Editorial

Introducing EbolaCheck: potential for point-of-need infectious disease diagnosis

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Introducing EbolaCheck: potential for point-of-need infectious disease diagnosis

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Abstract

The 2013-5 Ebolavirus disease (EVD) humanitarian crisis has spurred the development of laboratory-free, point of care (POC) nucleic acid testing (NAT) solutions. EbolaCheck is an international consortium of public health, academic and biotechnology industry stakeholders aiming to deliver clinical molecular diagnostic (MDx) standard of care (SOC) testing suitable for the West African milieu within 12 months. In this article the current status of the EbolaCheck platform is discussed in the context of the current regulatory framework. Future goals to achieve differential diagnosis of hemorrhagic fever disease from <5 microliters of whole blood samples (WBS) or mucosal biofluids, in a single tube process, in under 40 minutes and with minimal operator training are presented.

Keywords: Ebola, molecular diagnostics, nucleic acid testing, point of care, portable, non-profit.

Background: Clinical diagnosis of Ebolavirus disease

Ebolavirus disease (EVD) is a haemorrhagic fever disease (HFD) caused by members of the *filoviridae* family of RNA viruses. The filamentous Ebolavirus virion (~90 x 1000 nm) houses a 7 gene, ~19 kb genome packed in a nucleoprotein (NP) sheath. Transmission is mediated via the Ebolavirus transmembrane glycoprotein (GP) primarily via macrophage/monocytes. The glycoprotein also features immunomodulation, immune evasion and endothelial barrier disruption roles.[1] The monocytic tropism of Ebolavirus mediates proinflammatory responses during replication that amplify infectivity and pathology, collectively resulting in the internal haemorrhage and organ failure characteristic of the later stages of disease. [2,3]

Diagnosis is extremely difficult [2,4] as symptoms mimic other HFDs, flu, or gastrointestinal infections, which do not preclude Ebolavirus co-infection. [4,5] Transmission risk increases in line with symptom severity, mirroring viraemia;[6] pre-symptomatic patients are not considered contagious and may remain asymptomatic for up to 21 days. [3] Confirmation of Ebolavirus as the causal disease agent requires clinical molecular diagnostic (MDx) laboratory solutions. To date, USD$100, <8 hr long, transcription polymerase chain reaction (RT-PCR) nucleic acid tests (NAT) on RNA extracts from 3.5 ml of whole blood sample (WBS) are the method of choice. [7]

However, at the height of the EVD outbreak lack of capacity in West Africa required sample shipment overseas resulting in 3-5 day turnaround times and post-mortem diagnosis. [1,8] The need for a true point-of-need NAT was acute, yet no *in vitro* diagnostic (IVD) test had received regulatory clearance. 'Homebrew' assays were based on Trombley *et al.* (the 'Trombley' assays; United

States Army Medical Research Institute for Infectious Disease; USAMRIID) [9] or Panning *et al*. [10] These eventually received USFDA emergency use authorisation (EUA; EZ1 assay) or were made commercially available under the self-certification CE marking principles (Altona RealStar® Filovirus Screen), [11] respectively.

Molecular diagnostics for infectious diseases at the point of need

Following 9/11 and the subsequent airborne viral disease pandemics, efforts were made to develop decentralised, point of care (POC) NAT's [12]. The resulting solutions, however, were not designed with resource-limited settings in mind, [13] despite the ASSURED criteria espoused by the WHO. [14] Thus, the need for a safe, cheap, simple, robust, portable and battery operated solution remained, presenting an attractive development opportunity for emerging NAT technologies. However, clinical development costs, [13] convoluted intellectual property landscapes and industry doubts over outbreak duration and return on investment potential, presented substantial obstacles. Poignantly, despite corporate social responsibility opportunities, to date, all of the major diagnostics manufacturers that engaged in the Ebola response offered primer-probe kits for existing lab-based platforms, or developed 'cassette' kits for existing, closer-to-patient systems. Importantly, these cassette systems maintain for-profit pricing structures for low and middle-income countries, even following receipt of philanthropic donations in support of their development. The monetisation/investment barrier remains cornerstone to both regulator and non-government support organisation efforts. [15]

