since been developed (usually targeting NP, VP40, and GP proteins) either in flow-through and ELISA formats[18] or as rapid lateral flow immunoassays (LFIs), including commercially manufactured kits (**Table 1**). LFIs have known limitations in sensitivity, and antigen detection does not benefit from the amplification capacity of PCR assays. However, this may not necessarily translate into poor clinical sensitivity, depending on the patient population. In acutely ill patients, the clinical performance of an ELISA detecting Ebola antigen in serum gave results similar to PCR's in the limited studies in which both tests have been performed[13]. Saliva is an alternative sample matrix for both PCR and antigen detection, though the lim-

Box 2 Priorities for testing in West Africa

Fast, near-patient testing will be an important advance for Ebola disease control and management. But there remain several priorities for Ebola testing in West Africa, which we list below:

- Passive case finding among people reporting with symptoms
- Active case finding among symptomatic case contacts or in outbreak investigations
- Triage of patients in holding centers
- Identification of Ebola infection in corpses
- Confirmation of noninfectious status before release from ETC into the community
- Certification of noninfectious status to facilitate community reentry for suspects or prior patients
- Post-elimination surveillance
- Testing of children accompanying infected parents in ETCs
- Testing of patients seeking general care that requires physical examination, and especially, before procedures with risk of body fluid exposure (surgery, obstetrics)

Table 1 Example Ebola molecular assays on manual and automated platforms

Source/company	Assay name or type	Development status	Manual/automated
altona Diagnostics (Hamburg, Germany)	Ebolavirus RT-PCR Kit 1.0	FDA Emergency Use Authorization (EUA), WHO prequalification	Manual
Roche (Basel)	LightMix Modular Ebola Virus Zaire	FDA EUA	Manual
Shanghai ZJ Bio-Tech (Shanghai, China)	Ebola Virus (EBOV) Real Time RT-PCR Kit	CE mark for an *in vitro* diagnostic	Manual
US Centers for Disease Control (CDC)	CDC Ebola Virus NP Real-time RT-PCR Assay	FDA EUA	Manual
CDC	CDC Ebola Virus VP40 Real-time RT-PCR Assay	FDA EUA	Manual
US Department of Defense (DoD)	DoD EZ1 Real-time RT-PCR Assay	FDA EUA	Manual
Lucigen	ClariLight Ebola isothermal	In development	Simplified manual
Diagnostics For All	LAMP isothermal	In development	Simplified manual
Envirologix	DNAble isothermal	In development	Simplified manual
Alere (Waltham, MA, USA)	Alere q Ebola	In late development	Automated
Biocartis (Lausanne, Switzerland)	Rapid Ebola Virus Triage Test	In late development	Automated
BioMerieux (Craponne, France)	BioFire Defense FilmArray Biothreat-E test	FDA EUA	Automated
Cepheid (Sunnyvale, CA, USA)	Xpert Ebola	FDA EUA	Automated

ited data available suggest that the sensitivity of testing is lower, at least for living individuals[19].

Depending on their level of performance and degree of robustness, LFIs could have a large impact on testing strategies. Typically, LFIs, already heavily in use in developing countries for malaria and HIV, are used quite peripherally in the health system by nurses and local health workers. Ebola brings the additional challenge of biosafety, which may substantially erode the simplicity and low cost usually associated with LFI testing.

Unfortunately, developers of LFIs often have very limited access to clinical samples and attendant difficulty optimizing their assays or determining clear limits of detection. For assays with a limit of detection near the threshold of viral load commonly seen in new patients, enrollment bias can substantially affect the results of clinical studies. **Table 1** presents a list of LFIs for Ebola that are in late development or already in manufacture. There are substantial differences between some of the products in the selection and number of protein targets, the source of antibody, immunization strategy and buffer constitution. A comparative trial of a number of the LFIs for Ebola is planned during the first quarter of 2015.

