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Determining the Utility of Rapid Diagnostic Tests for Surveillance of  
Three Epidemic-Prone Diseases  
During Complex Emergencies: A Systematic Review

By

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By

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An abstract of  
A thesis submitted to the Faculty of the  
Rollins School of Public Health of Emory University  
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2016

## **Abstract**

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By Hannah Lofgren**

Complex emergencies (CEs) continue to occur in today's international climate, fueled by urbanization, transmigration, corruption and persistent economic and social inequalities. The societal breakdown and lack of authority characteristic of CEs provide opportunities for epidemics of infectious disease, increasing the morbidity and mortality during CEs. Rapid diagnostic tests (RDTs) capable of detecting these epidemics early and under harsh conditions can be useful tools for epidemic response teams in preventing and mitigating these epidemics, but little research has been done on their utility in CEs. This review examines commercially available RDTs for three epidemic-prone diseases of current global importance (cholera, dengue and Ebola) and evaluates their utility in CEs. Currently, there are promising RDTs that are commercially available or fast-tracked for development for these three diseases, but more research and development is needed to understand their implementation in CEs and improve their performance in low-resource settings. Finally, future research should also focus on creating testing algorithms to guide the use of dengue and Ebola RDTs for epidemic detection during CEs.

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## Definitions

**Complex emergency (CE)**- A humanitarian crisis in a country, region or society with total or considerable breakdown of authority resulting from internal or external conflict with increased morbidity and mortality that overwhelms local coping capacity and requires an international response. Also called a humanitarian emergency [1].

**Natural Disaster**- When a natural hazard impacts a population or area and may result in severe damage, destruction and increased morbidity and mortality that overwhelm local coping capacity. A natural disaster may have an acute onset (e.g. tsunami, floods) or slow onset (e.g. drought) [1].

**Epidemic**- An unusual increase in the number of cases of an *acute* infectious disease which already exists in the region or population concerned or the appearance of an infection previously absent from a region [1].

**Rapid Diagnostic Test (RDT)**- A diagnostic assay that is relatively simple to perform and interpret, rapidly provides results, requires limited training and allows for diagnosis at the community level [2].

-A diagnostic assay designed for use at the point-of-care (POC), and can be adapted for use in low-resource settings. An RDT is low-cost, simple to operate and read, sensitive, specific, stable at high temperatures, and works in a short period of time [3].

**Point of Care Test (POCT)**- A medical test performed at or near the site of patient care [4].

## Chapter 1: Introduction

Complex emergencies (CEs) have become a fixture on the world stage in recent decades. Factors such as urbanization, transmigration, perpetual economic and social inequalities, food and water insecurity, corruption and human rights violations, among others, will continue to contribute to the development of future CEs [5], as evidenced by the current crisis in Syria. CEs represent times of total upheaval and displacement, creating environments in which diseases may spread rapidly. CEs often occur in developing countries and while they are more prone to



epidemics of infectious disease than natural disasters, these events may occur concurrently [1], exacerbating existing health and infrastructure deficits. Infectious disease research and experience in CEs have shown that there are certain diseases that are more “epidemic-prone” than others [6], which require close surveillance during CEs in order to detect outbreaks before they become large-scale events resulting in high morbidity and mortality levels. International aid and response teams must therefore be equipped with reliable tools tailored to the unique CE context in order to diagnose and respond rapidly and efficiently to potential epidemics. Rapid diagnostic tests (RDTs) for epidemic-prone diseases are crucial in these efforts, but little research has been conducted on available tests for emergencies and their efficient and effective implementation in CEs.

### **1.1 Background and Significance**

CEs have increased in number in the decades since the Cold War [7]. According to the International Committee of the Red Cross, an average of five CEs occurred globally each year from 1975 to 1985 [7], while Spiegel *et al.* report that a total of 363 CEs were recorded from 1995 to 2004 [1]. After the fall of communism in the 1990s, the ensuing economic and social instability infiltrated many already weakened governments, as support from either superpower was no longer as accessible. This uncertainty often resulted in conflict and civil war [7, 8]. In addition, residual tensions and fragmented post-independence societies and economies in Africa devolved into increasing incidence of conflict from 1980-2000 [9]. Notable examples include the civil wars in Angola, Democratic Republic of the Congo (DRC), Rwanda, Afghanistan and Tajikistan.

These CEs share some important characteristics. First, most were contained within the borders of a single country, which can hinder international aid efforts when governments are

uncooperative towards external agencies [7, 10]. Second, civilians increasingly became targets in conflicts, either through ethnic cleansing or intentional destruction of resources, meaning that a much larger portion of the population was affected [7, 8]. Given this increased civilian involvement, a trend that continues to this day, CEs are also characterized by a large degree of population displacement, resulting in the establishment of rudimentary camps, often lacking basic sanitation and infrastructure, for internally displaced persons (IDPs) [7, 10]. In addition, the vast majority of CEs occur in impoverished countries already plagued by weak health infrastructure. Of the 363 CEs reported from 1995-2004, Spiegel *et al.* identified the 30 largest, based on total number of deaths recorded. Over half of the 30 largest CEs occurred in Africa and one-third occurred in Asia. CEs also tend to be lengthy events, with a median duration of 12.5 years [1].

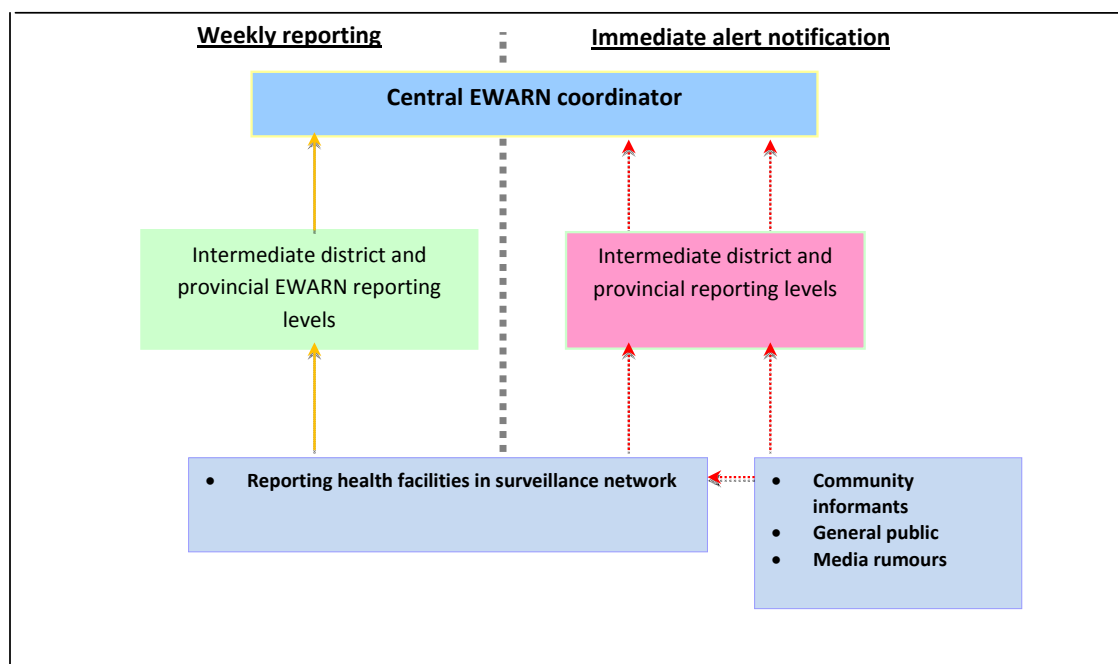
For these reasons, it is not surprising that CEs and epidemics of communicable diseases frequently overlap. Sixty three percent of the 30 largest CEs from this period also had an epidemic and nearly half of the 30 largest epidemics occurred during a CE [1]. This is also in contrast to natural disasters, only 23% of which involved an epidemic [1]. However, 87% of the largest CEs were further complicated by at least one natural disaster [1]. Thus, while epidemics are of greater concern during CEs than natural disasters, there is still significant concurrency between CEs and natural disasters, which is important for humanitarian aid and epidemic response teams to recognize in order to better tailor relief efforts.

CEs may also be defined by mortality levels that are significantly elevated above baseline levels [10]. These increased levels are most often due to communicable diseases, in addition to widespread malnutrition, and occur early in the acute phases of CEs [11]. In order to identify, compare and monitor CEs, Toole and Waldman have suggested a baseline crude mortality rate

(CMR) of 1 death per 10,000 people per day to indicate the acute phase of a CE, which may last anywhere from 1 to 12 months [12]. Once the CMR has dropped below 1 death per 10,000 people per day, the acute phase is considered over. The risk of a communicable disease epidemic occurring, as well as its severity, is directly related to population density and displacement during a CE [13], and more specifically, the living conditions and health status of the population [6], including any previous exposure to a given infectious agent. For example, CMRs recorded in the major CEs from 1991-2002 ranged from 0.2 among East Timor displaced persons in 2000, to an estimated 34.1-54.5 among Rwandan refugees in the DRC in 1994 [10]. CMRs were significantly elevated among Rwandan refugees in the DRC due to epidemics of cholera and shigellosis that resulted from poor water systems and poor sanitation because of the soil conditions in refugee camps of 600,000-800,000 persons [14]. These examples highlight the contextual nature of CEs and demonstrate the importance of taking all of the above factors into account when responding to CEs.

Due to the increased risk of epidemics during CEs, as well as the severely decreased capacity for or absence of public health surveillance by local authority, it is vital to install an early warning alert and response network (EWARN) [6] in the acute phase of a CE to enable timely detection of and response to these epidemics. An EWARN's primary goal is to closely monitor a CE for precursory signs of a potential epidemic's occurrence [6]. This is achieved through two principal data sharing elements: "*immediate alert*" and "*weekly reporting*" [6]. When an abnormal event that could potentially signal an epidemic is detected, this information must be shared on a district or provincial level or even directly with the central EWARN coordinator as an immediate alert. All other weekly, aggregated data are disseminated up the EWARN structure for analysis of trends and intervention impacts (Figure 1). An EWARN is

implemented by the local country's Ministry of Health, with assistance from the World Health Organization (WHO) and other bodies. It should not replace a local routine public health surveillance system, but instead supplement it to temporarily cover local surveillance deficits during an emergency.