Yet despite large industry indifference several academic groups and start up/spinout companies sought to address the POC clinical diagnostic need. However, they faced scepticism from some regulatory bodies regarding manufacturing capacity, quality assurance, commercial launch/support and distribution capability. [16] Thus, little consideration was given to post proof-of-principle, non-profit production and distribution opportunities similar to regulator-certified, generic pharmaceuticals supply chain models. Under normal circumstances this would appear appropriate considering the high risk to individual and public health on account of false positive or false negative misdiagnosis (WHO category 4 IVD classification). However, on August 2014 WHO declared the West African Ebola outbreak as a public health emergency of international concern (PHEIC). Interestingly, this motivated the FDA to enable EUA approvals; in contrast, the WHO demanded engagement through the full pre-qualification process. This diverged significantly from the documented successes with other WHO-listed, FIND Diagnostics-vetted, but academic-lead efforts to address neglected disease diagnostics need. The net result was limited performance validation facilitation (access to stored patient samples managed by the WHO) for innovations aiming to address the humanitarian need in the affected countries at the point of care, in lieu for questionable support to preferred lab-based platforms.

EbolaCheck: the team

The EbolaCheck consortium was formed in response to the August 2014 call of the Research for Health in Humanitarian Crises (r2hc) programme, managed by Enhancing Learning and Research for Humanitarian Assistance (ELRHA;

www.elrha.org). The Research for Health in Humanitarian Crises (R2HC) programme aims to improve health outcomes by strengthening the evidence base for public health interventions in humanitarian crises (visit www.elrha.org/work/r2hc for more information). The goal of the joint effort between University of Westminster, BioGene Ltd., Public Health England (PHE), USAMRIID, and the Kwame Nkrumah University of Science and Technology (KNUST) funded through R2HC is to deliver by November 2015 a novel point of need NAT solution for simple, rapid and safe patient triage for EVD anywhere in West Africa.

EbolaCheck: Key principles

EbolaCheck can be divided into four sub-systems: the NAT instrument, the EVD assay, the WBS reaction formulation and the reaction consumable. Together, they aim to replace the clinical MDx standard of care (SOC) with a rapid, point-of-need, sample-to-answer format.

Low cost suitable for West Africa

A simple, patent-protected, energy and engineering-efficient method enables rapid (<2 min), single-tube access to pathogen & host nucleic acids in biofluids with no need for microfluidics. Direct compatibility with standard, cryoprotectable RT-PCR biochemistries further reduces overall cost. Crucially, EbolaCheck will be available to support the on-going, WHO-declared, EVD humanitarian crisis in Africa at cost only.

Clinical standard of care reliability

The Trombley assay sets for Ebolavirus Zaire GP and NP [9] were migrated to EbolaCheck (Trombley+) to i) minimise delays, ii) avoid complex licensing negotiations and iii) on account of emerging field performance evaluation data. Multiplexed use of the Trombley+ assay sets also discriminate vaccinated from infected patients; NP is not found in the two most advanced EVD clinical vaccine candidates [17,18], a problem in on-going vaccination clinical trials pursued by other r2hc funded programmes (Gilbert S., personal communication). USAMRIID have demonstrated performance across 5 logs of viral RNA genome equivalents (GE) with 100% analytical specificity against 65 other pathogens and analytical sensitivities of 0.001 (NP) and 0.0001 (GP) plaque-forming units (PFU) per reaction. [9] The roughly 4,000 GE/PFU ratio observed under biosafety level 4 (BSL4) experimentation [19], suggests a lower limit of detection (LLOD) of 10 GE/reaction, or 10^4 GE/ml of WBS. Given typical time-to-presentation in autumn 2014 was >3 days post symptom onset, a LLOD goal of 10^4 GE/ml WBS was set for the Trombley+ assays on EbolaCheck. Present performance data on surrogate pseudoviral templates indicate 9 logs of quantitative linear dynamic range with a lower limit of quantification of 66 GE/reaction and LLOD of 6 GE/reaction, i.e. in line with our performance targets.

Simple, sample-to-result standard operating procedure.

The plethora of reports on 'simple' medical device misuse by end-users in the developed world underscore the importance of ensuring device reliability, particularly with category 4 IVD devices operated under significant duress, in environmentally challenging conditions. [13] The EbolaCheck standard operating procedure (SOP) consists of:

1) reagent unpacking and automated rehydration,

2) 5 microliter WBS collection by fingertip lancet puncture and MicroSafe® capillary collection,

3) Sample ejection into the rehydrated consumable,

4) lock and loading onto the EbolaCheck instrument, and

5) run initiation by touchscreen input.

Availability and status of the 8 random-access testing stations is visually identified on the front-facing touchscreen. Patient status is simply reported as positive, negative or problematic, with the latter indicating a need to repeat the test due to a failure. Full run kinetics, analytics and diagnostics can be accessed on-screen or over a WiFi connection.