In the meantime, control programs and NGOs caring for patients will be trying to decide on the potential role of these assays. The ReEBOV Antigen Rapid Test from Corgenix that detects VP40 (and which requires refrigerated transport and storage) has been recently listed as available for WHO procurement by the WHO prequalification team based on analytical and clinical studies and an assessment of manufacturing quality indicators. Analytical studies with Ebola virus spiked into whole blood showed a relatively high calculated limit of detection of 2.11×10^8 RNA copies/ml. In a set of 147 consecutive fresh venous whole blood samples and 146 frozen plasma specimens in Sierra Leone, the assay demonstrated 92% sensitivity and 85% specificity in comparison to RT-PCR. Data on other LFIs are pending. Even with imperfect performance, there may be a useful role for such assays, such as rapid triage of patients in transit centers (detecting and isolating the most contagious), but quick confirmatory testing for all positives will clearly be required. In settings of low prevalence such as is now seen in most districts of the affected countries, it could be predicted that the majority of positive tests will be false positives, potentially causing much social disruption and erosion of general confidence in diagnostic testing.

Molecular detection. PCR reagents used in past Ebola outbreaks were largely homebrew assays that were the standard in the sponsoring laboratory. During this outbreak, multiple different assays have been in use, including commercial kits, but for most companies have sought US FDA Emergency Use Authorization (FDA EUA) and developed substantial amounts of analytic data to support performance. The first six companies listed in **Table 2** market some of the assays in widest use in laboratories deployed in West Africa. None is yet regulated by the national authorities in West Africa. Not listed are several commercially available PCR-based Ebola assays, most regulated as for 'research use only' and without specific operational advantages over assays currently in use (in terms of speed, reagent robustness or simplicity of use).

Alternative approaches to simplify the testing process for nucleic acid amplification include isothermal chemistries that hold the promise of enhanced speed of testing, decreased susceptibility to inhibition and operation without a thermocycler. Lucigen (Shirley, NY, USA), Envirologix (Portland, ME, USA) and nonprofit Diagnostics for All (Cambridge, MA, USA)—three sponsors with simplified manual assays in development based on different isothermal amplification methods—are also listed in **Table 2**. Like many innovative developers, they face the challenge of how to address their lack of a large manufacturing capacity, no history of launching and supporting *in vitro* diagnostics, and no previous experience in building or establishing distribution capacity in Africa.

Over the past 10–15 years, several manufacturers have worked to develop fully automated PCR systems that have room-temperature-stable reagents and preloaded onto disposables

Table 2 Example commercial lateral flow immunoassays for Ebola antigen

Source/company	Assay name or type	Regulatory status
Alternative and Atomic Energy Commission (Paris)	Ebola eZYSCREEN	WHO PQ submitted
Chembio Diagnostics (Medford, MA, USA)	Lateral flow immunoassay	WHO PQ submitted
Corgenix (Denver)	ReEBOV Antigen Rapid Test	WHO PQ EA listed
InTec (Suzhou, China)	Lateral flow immunoassay	WHO PQ submitted
Orasure (Bethlehem, PA, USA)	Lateral flow immunoassay	WHO PQ submitted
SD Biosensor (Seoul, Korea)	SD Q Line Ebola Zaire Ag	WHO PQ submitted
Senova (Jena, Germany)	DEDIATEST—Ebola	WHO PQ submitted

PQ, prequalification.