*Figure 1. Data sharing in EWARNS [6]*

The WHO has compiled a set of guidelines for implementing an EWARNS, which also includes criteria for defining those diseases that pose the greatest risk to the population in a given CE. Although CEs may differ with respect to location, population and magnitude, there are certain communicable diseases that are more “epidemic-prone” [6] than others and thus occur with greater frequency across CEs. Historically, diarrheal diseases, acute respiratory infections, measles and malaria have been the largest contributors to higher morbidity and mortality levels in CEs, especially in Africa [10, 11]. In the acute phase of a CE, a risk assessment should be performed to identify the epidemic-prone diseases associated with that particular CE. According

to the WHO, in deciding whether a disease or syndrome is included in the EWARN system, the following should be considered:

- epidemic potential;
- ability to cause severe morbidity or death;
- international surveillance requirements (e.g. International Health Regulations and Public Health Emergencies of International Concern);
- availability of prevention and control measures;
- availability of reliable and meaningful case definitions and simple laboratory tests, where appropriate [6].

According to these criteria, acute flaccid paralysis (poliomyelitis), acute hemorrhagic fever syndrome, measles, suspected cholera or acute watery diarrhea, suspected shigellosis or acute bloody diarrhea, acute jaundice syndrome, suspected bacterial meningitis and confirmed malaria [6] are all common inclusions under EWARN. However, no more than 8-12 diseases or syndromes should be included in an EWARN, depending on the initial risk assessment performed in the acute phase of the CE.

An EWARN must not only detect possible signs of an emerging epidemic early, but also correctly identify the cause of the epidemic in a CE. Under EWARN, syndromes are used in place of diseases in order to increase the sensitivity of detection and therefore the likelihood of capturing all those who potentially may have the disease in question. This necessitates broad case definitions that are still capable of differentiating between diseases. Rapid, accurate diagnosis is especially crucial in CEs in order to distinguish epidemic-prone diseases from non-epidemic-prone diseases and inform an appropriate response. In addition, alert thresholds for many of these epidemic-prone diseases (e.g. cholera) are very low, meaning that even one case

can signal a potential epidemic with huge public health implications [6]. However, due to the similarity in symptoms of diseases included under these syndromes, accurate diagnosis can be difficult based on clinical symptoms alone, highlighting the need for laboratory confirmation. For example, acute jaundice syndrome, which involves yellowing of the skin and whites of the eyes, fatigue, vomiting and weight loss, among other symptoms, may result from a variety of infectious diseases, such as hepatitis A, B, or E, leptospirosis or yellow fever [15].

Due to limited infrastructure and resources available, as well as the need to respond quickly to potential epidemics, normal laboratory confirmation methods are generally not feasible or appropriate during CEs, making RDTs especially useful tools for international aid and epidemic response teams in this context. The term RDT is often used interchangeably with point of care test (POCT) in the literature and the difference between the two terms is often blurry or indistinguishable. While several definitions exist for POCT, all reinforce the fundamental idea that “point-of-care testing is done near the patient and leads to an expedited clinical decision” [16]. One key distinction between RDT and POCT seems to be that, in contrast to RDTs, POCTs do not require any specific technology for use in remote areas (e.g. battery- or solar-powered) or level of user training [16]. The key element in a POCT is simply the location in which it is performed, i.e., at or near the site of patient care. In the context of CEs, specifications such as these may be crucial to the usability of a test, rather than just the capability to test near the patient.

According to WHO’s Sexually Transmitted Disease Diagnostics Initiative, the best RDTs will comply with the ASSURED criteria, which was originally developed to improve RDTs for sexually transmitted infections:

1. Affordable;

2. Sensitive;
3. Specific;
4. User-friendly (simple to perform in a few steps with minimal training);
5. Robust and rapid (can be stored at room temperature and results available in <30 minutes);
6. Equipment free (or minimal equipment that can be solar powered);
7. Deliverable to those who need them [17].

In light of these criteria, which specify a POCT more applicable to the CE context, the term RDT will be used for this review. In sum, a POCT is an umbrella term that indicates any diagnostic test performed at or near the site of patient care. In contrast, RDTs can be conceptualized as a type of POCT that is associated with additional requirements to better adapt them to use in low-resource settings.

POCT technology began in 1962, with the advent of a faster technique to measure blood glucose levels, and gained momentum in subsequent decades with the first rapid pregnancy test in 1977 and portable methods used to measure electrolytes in emergency departments in the 1990s [16]. Since then, the field of POCT has grown exponentially, with a variety of POCTs available across medical disciplines. However, these tests have largely been designed for use in hospitals and clinics in developed countries. To date, POCTs for use in low-resource settings have focused on infectious diseases responsible for significant global mortality, such as HIV and malaria [16]. Although these tests have had a demonstrated impact on diagnosis of these diseases, they are still limited in some ways, including cost for these resource-limited countries [18].

In contrast, there has been limited research on commercial RDTs for most epidemic-prone diseases and their performance in CEs [19], perhaps because there appears to be little financial incentive for private companies to invest in this area [20]. However, the need for such research is great. In a survey of disaster care experts conducted by Kost *et al.*, respondents cited the lack of improved handheld diagnostic technologies capable of effectively withstanding the harsh conditions and limited resources inherent in CEs, with cholera and *E. coli* mentioned as priority diseases [21]. Similarly, to the author's knowledge, there are no published reviews on the performance of commercial RDTs for epidemic-prone diseases in CEs, nor guidelines for their use.

## **1.2 Statement of the Problem**

There is a need for further research on the availability and implementation of commercial RDTs for epidemic-prone infectious diseases in CEs in order to facilitate timely detection of and subsequent public health response to these diseases by epidemic response teams.

## **1.3 Statement of Purpose**

The purpose of this review is to synthesize and evaluate the characteristics, advantages, disadvantages, effectiveness and uses of commercially available RDTs for selected epidemic-prone infectious diseases by response teams in CEs in order to improve timely detection of and public health response to these diseases in complex emergency settings.

## **1.4 Research Objectives**

1. To determine a list of epidemic-prone diseases according to EWARN criteria and select a subset of the most globally important diseases for review, based on burden, mortality and morbidity caused and current relevance.



2. To review the available literature on commercially available RDTs for a subset of selected epidemic-prone diseases.
3. To evaluate the characteristics of commercially available RDTs
4. To identify steps for future research and expansion of RDT use in infectious disease surveillance and epidemic detection in CEs.

## **Chapter 2: Methods**

This study reviewed articles published in PubMed database on commercially available RDTs for selected epidemic-prone diseases. For this study, the list of epidemic-prone diseases, from which a subset of diseases was selected for review, was based on those diseases and syndromes most commonly integrated into WHO EWARN systems during CEs, which include:

- *acute flaccid paralysis (poliomyelitis only)*
- *acute hemorrhagic fever syndrome*
- *measles*
- *suspected cholera or acute watery diarrhea (AWD)*
- *suspected shigellosis or bloody diarrhea*
- *acute jaundice syndrome*
- *suspected bacterial meningitis*
- *confirmed malaria*
- *unusual cluster of events.*

The above diseases and syndromes were selected for inclusion in EWARN based on the following WHO criteria:

- A. epidemic potential;
- B. ability to cause severe morbidity or death;
- C. international surveillance requirements (e.g. International Health Regulations and Public Health Emergencies of International Concern);
- D. availability of prevention and control measures;
- E. availability of reliable and meaningful case definitions and simple laboratory tests, where appropriate [6].

The single diseases of poliomyelitis, measles, cholera and shigellosis were therefore included in the list of epidemic-prone diseases based on the above criteria alone. To select diseases pertaining to acute hemorrhagic fever syndrome, acute diarrheal disease, acute jaundice syndrome and bacterial meningitis, a modified Delphi group was used in order to reach consensus on a final disease list [22]. Two medical epidemiologists, one with an infectious diseases specialty, individually evaluated a list of diseases related to each syndrome (Appendix A) according to the above WHO criteria. A disease meeting at least criteria A, B, D and E was included in the list. A third expert was used as a tiebreaker in the event of disagreement on the inclusion of a disease.

Some additional diseases fitting the aforementioned criteria were excluded at the outset. Since this study seeks to fill gaps in synthesizing research on RDTs, malaria was excluded, as there already exists a breadth of literature on and periodic review of malaria RDTs [23, 24]. Acute respiratory infections (ARIs), including severe acute respiratory infection (SARI) and influenza, were also excluded for several reasons. Many ARIs in the form of upper respiratory tract infections resolve of their own accord and should therefore not be included in EWARN. Additionally, due to the non-specificity of symptoms of ARIs, EWARN is unlikely to collect

quality data on these conditions, so other surveillance systems should be used instead [6].

Influenza specifically was excluded because although many RDTs exist for influenza, these only detect types A and/or B, and cannot detect novel strains due to antigenic shift or drift, rendering them less useful in the context of CEs [25]. Unusual clusters of events were also excluded as their complexity is outside the scope of this review. Finally, although HIV and tuberculosis are diseases of potential importance in CEs, they were both excluded because of their low epidemic potential and the inability of EWARN to capture data relevant to their control, such as prevalence. This process of inclusion and exclusion resulted in the following final list of epidemic-prone diseases:

<b>Syndrome</b>	<i>Acute Jaundice Syndrome</i>	<i>Acute Hemorrhagic Fever Syndrome</i>	<i>Acute Diarrheal Disease</i>	<i>Bacterial Meningitis</i>	<i>Febrile Rash Syndrome</i>	<i>Acute Flaccid Paralysis</i>
<b>Disease</b>	Hepatitis A	Crimean Congo hemorrhagic fever	Cholera	<i>Neisseria meningitidis</i>	Measles	Polio
	Hepatitis E	Dengue fever	Enterohemorrhagic <i>Escherichia coli</i> /Shigellosis		Rubella	
	Leptospirosis	Ebola virus disease	Typhoid			
	Yellow Fever	Lassa Fever				
		Marburg virus				
		Rift Valley fever				
		West Nile virus				

**Table 1. Final list of epidemic-prone diseases considered in the study**

While all of the above diseases were initially examined for commercially available RDTs, in order to conduct more thorough research, for the purposes of this review, these diseases were further limited to three diseases that contribute globally to a large public health burden, are currently of high importance and thus perhaps in greatest need of RDTs; Ebola virus disease, due to the most recent outbreak in West Africa; dengue, due to rapidly increasing global distribution

and severity [26]; and cholera, due to its significant contribution to child [27] and adult mortality in developing countries [28], despite being generally neglected in research efforts [29].