Safety

The 5 microliter WBS requirement of EbolaCheck presents a significant risk reduction to both HCW and HFD patients compared to the closed system, 3.5 ml Vacutainer® Eclipse™ needle and Vacutainer® sample SOC protocol. Thermal cycling is expected to destroy EVD [20]; used, sealed consumables are nonetheless discarded as BSL4 clinical waste. The instrument is fully compatible with chlorine dioxide surface sterilisation [20] and designed against ingress of liquids or internal condensation [13]. Secure WiFi interface permits remote system checks, maintenance and full reaction data off-boarding. The random access stations also self-diagnose errors and automatically shut down to prevent misdiagnosis.

Speed

Tests with full personal protective equipment suggest the EbolaCheck HCW SOP takes under 2 minutes to complete by minimally trained individuals, with time to results in <40 minutes; real time reaction progression monitoring suggests high viraemia positive results could be called in as little as 20 minutes.

Portability

Field experience from in-country PHE response teams advised against easily removed, small-form designs, highlighting the need for higher throughput. The ruggedized, 8-well form maintains power supply independence through either mains and/or car battery/alternator power sources. Furthermore, energy consumption modelling indicates solar power supply to be achievable. Design for safety also achieves durability and reliable operation in savannah, coastal and jungle conditions, without corrosion or performance deterioration: simulated environment tests indicate the instrument can complete runs at temperatures as high as 50^{o}C with 98% humidity, and as low as -20^{o}C.

Development timeline

Prototype design, engineering and assay development was initiated in November 2014. Internal assay standards containing the Trombley assay targets were developed in MS2 phage icosahedron (Armored RNA®) [21] (commercially available) and lipid bilayer enveloped HIV pseudovirus [22] (open access) formats. Although 26nm and 80-100nm in size respectively, these represent a vast cadre of viral pathogens. Thus, BSL4 study requirements have been reduced to confirmatory studies using live Ebolavirus, and yielded data supporting EbolaCheck platform utility against other viral pathogens. BSL4 studies are thus limited to performance evaluation testing against the clinical SOC NAT Trombley assay on culture preparations of Ebolavirus and fresh WBS derived from non-human primate models of Ebolavirus infection. In-country testing with fresh or stored patient samples is not expected on account of continued outbreak decline and current WHO priorities to established technologies. However, at least 3 instruments will be tested in West Africa using mock sample preparations to confirm system operation, portability and reliability in urban, rural and remote environments.

Future directions

Our early data support multiplexed detection and quantification potential of 3-4 NAT targets in WBS on EbolaCheck. As positive [23] and detrimental [5] co-infections are common amongst EVD patients, expansion of multiplexing is necessary, but unlikely to exceed concomitant amplification capability need beyond 5 targets. Field data also indicate mucosal biofluids such as semen [24], ocular fluid [25] and breast milk [26] might be viral depots in convalescence. Interestingly, culturally acceptable alternatives such as saliva [27] and gingival-crevicular fluid [28], might also be of use for HFD diagnosis. Thus, demonstrating EbolaCheck compatibility with these mucosal biofluids will expand point of need monitoring and surveillance capability and introduce the opportunity for needle-free testing. Early feasibility studies indicate this may enable differential HFD diagnosis with minimal cost of goods increase.

Concluding remarks

Of the 9 EVD NATs that have received to date USFDA EUA, 3 involve complex cartridge/microfluidic systems. Only the 90 minute Cepheid Xpert® Ebola assay (May 2015) is reasonably priced for the West African milieu at ~US$20 per test, despite charitable backing. With a comparable assay cadre and LLOD to EbolaCheck, it features a 3 log, non-quantitative dynamic range in highly diluted WBS, requires sample pre-processing, multiple mechanical steps and a separate personal computer and barcode scanner. Despite >10,000 instruments placed worldwide this WHO-selected platform costs US$17,000-17,500 to eligible countries. Thus, per-unit scaled production costs are comparable to the current manufacturing cost of EbolaCheck prototypes and the Trombley+ EVD assays. The EbolaCheck consortium has demonstrated that humanitarian crises can motivate efforts to the significant potential benefit of those in need as well as leverage development opportunities for appropriately positioned technologies from socially responsible industry with commercial interests in the West. The EbolaCheck consortium is presently seeking charitable support towards scale-up production and delivery of the first differential HFD diagnosis solution, to be provided at cost for any future WHO-declared humanitarian crises.

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