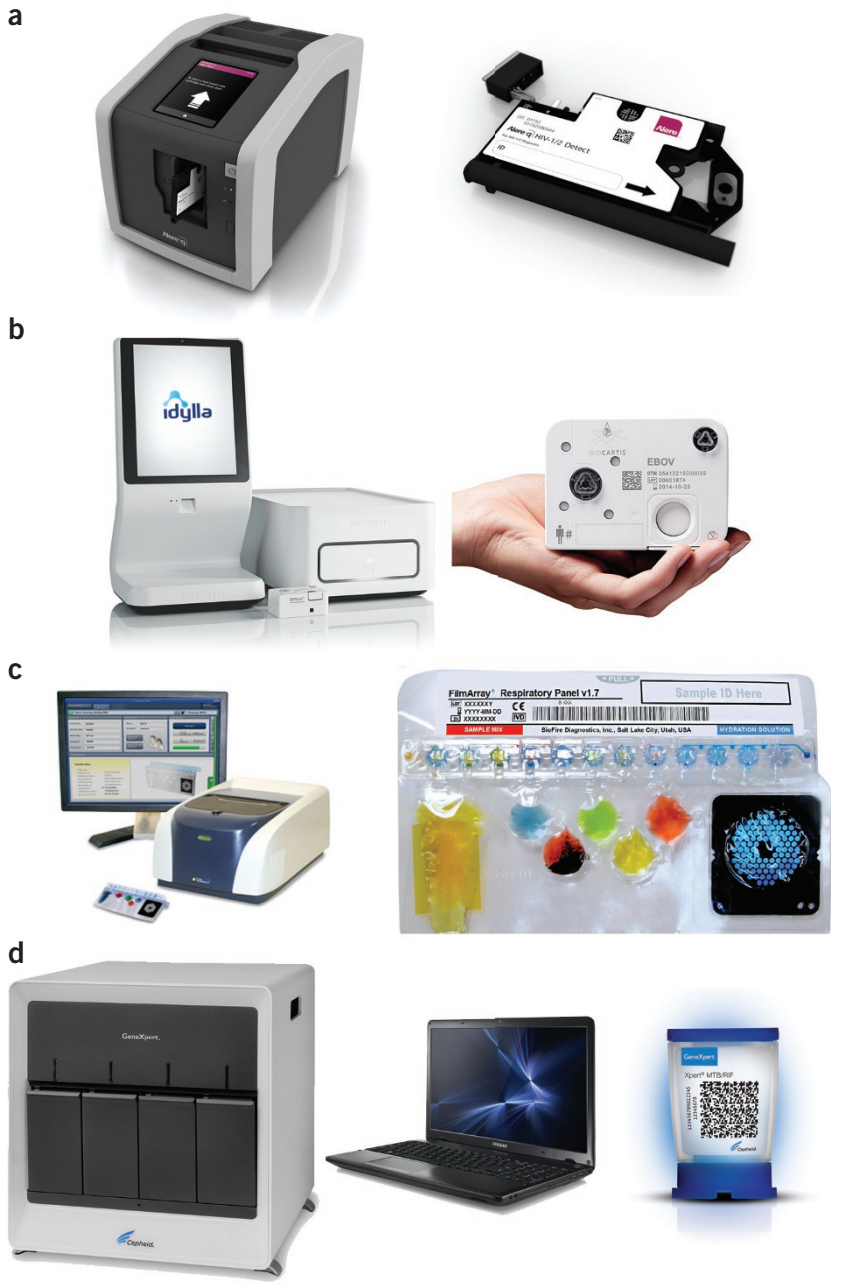

Figure 2 Selected *in vitro* diagnostic systems that have been or are close to commercialization. (**a**) Alere q is a portable 7-kg system that runs a single assay at a time per instrument, with options for battery or solar power. The cartridge has a built-in plastic capillary for blood collection directly from a finger prick, and all reagents are preloaded for automated processing, amplification and readout. There is an onboard computer and reporting can be wireless electronic. HIV assay already on the same platform marketed for use in Africa. Available Q3 2015. (**b**) Biocartis Idylla is a single cartridge per module with stackable modules. It accepts EDTA whole blood or plasma from venipuncture. Preloaded reagents detect both Ebola Zaire and Sudan. Requires separate computer console and device has wireless connectivity. First CE-marked assay on this platform (oncology) was launched in September 2014. FDA and CE-mark filing planned in 2Q15. (**c**) The BioFire FilmArray Biothreat E is a single assay instrument. Pouch is preloaded with temperature-stable reagents and accepts venipuncture whole blood (which requires a separate protease step) or urine. Electronic reporting not included. (**d**) Cepheid GeneXpert is a modular system with instruments running 1–64 tests in random-access mode. All reagents required are lyophilized on board. Developed to work from whole blood, finger-prick swab or oral swab with inactivation as the first step. Same instrument is in use in over 100 developing countries for tuberculosis and multidrug-resistant agent detection. Wireless electronic reporting. FDA EUA (Emergency Use Authorization).

