

Rapid On-Site Diagnosis of Filovirus Infections[®]

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Introduction

The current Ebola epidemic in Bundibugyo underscores the urgent need for diagnostic methods that can detect Ebola and Marburg hemorrhagic viral fevers at the time of patient presentation. Both Ebola and Marburg viruses are endemic in Uganda and both have caused substantial morbidity and mortality in Uganda in the past six months. In addition to the loss of life from epidemic spread of these virulent pathogens, there are substantial public health's, economic and social consequences that have resulted from these diseases. Methods currently in place for diagnosing Ebola and Marburg hemorrhagic fevers involve transport of blood samples from index cases to reference laboratories in either South Africa or Entebbe. Personal experience in Bundibugyo by this physician indicates that the use of reference laboratory facilities for diagnosing Ebola is not satisfactory. The basis for this conclusion is the recognition that a delay in diagnosis has a cascade of effects, such as inability to promptly institute disease-specific therapy, compromised implementation of public health measures at a time when an infected patient is most likely to disseminate the disease, unnecessary exposure of unaffected individuals to those with the disease as a result of preventative isolation and quarantine, and infectious hazards associated with transport of infectious biological specimens over long distances.

Diagnostic Strategies Currently Used

The definitive diagnosis of a viral hemorrhagic fever is based upon finding the pathogenic virus or its viral products in blood samples from an acutely ill patient. Immunoassay methods are the method of choice for the early detection of viral antigens in sick patients and are considered the best diagnostic test for Ebola and Marburg viruses because of their rapidity and robustness. Other diagnostic methods for diagnosing Ebola and Marburg hemorrhagic fevers, such as polymerase chain reaction (PCR) or detection of reactive antibodies, are less useful. PCR, though theoretically more sensitive than immunoassay detection of viral antigens, is subject to artifact and contamination. More importantly, genetic variation of viral DNA is a source of difficulty in ensuring that appropriate primers are applied to unknown samples. This problem occurred in the current Ebola Bundibugyo epidemic, as the PCR primer used to diagnose Ebola is not present in the current strain. The failure to identify a PCR primer by the reference laboratory in South Africa resulted in the incorrect conclusion that the "mysterious illness" in Bundibugyo was not Ebola. This in turn led to a substantial delay in diagnosis and resultant widespread consequences. The correct diagnosis of Ebola Bundibugyo using PCR required transport of specimens to the CDC Laboratory in Atlanta and involved the use of the most advanced techniques in molecular genetics that exist. Immunoassay detection of viral antigens, in contrast to PCR, are based upon a virion surface antigen from the Zaire strain which has been shown to be present in all Ebola strains, including Ebola Bundibugyo. Detecting the Ebola Zaire antigen readily identified the virus in patients from Bundibugyo. Assessing antibody levels specific for Ebola or Marburg virus is not a preferred method for diagnosing acute illness. The initial antibody response to Ebola or Marburg virus infection, a rise in IgM titers, does not occur until a patient is convalescing. As a result measurement of anti-Ebola or anti-Marburg IgM or the subsequent rise in anti-Ebola IgG does not provide timely information.^{1, 2}

Immunoassays for detecting viral antigens are based upon the use of antibody pairs. One antibody of a pair, termed the capture antibody, is affixed to a stationary substrate base and when exposed to viral antigen captures the virus at a site on the substrate. The second antibody of the pair is the signal-generating antibody. This antibody is connected to an enzyme such as peroxidase that can be detected through a colorimetric reaction or, alternatively, the antibody can be labeled with colloidal gold or colored latex beads. These pigmented antibodies collect at the site where the capture antibody has immobilized the viral antigen and result in an easily observed visual sign that literally shows that the viral antigen is present. Immunoassays which use a capture antibody bound to a nitrocellulose membrane and a signal antibody labeled with colored latex or gold particles are widely used, for they are simple to manufacture in bulk, inexpensive, small in size, disposable, easy to use, provide results in minutes, do not require trained personnel or laboratory equipment for test performance or interpretation, and produce sensitive and specific results in a broad range of field conditions. The best-known example of this type of rapid immunoassay is the home pregnancy test. These inexpensive, easy to use, disposable devices use two antibodies specific for human chorionic gonadotrophin (hCG), the pregnancy hormone. One antibody captures the hormone as it flows by capillary action along a porous membrane, while the other antibody identifies the hormone, once captured, through the appearance of a colored line.

Immunoassays for all Ebola virus strains and for Marburg virus exist. Monoclonal antibody pairs that can identify Ebola and Marburg virus surface antigens are widely available. Assay methods for these viruses currently in use are enzyme-linked immunosorbant assays are known by the acronym ELISA. ELISA methods require trained technical personnel and expensive laboratory equipment, so they must be performed in reference laboratories, as for example in the Institute for Virology Laboratory in Entebbe. As explained above, the logistics of transporting specimens from Bundibugyo to Entebbe have resulted in a delay in diagnosis. It is clear that there will continue to be outbreaks of Ebola and Marburg hemorrhagic fever in Africa and that effort to control future Ebola and Marburg epidemics will be hampered by relying on central laboratory diagnosis. As an experienced professor of internal medicine and endocrinology who understands the health care system in rural parts of Uganda, was working in Kikyu when the current Ebola epidemic started, and has been in Bundibugyo as recently as one week ago, I see a clear need for a new method to diagnose Ebola and Marburg hemorrhagic fevers in Uganda. If Uganda continues to rely on centralized diagnosis for the management of Ebola and Marburg virus, isolated outbreaks that affect small numbers of patients will have sufficient time to develop into epidemics.