**Cholera.** Cholera is an acute diarrheal disease caused by the bacterium *Vibrio cholerae* serogroups O1 and O139. Although many infected individuals are asymptomatic or experience mild diarrhea, in its severe form, cholera causes profuse diarrhea and vomiting, which can quickly lead to significant dehydration, and subsequently, death [30]. Without treatment, severe cholera has a case fatality rate of about 50% [31]. Cholera is transmitted through consumption of food or water contaminated by infected feces. For this reason, cholera occurs most frequently in low-resource areas with little infrastructure, poor hygiene and sanitation, reliance on surface sources for drinking water and crowded living conditions, all of which may be present during a CE. It is endemic in areas of Asia and Africa, and an estimated 2.4 billion people worldwide are at risk of cholera due to lack of access to adequate water and sanitation facilities [29]. In endemic areas, cholera largely affects children between 2 and 4 years of age, while all age groups are similarly affected in areas where cholera has been recently introduced [31].

In 2014, a total of 190,514 cholera cases were reported globally to the WHO, with a total of 2,231 deaths, a 47% increase from 2013 [29]. However, these numbers are estimated to be vastly under representative, potentially representing as little as 5-10% of the true global cholera burden [32]. These low estimates are due to poor surveillance, lack of diagnostic confirmation, underreporting for fear of financial impact from trade sanctions or decreased tourism and general neglect. While cholera was reported from all regions, Afghanistan, DRC, Ghana, Haiti and Nigeria collectively represented 84% of all cases in 2014 [29]. *V. cholerae* serogroup O1 continues to be the more prevalent strain, although serogroup O139, which was isolated in India

and Bangladesh in 1992, caused large outbreaks there and could threaten to initiate a new cholera pandemic [31].

Cholera diagnosis, although not required for the administration of treatment, is essential early on in the course of epidemics to reduce their magnitude, severity and length. The gold standard for cholera diagnosis is stool culture, but this method takes several days to produce results and requires a skilled technician, specialized reagents and a well-equipped laboratory [33]. All of these requirements make stool culture inadequate for cholera diagnosis during CEs. Among the acute hemorrhagic fever syndromes, two were selected: dengue and Ebola.

**Dengue.** Dengue is an acute febrile disease that is caused by any of the five dengue virus serotypes, the fifth of which was recently isolated in Malaysia in 2013 [34]. Despite a wide variety of clinical presentations, the WHO currently classifies dengue using two categories: dengue and severe dengue. Dengue begins with fever, myalgia, arthralgia, nausea and rash, among other possible symptoms. [35]. The majority of patients recover on their own, but a small proportion advance to severe dengue disease [26], which may be indicated by warning signs including abdominal pain, mucosal bleeding, persistent vomiting and lethargy. Severe dengue is then defined as progression to dengue shock syndrome (DSS) or dengue hemorrhagic fever (DHF) [35]. Primary dengue infection incurs subsequent protection against the infecting serotype, but secondary infection from the other four serotypes is possible.

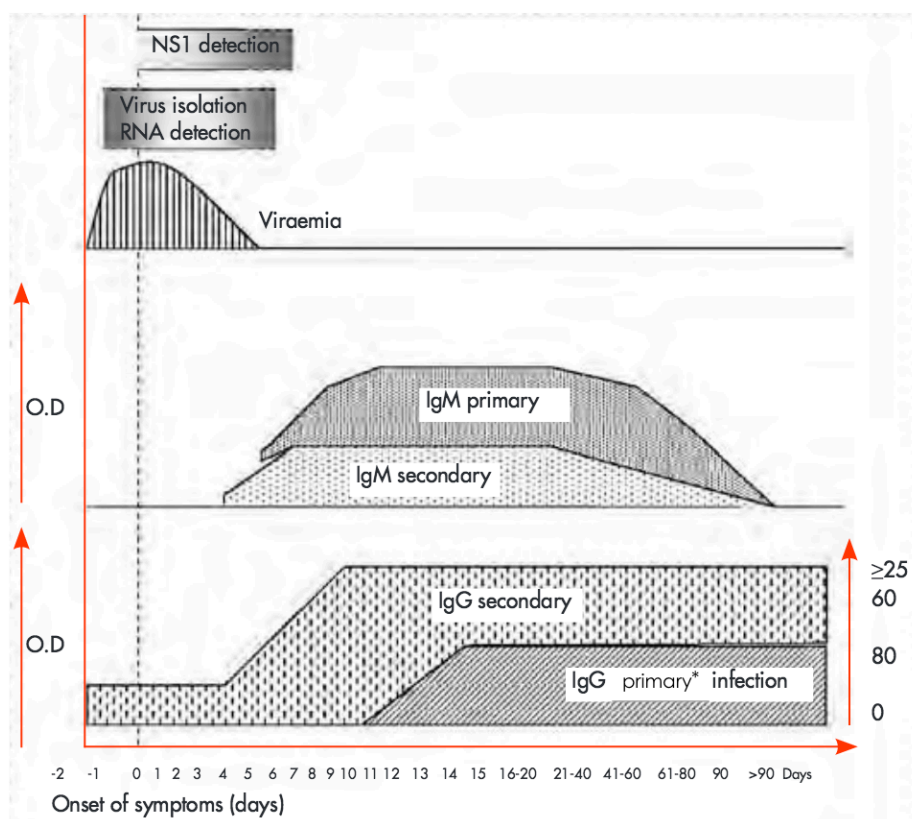
Dengue transmission has risen dramatically in the past few decades, largely due to increased human movement, urbanization, population growth, climate change and the expansion of the *Aedes* mosquitoes, the dengue vector, into new regions [26]. Because of this, dengue has been described as the “most prevalent and rapidly spreading mosquito-borne viral disease of human beings” [26] prior to the current chikungunya and Zika virus epidemics. Dengue is

currently endemic in more than 100 countries in Southeast Asia, the Americas, Africa, the western Pacific and eastern Mediterranean, placing roughly half of the world's population at risk for dengue infection [36]. Compounding this risk, many countries experience hyperendemicity of more than one dengue virus (DENV) serotype [26]. Recent studies estimate that 390 million dengue infections occur annually, with approximately 96 million of those manifesting clinically [26]. However, the exact global dengue burden is unknown, due to underreporting, lack of diagnosis, and misclassification. Dengue's widespread global distribution, particularly high prevalence in poor regions such as Southeast Asia and Africa and increasing transmission make it a disease of potential importance during CEs. Patterns of small-scale spatiotemporal clustering are also possible, meaning that one household infected with dengue may contribute disproportionately to substantial dengue propagation [26]. This feature has important implications for CEs, when large populations may be displaced and temporarily living in crowded conditions with little protective infrastructure.

Due to the duration of the virus and the timing of the appearance of the host responses during infection, dengue diagnosis relies on the detection of the dengue virus itself, its RNA, antigens, or antibodies, or a combination of these methods. Dengue virus, RNA and non-structural protein 1 (NS1) antigens may be detected from the onset of symptoms until day 5 or 6 of illness, after which their levels dramatically decrease [35, 37]. After days 3-5 of illness, IgM and IgG antibodies are detectable in increasing levels (Figure 2) [35]. IgA antibodies may appear simultaneously with or one day after IgM antibodies and decline rapidly until day 40 post symptom onset [35], but may appear much earlier than IgM or IgG in acute secondary DENV infection [38]. For the purposes of this review, the dengue gold standard reference diagnostic test was defined depending on the timing of the infection, as indicated above:

1. At least one: viral culture, reverse transcription-polymerase chain reaction (RT-PCR), NS1 Ag ELISA *and/or*
2. IgM/IgG capture ELISA

Although viral culture confirms dengue diagnosis, it is expensive, time-consuming and requires skilled lab technicians and advanced equipment. RT-PCR and ELISAs produce results in 1-2 days, but involve specialized equipment, which is costly and necessitates trained users [35].



**Figure 2. Detectable levels of dengue virus, RNA, NS1 Ag and IgG/IgM in primary and secondary infections according to day post symptom onset [35]**  
*\*Edit made by author*

**Ebola virus disease.** Ebola virus disease is a severe, acute illness characterized by the sudden onset of fever, malaise, myalgia and headache. In severe cases, subsequent symptoms can include vomiting, diarrhea, rash, multi-organ failure and internal and external hemorrhage [39]. Ebola virus disease is often fatal without early supportive care, with case fatality rates reaching

up to 88% [30]. Before 2013, Ebola virus disease was largely confined to central Africa, where outbreaks have occurred since discovery of the Ebola virus in 1976. The most recent Ebola virus disease outbreak in West Africa, the largest in history, demonstrated the ease with which infectious disease may cross international borders. As of March 27, 2016, there have been 28,646 cases (confirmed, probable and suspected) of Ebola virus disease and 11,323 deaths in 10 countries, with Guinea, Sierra Leone and Liberia hit the hardest [40]. Fruit bats are thought to be “natural hosts” of Ebola virus, which enters the human population through direct contact with these bats and other infected animals [39]. Human-to-human transmission occurs through direct contact with the blood or bodily fluids of an infected person. The gold standard for diagnosing Ebola virus disease is virus isolation or RT-PCR, however both methods require advanced lab equipment, skilled technicians and may take several days for results. Ebola virus disease’s sudden onset and quick progression especially demand functional rapid tests.

Once these three diseases were selected for further study, all searches in PubMed [41] were conducted using the same search string, modified only to specify the disease in question: *(disease X AND rapid AND (diagnosis OR diagnostic OR detection OR technique OR method OR methods OR test OR tests OR "point-of-care") AND sensitivity AND specificity) AND "last 10 years"[PDat] AND English[lang])*

Citations were exported to EndNote [42] and full text was obtained for all possible articles retrieved from each individual search string. Titles and abstracts were first reviewed for relevance and excluded if any established exclusion criteria were present. The full texts of those studies not excluded on the basis of title or abstract alone were then examined according to established inclusion and exclusion criteria. The inclusion criteria used to evaluate articles for this study included:



- Article discussed the commercial RDT of disease in question;
- Compared the RDT to the gold standard diagnostic test of disease in question
- Included RDT sensitivity and specificity rates and either the number of true positives of the RDT or the total number of RDT positives and negatives and total number of samples tested using the RDT
- Discussed location where test was performed (i.e. lab v. field)
- Published between January 2005 and December 2015
- Published in English and;
- Full text available through Emory or CDC Library systems

Articles that did not meet all of the above criteria were excluded.

For the purposes of this paper, an RDT was defined as a commercial portable test that could be performed at point-of-care, did not require any sample preparation and produced results in thirty minutes or less. In order to properly evaluate the strength and utility of a given RDT, articles that did not list and explain the calculation of an RDT's sensitivity or specificity rates were excluded. Articles that did not mention the site at which an RDT was or could be performed were also excluded, as this is a key characteristic in evaluating RDTs for use in CEs. Articles were limited to the past 10 years in order to capture the most up-to-date information on RDTs across the three diseases of interest. Non-English articles were also excluded due to the reviewer's language abilities.

