EDITORIAL

What are the challenges associated with testing for Ebola? A focus on the 2014 outbreak in West Africa

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Filoviruses, such as Ebola and Marburg viruses, are enveloped, negative-stranded RNA viruses that cause severe hemorrhagic fever in humans and nonhuman primates and are classified as biosafety level 4 agents. Past outbreaks of Ebola and Marburg hemorrhagic fever in humans were sporadic in central Africa resulting in cases numbering in the 10–100s each year. While outbreaks of both Ebola and Marburg have increased in frequency over the last decade, no one could have predicted the current Ebola outbreak, still ongoing in West Africa over a year after the index case [1,2]. Not only has this current outbreak occurred in countries where filoviruses have not previously been detected [3], but with over 10,000 deaths so far, this outbreak exceeds all others by an order of magnitude and has seen more cases than all previous documented Ebola outbreaks put together.

A clearly observed shortcoming of this outbreak has been the rapid isolation of cases to contain the outbreak and stop onward spread. In my opinion this was borne out of a fear and mistrust within the local communities who were

(in March/April 2014) still blaming the disease on ‘witchcraft’ and actively hiding sick patients from the outreach teams and contact tracing teams with whom I was working. Local authorities appeared unable to ‘sensitize’/educate their local populations enough to promote prompt presentation at isolation centers should they develop symptoms.

Hand-in-hand with quick and effective isolation of suspect patients [4] is the ability to rapidly test those patients to confirm or rule out Ebola virus infection as the cause of their symptoms [5]. This not only allows confirmation and isolation of those who are positive for the disease, but also allows others who present with similar symptoms (often caused by other infectious diseases including malaria) but test negative for Ebola virus infections to be discharged/transferred to the normal health-care system quickly, reinforcing trust in the community and minimizing the amount of time an Ebola-negative patient spends in the high-risk environment of the ‘suspect’ wing of an isolation ward. Repeated testing also allows some degree of viral

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load estimation and prognosis to be predicted and provides clinicians with the negative test(s) required to confidently discharge convalescent patients [4,6,7].

Rapid and reliable diagnosis of Ebola in a field setting presents many challenges to any virologist used to a world of regulated, multimillion pound high-containment laboratories with a host of sensitive, high-tech equipment to ensure accuracy and biosafety [8]. Attempt to recreate this environment in a tent in West Africa and many virologists could not be blamed for feeling a little uneasy. In my opinion many of these challenges are not necessarily associated with the scientific methodology itself but the logistics of conducting such testing outside of the normal laboratory setting.

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Containment & biosafety

The first and perhaps most obvious challenge is that it is not possible to replicate the engineering control measures used in regulated biocontainment laboratories. As such safe, bespoke methods of containing samples prior to inactivation must be designed and validated (as far as possible) that are appropriate for the conditions. Any containment system must be portable; able to be transported (along with the rest of the lab) in vehicles and light aircraft. They also need to have low power consumption, preferably with the option of being battery operated. Two types of containment are used; the first is to partition off a ‘hot room’ area and rely on personal protective equipment (PPE) – with lab workers donning full body suits, multiple gloves, gowns and a full face mask with positive pressure respirator – as the main layer of containment. A popular second method is to use a small glove and box system, such as that used by the European Mobile Laboratory [9]. This system consists of a Perspex box assembled around a rigid (often collapsible) frame with air-locked ports and rubber gauntlets/gloves. Airflow is by means of either a battery or mains operated air pump maintaining negative pressure within the box, with the expelled air filtered using commercial air filters. Although by no means a validated microbiological safety class III cabinet, these systems are based on the same principles, providing a robust layer of primary containment. Drawbacks of such a system are the restriction in dexterity and the challenges in ensuring all materials are thoroughly inactivated/decontaminated before removal. These issues are not insurmountable

but do require training of outbreak responders in the use of the equipment prior to deployment.

Assay selection

The physical conditions in which diagnosis is carried out in an outbreak setting puts more of an emphasis on the choice of suitable assays and reagents. As expected, the main assay platform for such diagnosis is real-time PCR due to its sensitivity and speed but also its simplicity or at least familiarity to the scientists in the field [5,10,11]. In addition labs should select assays that can easily be troubleshot in the field and therefore should not contain a complicated range of sensitive reagents. Cold chain systems both for storage, but particularly shipping, cannot be guaranteed so the use of lyophilized reagents with no cold chain requirements is certainly recommended. There are also more technical considerations to be made before the lab deploys with such assays, these include the use of multiplexed assays to allow differential diagnosis where required; the use of rigorous internal controls to quickly identify reagent degradation or indeed contamination.

Logistics

In my opinion the biggest issue is always logistics. Any scientist will know the amount of administrative and logistic support required to maintain a functioning laboratory with whole teams of lab managers, maintenance staff and contractors, suppliers, couriers, etc., none of which will come with you when you deploy the lab; it must be self sufficient. Added to this is the increased logistical burden for the lab staff that will need accommodation, food, transport and security. This is where organizations like WHO come into their own because if the lab is deploying under their Global Outbreak Alert and Response Network umbrella, much of this logistical demand should be taken care of. There is still a requirement for the lab users to consider their logistics chain; how will reagents and consumables be resupplied? How will the lab be powered? It is unlikely that there will be reliable mains power, so generators or invertors (run from vehicle engines) will be needed and equipment such as real-time PCR platforms chosen which can be reliably run on such power supplies. Scientists need to take out personal equipment themselves; will they be required (or would it be wise) to provide their own bedding, mosquito net or food? Do they have appropriate clothing and footwear? The

parent institution will probably be responsible for providing for any shortfalls in personal kit and equipment in addition to medication such as antimalarial prophylaxis and treatment for traveller's diarrhea, among others.

Personal specifications

Deploying as part of an outbreak response team may sound exciting – it certainly could be considered the 'front line of virology.' However, not every virologist is suited to such work, no matter how experienced in molecular detection techniques and/or high containment virology. Serious consideration should be given to how one would react to certain common situations from scientific-related issues such as whether you would be comfortable with the lack of formal containment and controls that you are used to in your parent laboratory; but also coping with the surroundings in which you will be working – can you withstand working long days in tropical heat/humidity? Are you comfortable to travel on poor quality roads where road traffic accidents are a real possibility? How will you cope with eating local food where you will have limited (if any) choice and even simple dietary requirements such as vegetarianism cannot be catered for? If you require long-term medication, how will you store this? What if your bags containing it gets lost, or stolen?

In summary, deploying to viral hemorrhagic fever outbreak response missions such as the ongoing devastating outbreak in West Africa, is essential to provide the rapid diagnosis and on-site expertise required to rapidly isolate positive patients and so contain the spread of the outbreak. However, laboratory work in such an environment poses many challenges and

considerations to individuals considering deploying. While many of these involve technical considerations and challenges around containment, sample processing and choices of assays, it poses even more nonscientific challenges, either logistical or personal. While spending a month in such a remote, intense location can be a rewarding experience – and it certainly is a privilege to be in such a position to help – it is not for everybody and can be disconcerting at times. Nowhere has this been more true than in the current Ebola outbreak in West Africa where hundreds of scientists have now deployed, but where the outbreak frustratingly continues. With new diagnostic labs continuing to open [12] and new patients continuing to be identified, it is clear that many more virologists will need to rise to these challenges in the coming months.

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