that allow integration of sample processing, amplification and detection. These systems tend to have disposables that are more expensive than the reagent costs of home-brew PCR, but which can be less expensive when nucleic acid extraction costs and human resources costs are also considered. Four companies have designed automated Ebola assays either already in manufacture or in late-stage development (**Fig. 2**). These systems all have the potential to be mobile in vans or other vehicles and are simple enough for use by laboratory or clinical staff with only limited training. All of these assays are designed to run in under 90 min, and some are engineered for electronic, real-time reporting. This feature alone has the capacity, if harvested by national programs and collaborating agencies, to transform not only the speed but also the accuracy of clinical result delivery, epidemiologic mapping and contact tracing. Moreover, electronic connectivity allows remote monitoring of instrument performance, highlighting areas where technical service or additional operator training is needed.

These automated and integrated molecular systems have the potential to transform and democratize molecular detection. With essentially push-button operation for many of these systems, locally trained technicians can perform PCR for pathogen detection with much greater reliability than is possible with manual testing. The performance of these systems may vary, but should be well documented by analytical results and are in most cases equal to, or more reliable than, results obtained with conventional manual RT-PCR testing. One instrument in this group, GeneXpert, has already proven its global utility for tuberculosis and drug resistance detection, and is the most widely used molecular testing system in developing countries.

Conclusions

The world health community has made tremendous strides in the control of many communicable diseases, but that battle is far from won. Weak health systems in many countries and increasingly mobile populations leave us at risk especially for outbreaks from emerging pathogens. In an interconnected world, existing and emerging pathogens do not stop at national borders. As people travel around the world with ever-increasing frequency, the risk of infections spreading out of control is greater than in earlier times. Today the battle that caught the world's attention is Ebola. But since the beginning of this century, multiple emerging infectious diseases have posed potential dangers to world health and have cost world economies untold billions of dol-

lars and wreaked havoc on the economic health of resource-constrained countries.

The ability of health organizations to deal with already challenging medical situations is further complicated by political and religious factors, population growth, especially in urban slum areas, and even climate change. Upheavals and population displacements in a number of conflict-rife areas such as the Middle East and in parts of Africa impose living conditions that also enhance the likelihood of the spread of diseases and infections. Political and religious conflict may also threaten laboriously constructed disease-control efforts, as exemplified by the Pakistan Taliban's battle against local childhood polio vaccination campaigns.

The fight against Ebola has harnessed a tremendous amount of good will from the diagnostics industry, which can expect little revenue from tackling this disease. Our organization, FIND, is tracking an amazing 70-plus diagnostic companies that have Ebola products in their R&D portfolios, despite the fact that for most of these companies, external funding has not been available to defray investment costs. Adding to financial concerns for companies are lack of diagnostic standards, unavailability of samples, special biosafety considerations, and lack of clarity about who would purchase systems and what level of performance would trigger public uptake.

One important challenge for the implementation of these technologies is the cost. This holds true for the current assays in use. In an outbreak setting, cost considerations are minimized because of the huge social and financial cost of an uncontrolled epidemic. In an op-ed piece in *The New York Times*, Jim Kim, president of the World Bank, noted that in Senegal, the cost to treat one patient and track all of his contacts was >$1 million[20]. In such a setting, the focus shifts from the cost of testing, but to how much accurate testing can save, especially in lives. Once the epidemic phase is over, however, there is the very real challenge of making use of the laboratory capacity that has been installed, whether it is on complex manual systems or on automated systems with fixed consumables cost. The diagnostic costs that can be tolerated for an outbreak are not the same as those that are tolerated for the care of endemic diseases. The expenditure of $100 to detect a case of Ebola early is trivial if it avoids even a single secondary case. Once the epidemic is brought to a halt, the cost of Ebola detection must compete with other diagnoses, including malaria, HIV and diabetes, all of which can be done for under $1.00 in consumables. Translating national and international expenditures on Ebola control into sustainably strengthened health systems will be very difficult. A snapshot of the challenge is seen in Liberia, which has only 0.032 laboratory workers per 1,000 population, spends <30 cents per capita per day on public health[21] and has a reported incidence of malaria of 1.2 million cases per year[22].