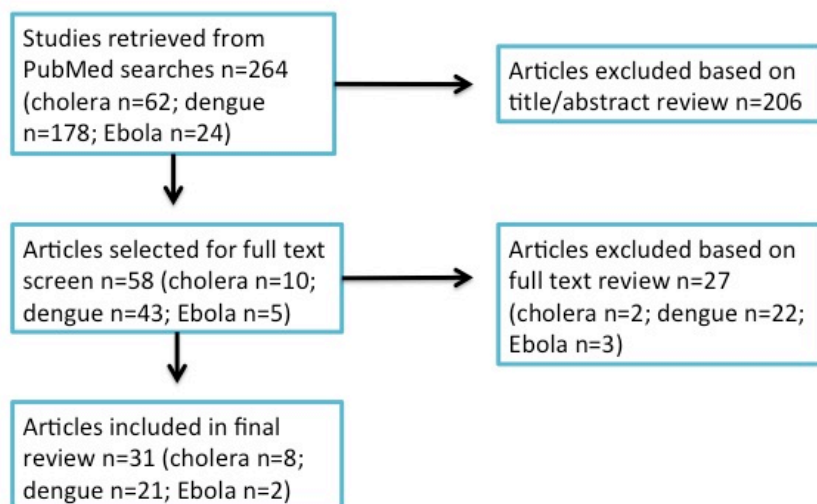
Relevant data were obtained from each article meeting the inclusion criteria and organized in a Microsoft Excel [43] spreadsheet. Extracted data included name of first author, publication year, disease of interest, RDT name and manufacturer, RDT format, analyte (bacteria, virus, antigen, etc.), specimen(s) required, sensitivity and specificity rates, positive and

negative predictive values, gold standard diagnostic method to which the RDT was compared, time required for results, location of RDT, storage criteria and cost.

IRB approval was not required for this study, as it did not involve human subjects research.

### **Chapter 3: Results**

The PubMed database [41] retrieved a total of 264 articles across the cholera (n=62), dengue (n=178) and Ebola (n=24) search strings. All articles were logged in an Excel [43] spreadsheet and all citations were exported into Endnote [42]. Article titles and abstracts were evaluated first for any excluding criteria, such as the study of non-rapid tests, non-human samples, or diseases other than those included in the present study, which resulted in the exclusion of 206 articles. The full text of the remaining 58 articles was evaluated according to the inclusion and exclusion criteria, resulting in a final list of 31 articles for inclusion in the study (Figure 2). Overall, articles were most often excluded because the diagnostic test in question did not qualify as an RDT, reference test(s) used were not considered the gold standard diagnostic test for the disease, or there were not sufficient data reported on methodology or RDT results (n=230).



*Figure 3. Flow chart of article selection process*

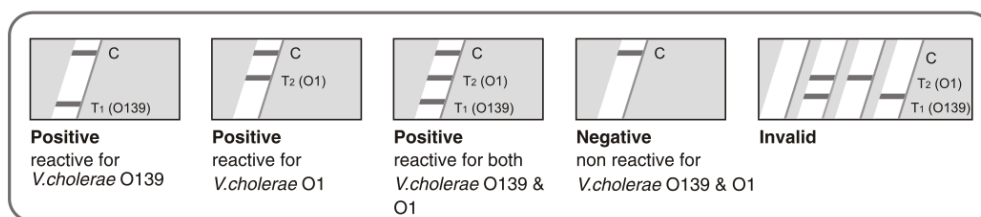
### 3.1 Cholera

Eight cholera RDT articles were selected for inclusion in the study. Three RDTs were evaluated across the eight studies, in addition to the prototype version of one of the commercial tests. These included the Institut Pasteur (IP) prototype, now known as Crystal VC® [44], the Sensitive Membrane Antigen Rapid Test™ (SMART) [45] and Medicos. Crystal VC® is a lateral flow, immunochromatographic dipstick test that is designed to detect both serogroups *Vibrio cholerae* O1 and *Vibrio cholerae* O139. To use Crystal VC®, the sample is placed into a test tube, the dipstick is inserted vertically and results are read after 15-20 minutes. Bands appearing at the control and test lines indicate a sample positive for *Vibrio cholerae*, whereas a single band at the control line indicates a negative sample (Figure 3) [44]. The manufacturer-reported sensitivity and specificity of Crystal VC® for detection of *Vibrio cholerae* O1 are 88-100% and 61-87.3%, respectively. For detection of *Vibrio cholerae* O139, the manufacturer reports a sensitivity of 99% and a specificity of 96% [44]. The SMART™ reviewed in this study

has since been replaced by an updated version called SMART™ II, which uses a monoclonal antibody based lateral flow design instead of the flow through, monoclonal antibody-polyclonal antibody sandwich utilized in SMART™ [45]. Only SMART™ is included in this study; however, due to this replacement, manufacturer-reported sensitivities and specificities are no longer available for SMART™. No information on Medicos or its manufacturer was available. Characteristics of these RDTs are shown in Table 2.

RDT	RDT Format	Detecting	Manufacturer	Sample	Read Time	Storage	Cost per Test (USD)
<b>Crystal VC® [44]</b>	Dipstick	<i>Vibrio cholerae</i> O1, O139	Span Diagnostics, Ltd. (India)	Watery stool	15 minutes	Between 4 and 30 °C	2.57
<b>SMART™ [46]</b>	Sandwich assay	<i>Vibrio cholerae</i> O1 antigen	New Horizons Diagnostics (USA)	Watery stool	15 minutes	Refrigeration for long term storage	14
<b>Medicos [46]</b>	Dipstick	<i>Vibrio cholerae</i> O1	Advanced Diagnostics, Inc. (USA)	Watery stool	10 minutes	Refrigeration for long term storage	4

**Table 2. Characteristics of cholera RDTs included in the review.**



**Figure 4. Possible test results using Crystal VC® [44]**

The IP prototype/Crystal VC® was the RDT most often studied and was included in all eight articles. SMART™ and Medicos were each included in one study. Study sites spanned three WHO regions and included Bangladesh (n=2), India (n=1), DRC (n=1), Mozambique (n=1), Zanzibar (n=1), Guinea-Bissau (n=1) and Haiti (n=1). In the majority of studies, RDT evaluations were conducted at hospitals or cholera treatment centers (5/8) while the remaining evaluations were completed in research laboratories. All studies were conducted during a cholera

outbreak or normal cholera season. All studies used stool culture as the gold standard diagnostic reference, except one study (Harris *et al.*, 2009), which used PCR due to unforeseen issues with samples during shipment. Sample sizes ranged from n=101 to n=644. All studies used watery stool samples, except for Kalluri *et al.* 2006, which used an aliquot of whole stool.

Three studies (Kalluri *et al.* 2006, Ley *et al.* 2012 and Page *et al.* 2012) compared the performance of the RDT by the skill level of the RDT user using all study samples or a subset of samples (Ley *et al.* 2012). Comparisons were made between skilled laboratory technicians with advanced training in microbiology techniques and clinicians, field technicians and community health workers with at least primary education, but no laboratory training. Non-laboratory RDT users were either trained by senior microbiologists to use the RDT (Kalluri *et al.* 2006); received several training sessions and practiced using the RDT beforehand (Ley *et al.* 2012); or were simply provided with the manufacturer's instructions (Page *et al.* 2012). Sensitivities and specificities reported for the three RDTs across the eight studies are shown in Table 3. The lowest sensitivity for any RDT was 58% among field technicians using SMART™ and the highest was 97% reported using Crystal VC®. The lowest specificity was 49.2% with Crystal VC® and the highest was 95% among field technicians using SMART™. For Crystal VC®, PPVs ranged from 47-89.4% and NPVs ranged from 71.8-94.4%. Kalluri *et al.* reported 148 total indeterminate readings across the three RDTs and these were excluded from the final analysis.

RDT	Author	Year	Country	Sample Size	RDT Performance Location	RDT Performed by	Sensitivity (95% CIs)	Specificity (95% CIs)
Medicos	Kalluri, <i>et al.</i> [46]	2006	Bangladesh	N=304	Laboratory	Field technician	84% (77-91)	79% (73-85)
Medicos	Kalluri, <i>et al.</i>	2006	Bangladesh	N=304	Laboratory	Lab technician	88% (81-94)	80% (73-95)
SMART™	Kalluri, <i>et al.</i>	2006	Bangladesh	N=304	Laboratory	Field technician	58% (46-71)	95% (91-98)
SMART™	Kalluri, <i>et al.</i>	2006	Bangladesh	N=304	Laboratory	Lab technician	83% (75-90)	88% (82-93)

<b>IP Prototype</b>	Kalluri, <i>et al.</i>	2006	Bangladesh	N=304	Laboratory	Field technician	93% (87-97)	67% (60-74)
<b>IP Prototype</b>	Kalluri, <i>et al.</i>	2006	Bangladesh	N=304	Laboratory	Lab technician	94% (88-98)	76% (70-82)
<b>IP Prototype</b>	Wang, <i>et al.</i> [47]	2006	Mozambique	N=391	Cholera treatment center	Lab technician	95% (91-99)	89% (86-93)
<b>Crystal VC®</b>	Harris, <i>et al.</i> [48]	2009	Guinea-Bissau	N=101	Hospital ward	Study authors	97% (NS)	71-76% (NS)
<b>Crystal VC®</b>	Mukherjee, <i>et al.</i> [49]	2010	India	N=212	Laboratory	Lab technician	91.7% (NS)	72.9% (NS)
<b>Crystal VC®</b>	Ley, <i>et al.</i> [50]	2012	Zanzibar	N=622*	Cholera treatment camps	Lab technician	93.1% (88.7-96.2)	49.2% (44.3-54.1)
<b>Crystal VC®</b>	Ley, <i>et al.</i>	2012	Zanzibar	N=67**	Cholera treatment camps	Lab technician	87.5% (73.2-95.8)	74.1% (53.7-88.9)
<b>Crystal VC®</b>	Ley, <i>et al.</i>	2012	Zanzibar	N=67**	Cholera treatment camps	Health workers	90% (76.3-97.2)	55.6% (35.3-74.5)
<b>Crystal VC®</b>	Page, <i>et al.</i> [51]	2012	DRC	N=256	Cholera treatment center	Lab technician	92.2% (86.8-95.9)	70.6% (60.7-79.2)
<b>Crystal VC®</b>	Page, <i>et al.</i>	2012	DRC	N=256	Cholera treatment center	Clinician	92.2% (87.6-96.4)	60.4% (50.2-70)
<b>Crystal VC®</b>	Boncy, <i>et al.</i> [52]	2013	Haiti	N=644	Laboratory	Lab technician	95% (NS)	80% (NS)
<b>Crystal VC®</b>	George, <i>et al.</i> [53]	2014	Bangladesh	N=125	Hospital ward	Study personnel	65.6% (52.7-77.1)	91.8% (81.9-97.3)

**Table 3. Measures of test performance for the three cholera rapid diagnostic tests included in the review**

*NS Not Stated*

*\*Overall measures*

*\*\*Subset compared to RDT performed by health workers*

### 3.2 Dengue

Twenty-one articles on dengue RDTs were selected for inclusion in the study. Study sites were concentrated in Latin America and the Caribbean (Mexico n=2, Martinique n=1, French Guiana n=1, Venezuela n=1, Brazil n=1) and Asia (Vietnam n=4, Bangladesh n=2, Cambodia n=2, Singapore n=2, Thailand n=1, Sri Lanka n=1, Laos n=1 and Malaysia n=1). In addition, one study (Pal *et al.* 2015) was a multi-site study located in both the Americas (Peru, Venezuela, USA) and Asia (Cambodia). A total of 15 dengue RDTs were evaluated across the included studies (Tables 4, 5), however only 9 appear to be commercially available at the time of writing (April 2016). Furthermore, one of these 9 commercially available dengue RDTs (Dengucheck™)

has been updated so that the current version is different than what was evaluated in the included studies.