Rapid, On-Site Testing

Based upon conversations with Pierre E. Rollin MD, Special Pathogens Branch, National Center for Infectious Disease, CDC, it is clear that a self-performing lateral flow immunochromatographic assay to rapidly detect Ebola and Marburg viral antigens in the field at the time of patient presentation can be developed. The advantages of this method for diagnosing Ebola and Marburg hemorrhagic fevers are that tests can be performed in district hospitals and health centers by nurses, clinical officers, and lab technicians while they are evaluating a sick patient and results can be obtained within a matter of minutes. Additionally, cell phones can be used to image lateral flow test results and transmit the results with date, time, and GPS locators to a public health database. This would allow real-time epidemiologic mapping of Ebola cases. Based upon my personal experience, cell phone coverage in Bundibugyo is excellent. The practical advantages of lateral flow, point-of-care Ebola and Marburg assays combined with cell phone data imaging and transmission are obvious and substantial. Using cell phones to record and transmit test results in real time represents a transformational use of mobile phone technology. In the case of the current Ebola epidemic, the use of this technology would have identified Ebola hemorrhagic fever as early as August, when the first two cases came to Kikyu Health Center and were incorrectly diagnosed as “food poisoning”.

Ebola / Marburg Rapid Test Specifications

Based upon my decades of work in the field of immunoassay development and discussions with one of the world's expert on Ebola, Dr. Pierre Rollin from the C.D.C., I am very confident that a rapid, on-site, disposable, self-performing immunoassay for detecting Ebola and Marburg virus antigens in acutely ill individuals can be developed, validated, and manufactured in bulk within a six month period. The manufacturing and performance specifications for the Ebola and Marburg tests that I propose developing are

1. the test will be intended for point of care use at the time that a patient with signs or symptoms consistent with possible Ebola or Marburg virus infection presents to a health care provider
2. the test will provide identification of Ebola or Marburg viral antigens in blood
3. the test will
 - a. use 50 microliters of capillary blood obtained by a fingerstick
 - b. provide results in minutes
 - c. be designed for use in field conditions as are found in areas such as Kikyo, Bundibugyo
 - d. when mass-produced, the cost for each test should be less than \$5.00 per test.
 - e. tests will be individually packaged and have a shelf life of not less than two years.

Risks

Although I am confident that I can successfully carry out the proposed immunoassay development, as I have successfully completed a number of similar projects, responsible project planning requires that I consider potential pitfalls that might interfere with the proposed plan of action. These include the following:

1. Difficulty sourcing antibodies and antigens.
2. Test sensitivity, i.e. lower limit of viral antigen detection, is unable to identify extremely low levels of viral antigen in human blood.
3. Costs of manufacture exceed \$5.00 per test.

Additional Considerations

One of the reasons why on-site rapid immunoassays for Ebola and Marburg have not been developed is because the potential market is small and the population at risk has very limited financial resources for purchasing tests. This economic constraint is similar to that encountered with drug development for neglected tropical diseases. However, the economic consequences of Ebola and Marburg epidemics are substantial. For example, the loss in tourist revenue due to fear of contracting a fatal hemorrhagic fever has a major affect on significant source of revenue for Uganda. Prompt diagnosis will clearly help controlling disease outbreaks before they spread, become epidemics, and become world press news items.

Another aspect of endemic African Ebola that gets less attention than human is that it is an epidemic that primarily affects non-human primates. For example, over 80% of western gorillas in certain regions of West Africa have died from Ebola virus and a sick chimpanzee was the source of a prior Ebola epidemic in Gabon. Uganda derives substantial revenue from gorilla and chimp trekking, and loss of gorilla and chimp populations from Ebola will result in a

loss of tourist revenue. Rapid tests for Ebola will have the same benefit in animal populations as they do in humans. In addition to the benefits such tests have on the medical care of humans; they also represent a proactive step to protecting Uganda's chimp and gorilla populations, natural resources that generate significant income for Uganda.

Rapid tests for Ebola and Marburg may be patentable and, as intellectual property, could represent a source of revenue from licensing fees, royalties, or sales. I have considerable aspect in this aspect of diagnostic test development. Additionally, it may be possible, through a joint venture with an immunoassay test manufacturer, to leverage purchase of tests for Ebola and Marburg to obtain discounted prices on other diagnostic tests currently purchased by the Ugandan government, as for example rapid tests for HIV, malaria, tuberculosis, cholera, hepatitis, and glucose.

Lastly, the public relations benefits Uganda would accrue as a result of developing new biotech methods to counter tropical diseases unique to this environment should be considered. Setting up a new biotech program in Uganda would be a first for sub-Saharan Africa. The benefits from such forward thinking and action could be substantial, both in terms of enhancing Uganda's international reputation and attracting investments from abroad.

Summary and Conclusions

There is an urgent need for methods to diagnose Ebola and Marburg hemorrhagic fevers in Uganda that can be used in the field to provide rapid results. Based upon my professional expertise in the field of immunoassay test development, I am confident that on-site, rapid, self-performing diagnostics tests based upon a lateral-flow, immunochromatographic assay format can be developed and mass-produced within 6 months. I am willing to lead this lead this project to develop methods for the early detection of Ebola and Marburg hemorrhagic fevers.

Literature Cited

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