The Ebola outbreak has revealed long-standing weaknesses not only in health systems in West Africa, but also in our global capacity to respond early in epidemics with effective diagnostic capacity. After multiple successive outbreaks of emerging infections, from severe acute respiratory syndrome, to animal influenza strains jumping to humans, to Middle East respiratory syndrome, we still do not have effective mechanisms in place to rapidly respond to the critical diagnostic needs posed by epidemics.

There are multiple reasons for the lack of diagnostic preparedness against infectious agents, but the overarching problem is the lack of a financed global strategy that can be implemented ahead of, rather than during, disease outbreaks. Such a strategy would need to address the following: first, the need for coordinated interaction between sentinel field laboratories to detect outbreaks and collect samples, and reference centers with advanced identification and sequencing capacity; second, the need for a politically palatable and ethical platform for the rapid sharing of critical reagents and clinical specimens; and third, the need for assay development, regulatory approval and implementation planning in anticipation of outbreaks. Epidemic diseases, especially when they involve impoverished populations, hold little return on investment for diagnostic companies—markets are fragmented, resource-poor and fleeting. Without a mechanism to fund targeted assay development to meet such needs, the tremendous capacity of the multi-billion-dollar diagnostics industry will remain an untapped resource.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. WHO Ebola Situation Report–15 (April 2015). http://apps.who.int/ebola/current-situation/ebola-situation-report-15-april-2015
2. WHO Ebola response roadmap: situation report 1–29 August 2014. http://apps.who.int/iris/bitstream/10665/131974/1/roadmapsitrep1_eng.pdf?ua=1
3. Bolkan, H.A., Bash-Taqi, D.A., Samai, M., Gerdin, M. & von Schreeb, J. *PLoS Curr.* **6,** (August 12 2014) pii:dis.8b3c24217d80f3975618fc9d9228a144. doi: 10.1371/currents.dis.8b3c24217d80f3975618fc9d9228a144.
4. Fink, S. Ebola drug aids some in a study in West Africa. *The New York Times*, Feb. 4, 2015.
5. Cho, K.S. & Yoon, J. *Epidemiol. Health* **36**, e2014004 (2014).
6. McBride, W.J., Buikstra, E. & FitzGerald, M. *Aust. N. Z. J. Public Health* **34**, 5–10 (2010).
7. Priest, P.C., Duncan, A.R., Jennings, L.C. & Baker, M.G. *PLoS ONE* **6**, e14490 (2011).
8. Kuan, M.M., Lin, T., Chuang, J.H. & Wu, H.S. *Int. J. Infect. Dis.* **14**, e693–e697 (2010).
9. Bitar, D., Goubar, A. & Desenclos, J.C. *Euro Surveill.* **14**, 19115 (2009).
10. Becquart, P. *et al. PLoS ONE* **5**, e9126.
11. Heffernan, R.T. *et al. J. Infect. Dis.* **191**, 964–968 (2005).
12. Leroy, E.M. *et al. Lancet* **355**, 2210–2215 (2000).
13. Towner, J.S. *et al. J. Virol.* **78**, 4330–4341 (2004).
14. Schieffelin, J.S. *et al. N. Engl. J. Med.* **371**, 2092–2100 (2014).
15. Ksiazek, T.G. *et al. J. Clin. Microbiol.* **30**, 947–950 (1992).
16. Ksiazek, T.G. *et al. J. Infect. Dis.* **179** (suppl. 1), S192–S198 (1999).
17. Ksiazek, T.G. *et al. J. Infect. Dis.* **179** (suppl. 1), S177–S187 (1999).
18. Saijo, M. *et al. J. Med. Virol.* **76**, 111–118 (2005).
19. Formenty, P. *et al. Clin. Infect. Dis.* **42**, 1521–1526 (2006).
20. Kim, J.Y. The path to zero Ebola cases. *The New York Times*, Dec. 11, 2014.
21. WHO country statistics. http://www.who.int/countries/lbr/en/
22. WHO. World Malaria Report, 2014. http://www.who.int/malaria/publications/world_malaria_report_2014/en/
23. Leroy, E.M. *et al. J. Med. Virol.* **60**, 463–467 (2000).