Thus, in an effort to provide the most pertinent information, only the 8 RDTs evaluated in the included studies that are currently commercially available in the same format are discussed. These 8 RDTs are designed to detect dengue virus serotypes 1-4 through single detection of the NS1 antigen (n=2) or dengue-specific intestinal IgA antibody (n=1); or combined detection of dengue-specific IgG/IgM antibodies (n=4) or both NS1 antigen and IgG/IgM antibodies (n=1). The NS1 Ag STRIP was the RDT most often studied, included in 9 studies, followed by the BIOLINE Dengue Duo in 8 studies. Sensitivities and specificities reported for the 8 RDTs across the 21 studies are shown in Tables 6-8.

	RDT	RDT Format	Detecting	Manufacturer	Sample	Read Time	Storage	Cost per Test (USD)
Single test	NS1 Ag STRIP	Wick style strip	NS1 Ag	BioRad (USA)	S/P	15 minutes	2-8°C	NS
	PanBio Early Rapid Kit	Lateral flow cassette	NS1 Ag	Alere (Australia)	S/P/WB	15-20 minutes	1-30°C	NS
	ASSURE® IgA	Reverse flow wick style assay	IgA	MP Biomedicals (USA)	S/P/WB	20 minutes	2-28°C	NS
Combo test	Denguecheck™*	Lateral flow	IgM	Tulip Group (India)	S/P/WB	15 minutes	4-30°C	NS
	BIOLINE Dengue IgG/IgM	Lateral flow	IgG/IgM	Standard Diagnostics, Inc. (Korea)	S/P/WB	15-20 minutes	1-30°C	NS
	IMMUNOQuick®	Wick style cassette	IgG/IgM	Biosynex (France)	S/P/WB	10 minutes	2-30°C	NS
	PanBio Duo Cassette	Lateral flow dual cassette	IgG/IgM	Alere (Australia)	S/P/WB	15 minutes	2-30°C	NS
	Core™ Dengue	Lateral flow	IgG/IgM	Core Diagnostics (UK)	S/P/WB	15 minutes	4-30°C	NS
	BIOLINE Dengue Duo	Lateral flow dual cassette	NS1 Ag, IgG/IgM	Standard Diagnostics, Inc. (Korea)	S/P/WB	15-20 minutes	1-30°C	NS

**Table 4. Characteristics of commercially available dengue RDTs included in the review.**

**S=serum; P=plasma; WB=whole blood**

**NS not stated**

**\*Currently marketed as Denguecheck™ Combo, which tests for both NS1 Ag and IgG/IgM antibodies and therefore excluded from analysis.**

	RDT	RDT Format	Detecting	Manufacturer
<b>Single Test</b>	Smartcheck	Lateral flow	IgM	GlobalEmed (USA)
	VScan	Lateral flow	IgM	Minerva Biotechnology (USA)
<b>Combo Test</b>	Dengue IgG/IgM Rapid Test Device	Lateral flow	IgG/IgM	Abon Biopharm (China)
	Dengue Fever IgG/IgM Combo	Strip	IgG/IgM	Merlin Labs (USA)
	Dengue Fever IgG/IgM Combo	Lateral flow	IgG/IgM	Teco Diagnostics (USA)
	Diazyme Combo	Lateral flow	IgG/IgM	Diazyme Laboratories (USA)

**Table 5. Characteristics of dengue RDTs not commercially available or for which manufacturer website does not exist and therefore excluded from analysis. Information regarding sample, read time, storage and cost were unavailable.**

RDT	Detecting	Author	Year	Country	Sample Size	RDT Performance Location	Specimen Timing (days pso)	Sensitivity (95% CIs)	Specificity (95% CIs)
<b>NS1 Ag STRIP</b>	NS1 Ag	Dussart, <i>et al.</i> [54]	2008	French Guiana	N=320	Laboratory	1->7	76.1% (70.6-81.0)	100% (92.6-100)
	NS1 Ag	Hang, <i>et al.</i> [55]	2009	Vietnam	N=138	Hospital	1-6	72.8% (64.1-80.3)	100% (91.6-100)
	NS1 Ag	Ramirez, <i>et al.</i> [56]	2009	Venezuela	N=147	Laboratory	2-6	67.8% (57.4-76.7)	94.4% (80.9-99.4)
	NS1 Ag	Najioullah, <i>et al.</i> [57]	2010	Martinique	N=537	Laboratory	0-8	49.4% (43.2-55.6)	100%
	NS1 Ag	Queiroz Lima, <i>et al.</i> [58]	2010	Brazil	N=450	Laboratory	1-9	89.60%	99.10%
	NS1 Ag	Tricou, <i>et al.</i> [59]	2010	Vietnam	N=292	Hospital	0-7	61.6% (55.2-67.8)	100% (93.8-100)
	NS1 Ag	Chaterji, <i>et al.</i> [60]	2011	Singapore	N=354	Laboratory	0-3	77.3% (69.8-83.6)	100% (98.5-100)
	NS1 Ag	Blacksell, <i>et al.</i> [61]	2011	Sri Lanka	N=259	Laboratory	Median: 5	58.6% (48.2-68.4)	98.8% (95.6-99.9)
	NS1 Ag	Tuan, <i>et al.</i> [62]	2015	Vietnam	N=5707	Hospital	0-3	70.4% (68.2-72.6)	99.2% (98.9-99.5)
<b>PanBio Kit</b>	NS1 Ag	Blacksell, <i>et al.</i>	2011	Sri Lanka	N=259	Laboratory	Median: 5	58.6% (48.2-68.4)	92.5% (87.3-96.1)
	NS1 Ag	Fry, <i>et al.</i> [63]	2011	Vietnam	N=298	Laboratory	1-4	69.2% (62.8-75.6)	96% (92.2-99.8)
<b>ASSURE® IgA</b>	IgA	Ahmed, <i>et al.</i> [38]	2010	Bangladesh	N=204	Laboratory	A&C	A: 86% C: 99.4%	A: 99.2% C: 92%



IgA	Tan, <i>et al.</i> [64]	2011	Samples from Bangladesh	N=914	Laboratory	Acute	86.7% (81.7-90.8)	86.05% (83.2-88.6)
IgA	Hernandez, <i>et al.</i> [65]	2012	Mexico	N=225	Laboratory	0-5	61% (53.7-68.3)	85.1% (74.9-95.2)

**Table 6. Measures of test performance for dengue RDTs detecting NS1 Ag or IgA antibody A&C acute and convalescent NS not stated**

RDT	Detecting	Author	Year	Country	Sample Size	RDT Performance Location	Sample Timing (days pso)	Sensitivity (95% CIs)	Specificity (95% CIs)
<b>PanBio Duo Cassete</b>	IgG/IgM	Blacksell, <i>et al.</i> [66]	2006	Thailand	N=491	Laboratory	A&C	65.3% (59.9-70.5)	97.6% (93.9-99.3)
	IgG/IgM	Blacksell, <i>et al.</i> [67]	2007	Laos	N=151	Hospital	A&C	21.7% (15.2-28.2)	96.3% (93.4-99.3)
	IgG/IgM	Blacksell, <i>et al.</i>	2011	Sri Lanka	N=259	Laboratory	Median: 5	70.7% (60.7-79.4)	80% (73-85.9)
<b>Core™</b>	IgG/IgM	Pal, <i>et al.</i> [68]	2015	Multiple	N=1108	Clinic, hospital	4-14	92.1% (87.8-95.2)	62.2% (54.5-69.5)
	IgG/IgM	Blacksell, <i>et al.</i>	2006	Thailand	N=491	Laboratory	A&C	22.9% (18.3-27.6)	98.8% (95.7-99.9)
	IgG/IgM	Blacksell, <i>et al.</i>	2007	Laos	N=151	Hospital	A&C	13% (7.7-18.4)	98.8% (97-100)
<b>BIOLINE</b>	IgG/IgM	Blacksell, <i>et al.</i>	2006	Thailand	N=491	Laboratory	A&C	21.8% (17.4-26.7)	98.8% (95.7-99.9)
	IgG/IgM	Blacksell, <i>et al.</i>	2007	Laos	N=151	Hospital	A&C	10.2% (5.3-15)	96.3% (93.4-99.4)
<b>IMMUNOQuick®</b>	IgG/IgM	Blacksell, <i>et al.</i>	2011	Sri Lanka	N=259	Laboratory	Median: 5	79.8% (70.5-87.2)	46.3% (38.3-54.3)

**Table 7. Measures of test performance for dengue RDTs detecting IgG/IgM A&C acute and convalescent**

RDT	Detecting	Author	Year	Country	Sample Size	RDT Performance Location	Sample Timing (days pso)	Sensitivity* (95% CIs)	Specificity* (95% CIs)
<b>BIOLINE Dengue Duo</b>	NS1 Ag, IgG/IgM	Wang, <i>et al.</i> [69]	2010	Malaysia	N=265	Hospital	Acute	88.7% (84.0-93.3)	98.8% (96.3-100)
	NS1 Ag, IgG/IgM	Tricou, <i>et al.</i>	2010	Vietnam	N=292	Hospital	0-7	83.7% (78.4-88.1)	97.9% (88.7-99.9)
	NS1 Ag, IgG/IgM	Blacksell, <i>et al.</i> **	2011	Sri Lanka	N=259	Laboratory	Median: 5	92.9% (83.9-97.1)	88.8% (82.8-93.2)
	NS1 Ag, IgG/IgM	Andries, <i>et al.</i> [70]	2012	Cambodia	N=157	Hospitals	0-7	85.7% (78.4-91.3)	83.9% (66.3-94.5)
	NS1 Ag, IgG/IgM	Sanchez-Vargas, <i>et al.</i> [71]	2013	Mexico	N=397	Health centers, hospital	A&C	90.7% (87.2-94.1)	89.7% (82.7-96.6)
	NS1 Ag, IgG/IgM	Gan, <i>et al.</i> [72]	2014	Singapore	N=244	Hospital	1-14	93.9% (88.8-96.8)	92% (81.2-96.9)
	NS1 Ag, IgG/IgM	Pal, <i>et al.</i>	2015	Multiple	N=1108	Clinic, hospital	0-14	87.3% (84.1-90.1)	86.8% (83.9-89.3)
	NS1 Ag, IgG/IgM	Carter, <i>et al.</i> [73]	2015	Cambodia	N=337	Hospital	Mean: 4.4	57.8% (45.5-69.4)	85.3% (80.3-89.5)

**Table 8. Measures of test performance for dengue RDTs detecting NS1 Ag and IgG/IgM A&C acute and convalescent**  
*\*Numbers reported here are combined measures, i.e. any one test as positive*  
*\*\*NS1 Ag and IgM measures only*

### 3.2.1 IgG/IgM Detection RDTs

DENV-specific IgG and IgM antibodies are produced by the host in response to DENV infection. As previously discussed, in primary DENV infection, IgM antibodies begin to appear around days 3-5 post symptom onset, peak around two weeks and begin to decline to undetectable levels for the next 2-3 months. In contrast, IgG antibodies emerge around day 14 post symptom onset and may persist for life. In secondary DENV infection, IgG levels remain high, while IgM levels are much lower than in primary DENV infection [35]. RDTs designed to detect these antibodies are therefore intended to distinguish between primary and secondary DENV infection [35].

**PanBio Duo Cassette.** The PanBio Duo Cassette is a lateral flow immunochromatographic test. The reported sensitivities ranged widely from 21.7% to 92.1%, although no difference was reported among DENV serotypes [68]. In separate studies, Blacksell *et al.*, 2007, 2011 demonstrated that the PanBio Duo Cassette could not distinguish with high accuracy between primary and secondary DENV infection. Reported specificities were higher, ranging from 62.2% to 97.6%.

**Core™ and BIOLINE.** The Core™ and BIOLINE IgG/IgM RDTs are lateral flow immunochromatographic tests. Both tests demonstrated poor sensitivity with the Core™ detecting 13% and 22.9% of DENV infections in the two studies in which it was evaluated and BIOLINE detecting 10.2% and 21.8% of DENV infections. Specificities for these tests were

much higher. Core™ specificity was observed at 98.8% in both studies, while the BIOLINE was observed at 96.3%.

**IMMUNOQuick®.** IMMUNOQuick® is a wick style cassette. The observed sensitivity was 79.8% and the specificity was much lower at 46.3%. Blacksell et al., 2011 also reported IMMUNOQuick® as having the highest cross-reactivity with non-dengue tropical illnesses out of the six RDTs included in their evaluation.

### 3.2.2 NS1 Ag Detection RDTs

NS1 is a highly conserved glycoprotein that is found in the sera of dengue-infected patients during the acute phase of the disease. NS1 levels are highest in the first few days post symptom onset, but persist up until about day 9 [69]. Highly sensitive and specific RDTs detecting the NS1 Ag thus present a promising opportunity for early dengue diagnosis.

**NS1 Ag Strip.** The NS1 Ag STRIP, the first test designed for NS1 Ag detection, is a wick-style, lateral flow immunochromatographic test. The strip is placed vertically in a test tube containing the sample and results are read after 15 minutes of incubation [74], making it an easy to use test [54, 58]. Reported sensitivities for the NS1 Ag STRIP ranged widely from 49.4% to 89.6% across the 9 studies in which it was evaluated. While some studies reported equal sensitivities across DENV serotypes using the NS1 Ag STRIP (Dussart *et al.*, 2008, Najjoullah *et al.*, 2010), several studies noted significant differences based on DENV serotype. Tuan, *et al.*, 2015 observed decreased sensitivity for DENV-2 (46.4%) compared to DENV-1, -3 and -4 (75-85%) [62]. Similarly, Hang, *et al.*, 2009 reported significantly lower detection of DENV-2 (55%) compared to DENV-1 (98%), as did Ramirez *et al.*, 2009 [55, 56]. Many studies also observed better sensitivity with the NS1 Ag STRIP in detecting primary versus secondary DENV infection

[37, 54, 55, 57]. Specificities for the NS1 Ag STRIP were better, ranging from 94.4% to 100% and did not differ significantly among serotypes.

**PanBio Early Rapid Kit.** The PanBio Early Rapid Kit is an in vitro, immunochromatographic test in a lateral flow cassette format. The sensitivities reported with this test ranged from 58.6% to 69.2%. Fry *et al.*, 2011 observed a peak in the Early Rapid Kit's sensitivity at 75% on days 2 and 3 post symptom onset [63]. Reported specificities were higher, ranging from 92.5% to 96%, although Blacksell *et al.*, 2011 reported some cross-reactivity with chikungunya, scrub typhus, Q fever and leptospirosis [61]. There was high inter-user agreement observed with the Early Rapid Kit [37, 61].

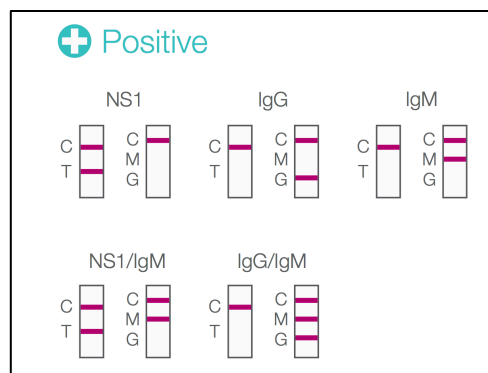
### 3.2.3 IgA Detection RDTs

**ASSURE® IgA.** The ASSURE® IgA test is a reverse flow immunochromatographic test and the first test developed to detect the dengue-specific IgA antibody. The use of IgA as a method for early dengue detection has only recently been explored, but studies suggest IgA may appear before or simultaneously with IgM antibodies during DENV infection [38, 64]. In acute blood samples, the sensitivity of the ASSURE® IgA test ranged from 61% to 86.7% across the three studies in which it was evaluated, but increased to 99.4% in convalescent samples as measured by Ahmed, *et al.*, 2010. Both Ahmed *et al.* 2010 and Hernandez *et al.* 2011 observed improved sensitivity of the test in secondary DENV infections (92.6% and 82.1%) compared to primary infections (72.4% and 45.4%). Specificities ranged from 85.1% to 99.2% in acute samples, and were reported at 92% in convalescent samples.

### 3.2.4 Combined NS1 and IgG/IgM Detection RDT

**BIOLINE Dengue Duo.** In addition to their BIOLINE IgG/IgM RDT, Standard Diagnostics also manufactures the BIOLINE Dengue Duo. This combination test is an

immuno-chromatographic duo cassette assay designed to detect both NS1 antigen and IgG/IgM antibodies in an attempt to broaden the window of DENV detection throughout the clinical disease phases. Both the NS1 Ag and IgG/IgM cassettes have separate but identical test strips and all possible positive results are shown in Figure 4. A test is considered positive for DENV if either cassette reads positive. However, as discussed earlier, the timing of the test specimen is important in determining which test cassette to use, since NS1 Ag is generally detectable until day 5 or 6 post symptom onset, whereas IgG/IgM appears in increasing levels around days 3-5 post symptom onset. Across the 8 studies in which it was evaluated, the overall sensitivities of the test ranged from 57.8% to 93.9%. Sensitivities were generally reported as similar between primary and secondary DENV infection [71, 72] or higher in primary DENV infection [68, 69]. No statistically significant differences using the BIOLINE Dengue Duo were noted by DENV serotype [68-70, 72]. Unsurprisingly, nearly all studies reported improved sensitivity by combining the NS1 Ag test with the IgG/IgM test, versus using them separately. However, Carter *et al.*, 2015 observed a lower sensitivity (57.8%) than previous studies potentially due to high rates of co-infections, including Japanese encephalitis, rickettsioses, and a variety of bacterial infections, in the pediatric study population. Observed overall specificities ranged from 83.9% to 98.8%.



**Figure 5. Potential positive test results using BIOLINE Dengue Duo RDT [75]**

### 3.3 Ebola virus disease

In total, only four articles on Ebola virus disease RDTs were included in the study, including two evaluations of RDTs in development, one review and modeling scenario and one special report. Currently, there are no commercially available RDTs for the detection of Ebola virus, although the U.S. FDA issued an Emergency Use Authorization in July 2015 to allow the use of the OraQuick® Ebola Rapid Antigen Test during the ongoing outbreak in West Africa [76]. No evaluations of this test were found in this study's literature search. Several RDTs are also under development, including the ReEBOV Antigen Rapid Test kit (Corgenix Inc, USA), which was recently approved for emergency use by the WHO, and an in-house RDT developed by the Defense Science and Technology Laboratory (DSTL) in the UK. In light of the most recent Ebola virus disease outbreak in West Africa, the ReEBOV Test kit was chosen for evaluation by the Foundation for Innovative New Diagnostics, a nonprofit dedicated to developing diagnostics for diseases of poverty.

RDT	RDT Format	Detecting	Manufacturer	Sample	Read Time	Storage	Cost (USD)
ReEBOV Antigen Rapid Test kit	Dipstick	Ebola virus VP40 matrix protein antigens	Corgenix Inc. (USA)	P/WB	5-15 minutes	Must be kept at 4°C	Not yet commercially available
Defense Science and Technology Lab (DSTL) RDT	Lateral flow	Ebola virus antigens	DSTL (UK)	Capillary blood	20 min	NS	Not yet commercially available

*Table 9. Characteristics of Ebola virus RDTs included in the review.  
NS not stated*

RDT	Author	Year	Country	Sample Size	RDT Performance Location	RDT performed by	Sensitivity (95% CIs)	Specificity (95% CIs)
<b>ReEBOV Antigen Rapid Test kit</b>	Broadhurst, <i>et al.</i>	2015	Sierra Leone		Ebola treatment/holding centers	MOH phlebotomists	100% (87.7-100)	92.2% (83.8-97.1)
<b>Defense Science and Technology Lab (DSTL) RDT</b>	Walker, <i>et al.</i>	2015	Sierra Leone		Ebola holding centers	Trained clinical staff	100% (78.2-100)	92%-97% (85.8-99.1)*

*Table 10. Measures of test performance for the two Ebola virus rapid diagnostic tests included in the review*

**ReEBOV Antigen Rapid Test kit.** The ReEBOV Antigen Rapid Test kit is a dipstick immunoassay designed to detect Ebola virus protein antigens in whole blood or plasma. While it does not require external equipment or advanced user training, it does require a cold chain [77]. In a study performed in Sierra Leone during the most recent outbreak, the ReEBOV Test had a sensitivity of 100% and specificity of 92.2% against an RT-PCR reference in both POC and laboratory-based settings [77]. PPV scores were 82.4% and 71.4% in POC and the laboratory, respectively and NPV scores were 100% in both settings.

**DSTL RDT.** The RDT under development at the DSTL is a lateral flow assay used to detect Ebola virus antigens in capillary blood. It requires minimal training and results are read after 20 minutes [78]. The DSTL RDT was also tested during the most recent Ebola virus disease outbreak in Sierra Leone against an RT-PCR reference standard. Sensitivity was observed at 100% and specificities ranged from 92% to 97%, depending on variations in interpretations [78].

## Chapter 4: Discussion and Conclusion

### 4.1 Discussion

**Recommendations.** Cholera, dengue and Ebola are epidemic prone diseases with the potential to cause high morbidity and mortality. This potential increases when epidemics of these diseases occur during CEs, demanding diagnostic tests capable of detecting these diseases early and in resource-constrained settings to prevent widespread transmission. In order to achieve this, RDTs for use in CEs should ideally comply with the ASSURED criteria. There is extensive research being done on the numerous commercially available dengue RDTs to identify those that best meet these criteria, while research has stagnated on cholera RDTs and is just beginning for Ebola virus. Currently, there are several RDTs for these diseases that are commercially available or fast-tracked for rapid development that show promise in meeting the ASSURED criteria and becoming useful tools for disease surveillance and epidemic prevention during CEs: Crystal VC® for cholera, BIOLINE Dengue Duo for dengue and the ReEBOV Antigen Rapid Test kit for Ebola virus. The following assesses the ASSURED criteria for one cholera, one dengue and one Ebola RDT.

#### **Crystal VC®**

**Affordable.** According to the manufacturer website, 50 Crystal VC® tests can be purchased for 8500 rupees [44]. At the current USD exchange rate (April 16, 2016), this calculates to \$2.55/test. However, George *et al.*, 2014 reported that 10 tests can be purchased for \$19 USD (\$1.90/test) [53].



**Sensitive.** The ease with which cholera spreads and its potential severity require a highly sensitive diagnostic test [48]. Sensitivities for Crystal VC® were generally quite good, ranging from 87.5%–97%, with the exception of one study (George *et al.*, 2014) where sensitivity was much lower at 65.6%. The study authors are uncertain why they observed a low sensitivity, since all patients in the study presented with moderate to severe dehydration and likely had high bacterial loads, which should make the detection of *Vibrio cholerae* easier [53].

**Specific.** Specificity is equally important in cholera surveillance, given the stigma surrounding a cholera diagnosis, to prevent unnecessary panic and use of resources [48], especially in settings where resources are already scarce. Unfortunately, specificities for Crystal VC® were generally lower than the sensitivities and ranged from 49.2% – 91.8%. Ley *et al.*, 2012 observed a specificity of 49.2% among patients presenting to cholera treatment centers during a cholera epidemic in Zanzibar. Field workers performed the test outdoors in daylight after receiving training and a practice session and were visited frequently to ensure proper test use. In contrast, a subset (n=67) of total samples (n=622) was tested in the lab and the reported specificity was 74.1%. The authors concluded that the most likely explanation was the over-interpretation by field workers of faint test lines visible in daylight but not indoors. Similarly, during an epidemic in DRC, Page *et al.*, 2012 observed a specificity of 60.4% among tests performed at cholera treatment centers by nurses and doctors untrained in use of the test. Although the reported specificity among tests performed by lab technicians was higher (70.6%), this difference was not statistically significant.

**User-friendly.** Crystal VC® requires very few steps to complete and it was considered an easy to use test [48, 52]. However, both Ley *et al.*, 2012 and Page *et al.*, 2012 demonstrated that user training may be important, especially for non-laboratory personnel, to achieve the most accurate

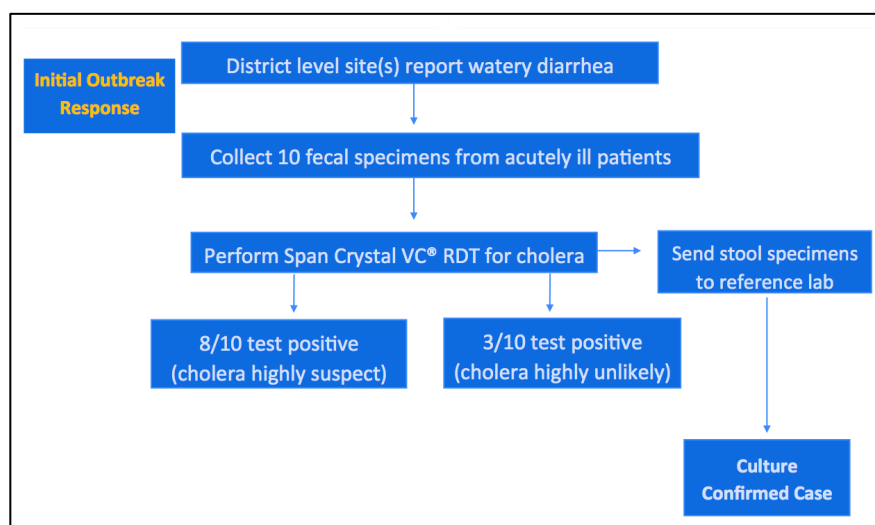
results. In addition, Mukherjee *et al.*, 2010 recommended a short training and demonstration of Crystal VC® after experiencing faint test lines subject to individual interpretation.

**Robust and rapid.** Crystal VC® can be stored between 4° and 30 °C and under high humidity, since test strips are packaged in waterproof pouches [50]. All studies reported read times under 20 minutes.

**Equipment free.** All necessary equipment is included in the test kit.

**Deliverable to those who need them.** Crystal VC® is a portable and handheld test.

In sum, Crystal VC® is a sensitive RDT with moderate specificity. Although it requires few steps to perform, user training is important to ensure proper interpretation and inform users of potential interfering factors, such as lighting. Currently, Crystal VC® is best used as a screening tool alongside a defined testing algorithm (Figure 5) during suspected epidemics in low-resource settings, especially during the start of an epidemic [47-49, 53]. When cases of watery diarrhea are reported and cholera is suspected, samples from 10 different patients should be tested with Crystal VC®. If 8 or more samples test positive for cholera, it is highly likely that a cholera epidemic is underway and action can be taken to halt it. In contrast, if 3 or fewer samples test positive for cholera, it is highly unlikely that a cholera epidemic is occurring.



*Figure 6. Testing algorithm for cholera RDTs for epidemic surveillance during CEs. Courtesy of Susan Cookson and Michelle Parsons, CDC.*

### **BIOLINE Dengue Duo**

**Affordable.** Despite being commercially available, the manufacturer website does not state costs for the BIOLINE Dengue Duo test. Elsewhere it was reported to cost 400 rupees per test [79].

Using the current exchange rate (April 16, 2016), this amounts to 6.00 USD per test, which could be prohibitively expensive in many low-resource settings. The BIOLINE Dengue DUO is sold in kits containing 10 or 25 tests.

**Sensitive.** Definitive dengue diagnosis is not possible without serological testing, since there is significant overlap in clinical symptoms among dengue and other infections prevalent in the same regions, such as malaria, leptospirosis and typhoid fever. Overall reported sensitivities of the BIOLINE Dengue Duo for all 4 DENV serotypes were generally high, ranging from 83.7%–93.9%, with the exception of Carter *et al.*, 2015, who reported a sensitivity of 57.8%, partially due to high rates of viral and bacterial coinfection among the study population. This is important to consider, as coinfection with other infectious diseases may be present in vulnerable populations in low resource contexts. The combination of NS1 Ag and IgG/IgM detection capabilities allows for an increased window of detection post symptom onset, detecting both acute dengue infection and more severe disease as well.

**Specific.** A dengue RDT with high specificity is needed to exclude those without DENV infection. This is important in order to gauge the size of the epidemic in question and concentrate resources on those who truly have dengue, especially when the RDT itself is expensive.

Observed overall specificities for this RDT were also high, ranging from 83.9% – 98.8%.

**User-friendly.** Both the NS1 Ag and IgG/IgM tests are each completed in less than 3 steps.

Regarding RDT training, the majority of studies (8/10) evaluating the BIOLINE Dengue Duo used trained laboratory technicians to perform the test, none of whom encountered difficulties in its use. Of the remaining 2 studies, only Andries *et al.*, 2012 reported differences in RDT performance between hospital POC and the laboratory. However, since visual interpretation of test lines can be subjective, it may be useful to provide a brief training prior to implementation.

**Robust and rapid.** The BIOLINE Dengue Duo RDT can be stored between 1 and 30 °C and all read times were reported under 20 minutes.

**Equipment free.** All necessary equipment is included in the test kit.

**Deliverable to those who need them.** BIOLINE Dengue Duo is portable and handheld.

In conclusion, using NS1 Ag and IgG/IgM assays in combination increases the capability for DENV detection across the spectrum of disease. The NS1 Ag assay may be particularly useful in early detection of DENV, which is crucial in surveillance and mobilization of resources to arrest epidemics during CEs. However, a testing algorithm like the one for cholera must be developed for dengue to guide the use of this RDT in epidemic detection during CEs.

Furthermore, the cost of the BIOLINE Dengue Duo may limit its widespread use in low resource settings. Given the high price of this RDT, testers should also be trained prior to use to avoid wasting tests due to inaccurate execution.

### **ReEBOV Antigen Rapid Test kit**

**Affordable.** The ReEBOV test is not currently commercially available.

**Sensitive.** Diagnosis of Ebola virus disease on clinical presentation alone is unreliable since many diseases with similar clinical presentations are widespread in West Africa, such as Lassa fever, Rift Valley fever and malaria. High sensitivity is therefore crucial in an RDT for Ebola

virus. Furthermore, lack of sensitivity can result in infected patients returning to the community or being sent to non-Ebola virus disease treatment centers and exposing others [80]. This test demonstrated excellent sensitivity of 100% using both fingerstick samples at POC and whole blood samples in the lab. This is important because fingerstick samples reduce the tester's risk of exposure, compared to venous samples.

**Specific.** High specificity is also desirable in an RDT for Ebola virus disease to avoid admitting non-Ebola virus disease patients to an Ebola treatment center, where they risk exposure. The ReEBOV test had a high observed specificity of 92.2% using fingerstick samples at POC and whole blood samples in the lab.

**User-friendly.** Broadhurst *et al.*, 2015 reported that “technicians responsible for routine phlebotomy” from Sierra Leone’s Ministry of Health were able to execute the test with little training. There are few steps involved, but considering the risk of exposure inherent in handling patient specimens, it is likely that training will be required prior to using the ReEBOV test, especially for non-laboratory personnel.

**Robust and rapid.** The ReEBOV test must be used at room temperature (18-30°C) and stored at 4 °C, requiring a cold chain, which could be prohibitive in some locations. Results are read within 15-25 minutes.

**Equipment free.** This RDT needs no external equipment, but additional supplies not provided in the kit are required, including a precision pipettor and deionized water. Broadhurst *et al.*, 2015 achieved their results using bottled water, although this may also be difficult to obtain during a CE. Additionally, it’s important to note that any Ebola virus RDT will also require personal protective equipment for use.

**Deliverable to those who need them.** The ReEBOV test is portable and handheld.

Although the ReEBOV Antigen Rapid Test kit demonstrated significant potential in the early detection of Ebola virus in the West Africa outbreak, these are results from one point in a single epidemic. Broadhurst *et al.*, 2015 note that the patients included in their study presented to the treatment center 1-16 days post symptom onset, with a mean of 4 days, so it is unclear how this test may perform earlier in the disease course. Additional research and in different populations is therefore essential in order to better understand the utility of this RDT and to design an appropriate testing algorithm to detect future epidemics. Furthermore, the RT-PCR reference standard assay used was also considered imperfect, potentially inflating the sensitivity and underestimating the specificity of the RDT. Finally, a cold chain could be problematic in some contexts.

**Limitations.** There are many factors to consider in creating a well-designed evaluation of an RDT, including objectives, study design, site location, population, sample size and diagnostic reference standard, among others. All of these factors can also have an effect on the evaluation of an RDT. It's important to note that the objectives of most of the studies included in the review were to determine the utility of the RDTs strictly for clinical management, rather than surveillance and/or epidemic detection. With individual clinical management as the goal, the entire study is subsequently designed with this in mind. As much as possible, any evaluation of an RDT should be conducted under all the conditions in which it is likely to be used. While none of the included studies evaluated RDTs during a CE, both Ebola studies and half of the cholera RDT studies were conducted during outbreaks, so these studies placed a greater emphasis on evaluating the RDT in a context with more urgency, characteristic of a CE. Additionally, all cholera and Ebola studies were prospective and used fresh samples, although only a few cholera studies and neither Ebola study utilized non-laboratory personnel in evaluating the RDTs.

In contrast, the dengue RDT studies reflected wide variability on study design, inclusion criteria for samples, and diagnostic reference standard used. Only three dengue RDT studies were described as taking place during outbreaks and many used archived specimens in major research laboratories. While archived specimens have advantages in terms of speed, cost and convenience, there is a risk that the quality of the specimens will have deteriorated over time if not stored properly [81]. In addition, using stored specimens with known disease status could result in inflated measures of diagnostic accuracy when samples have been collected from the sickest and healthiest patients, who may not be representative of the general population [81]. Furthermore, a limited number of dengue RDT studies employed community health workers, providers or other non-laboratory personnel in their RDT evaluations. Since these groups are most likely to be administering the RDT to the patient, it is vital that they are included in evaluations of the RDT. In sum, prospective field trials involving healthcare personnel are particularly crucial in evaluating RDTs, especially given the potential for variability involved in interpreting test results with the naked eye [68].

Studies of cholera, dengue and Ebola RDTs cited the imperfection of the gold standard diagnostic test. Without a 100% sensitive and specific gold standard diagnostic test, estimates of the sensitivity and specificity of the RDT will be inaccurate [81]. Page *et al.*, 2012, noted the lower specificity in single stool cultures for cholera diagnosis and recommended adding PCR to the diagnostic reference algorithm or utilizing statistical approaches, such as a Bayesian latent class model, to account for this imperfection during analysis. Despite WHO recommendations for a composite dengue gold standard diagnostic, comprising a combination of virus isolation, PCR, antigen detection and serology, there was wide variation in the gold standard used among the included dengue RDT studies and in the broader dengue literature. Consensus on a gold

standard for dengue diagnosis will improve the comparability of dengue RDT evaluations and provide a more accurate body of knowledge. In addition, Broadhurst *et al.*, 2015, observed a lower than expected sensitivity of the reference RT-PCR assay for Ebola, thus prohibiting an accurate interpretation of the ReEBOV RDT performance.

Finally, the choice of inclusion and exclusion criteria of the current study may have introduced additional bias. Limiting the search to the PubMed database, albeit one of the largest repositories for biomedical literature, may have excluded other potentially pertinent articles not offered by PubMed. Similarly, the search string used may not have captured all relevant articles in PubMed and limiting the search to articles in English could have excluded studies conducted by researchers from other countries.

**Gaps.** Relatively few studies evaluating cholera RDTs were retrieved, which may reflect the disproportionate attention given to cholera relative to its impact [28]. Twenty four RDTs have been developed for cholera over the years, but poor initial performance and limited field trials have left few in use today [33]. Furthermore, the included studies focused almost exclusively on Crystal VC®, despite the availability of other tests, such as SMART™ II. Although there are currently no commercially available RDTs for Ebola and very few evaluations of RDTs under development have been published, the most recent outbreak has stimulated substantial research in this area. Of note, no studies evaluating dengue RDTs included in the review were conducted in Africa, despite substantial recent increases in dengue transmission there [26] and a dengue burden higher than that of the Americas [82]. While the dearth of dengue RDT studies in Africa may be a result of the author's inclusion and exclusion criteria, it is more likely due to unrecognized dengue risk there.



## 4.2 Conclusion

Today's global environment of rapid urbanization, extreme income disparities, pervasive social inequalities and corrupt governance suggest the occurrence of CEs is likely to continue in the future. The confluence of factors present in CEs, such as societal and governmental breakdown, lack of infrastructure and susceptible populations can create a perfect storm for infectious disease epidemics to occur. Affordable, accurate and robust RDTs capable of performing well in austere settings are crucial to assisting response teams in detecting epidemics early and provoking subsequent resource mobilization and intervention to prevent widespread morbidity and mortality. The most recent outbreak of Ebola in West Africa exposed this necessity particularly harshly. For cholera, dengue and Ebola, three epidemic-prone diseases of global concern, there are promising RDTs currently on the market or in development. However, even the best RDT choices available for these diseases are still limited by cost, performance variability and additional equipment requirements. Furthermore, research on RDTs for these diseases is marred by inconsistent study design and paucity of studies, especially those conducted under field conditions, making definitive conclusions on their performance and feasibility in CEs difficult. In the future, more research and development on cholera, dengue and Ebola RDTs, guided by standardized methods, will play a significant role in preventing the devastating effects of outbreaks of these diseases during CEs.

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## Appendix A

**Full list of diseases evaluated by Delphi group for inclusion in review based on WHO EWARN criteria. Disease must meet at least criteria A, B, C & E for inclusion.**

Syndromes	WHO EWARN Criteria				
	A	B	C	D	E
<b>Acute Jaundice Syndrome [15]</b>	<b>Epidemic Potential</b>	<b>Ability to cause severe morbidity or death</b>	<b>International surveillance requirements</b>	<b>Availability of prevention and control measures</b>	<b>Availability of case definitions and lab tests</b>
<b>Hepatitis A</b>	X	X		X	X
<b>Hepatitis B</b>	X			X	X
<b>Hepatitis C</b>				X	X
<b>Hepatitis E</b>	X	X	X	X	X
<b>Leptospirosis</b>	X	X		X	X
<b>Yellow fever</b>	X	X	X	X	X
<b>Acute Diarrheal Disease [30]</b>					
<b>Campylobacter</b>				X	X
<b>Cholera</b>	X	X	X	X	X
<b>Cryptosporidium</b>	X	X			
<b>Giardiasis</b>	X			X	X
<b>Norovirus</b>	X			X	X
<b>Rotavirus</b>	X			X	X
<b>Salmonellosis</b>	X			X	X
<b>EHEC/Shigellosis</b>	X	X	X	X	X

<b>Typhoid</b>	X	X	X	X	X
<b>Acute Hemorrhagic Fever [83]</b>					
<b>Crimean Congo H.F.</b>	X	X		X	X
<b>Dengue fever</b>	X	X		X	X
<b>Ebola</b>	X	X	X	X	X
<b>Hantavirus</b>		X			X
<b>Kyasanur Forest disease virus</b>		X		X	X
<b>Lassa fever</b>	X	X	X	X	X
<b>Lujo disease</b>		X	X	X	
<b>Marburg virus</b>	X	X	X	X	X
<b>Omsk H.F.</b>			X	X	X
<b>New World arenaviruses (Nipah, Hendra viruses)</b>		X	X	X	X
<b>Rift Valley fever</b>	X	X	X	X	X
<b>West Nile virus</b>	X	X	X	X	X
<b>Acute Flaccid Paralysis [6]</b>					
<b>Polio</b>	X	X	X	X	X
<b>Bacterial Meningitis [30]</b>					
<b>E. coli</b>		X			X
<b>Group B streptococcus</b>		X			X
<b>Haemophilus influenzae type b</b>		X		X	X
<b>Listeria</b>	X				
<b>Neisseria meningitidis</b>	X	X	X	X	X
<b>Streptococcus pneumoniae</b>		X		X	X
<b>Staphylococcus</b>		X			X
<b>Febrile Rash Syndrome [84]</b>					

<b>Kawasaki disease</b>		X			
<b>Measles</b>	X	X	X	X	X
<b>Roseola</b>	X				
<b>Rubella</b>	X	X		X	X
<b>Scarlet Fever</b>		X			
<b>Varicella</b>	X			X	X