PDC
PARTNERSHIP FOR DENGUE CONTROL

Workshop Report

New and Innovative Approaches to Laboratory Diagnosis of Zika, Dengue and other Arboviruses

LES PENSIÈRES CENTER FOR GLOBAL HEALTH, ANNECY, FRANCE
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Table of contents

Foreword

Despite advances in diagnostics for flaviviral infections, there is a critical need for more sensitive, specific, easy-to-use and affordable tests for point of care (POC) diagnosis, multiplex virologic and serologic assays to differentiate infections with co-circulating viruses of public health significance, e.g. Dengue, Zika, yellow fever and chikungunya, and methods for viral quantification and genomic characterization. New diagnostic methods continue to be innovated, such as microfluidics, paper-based diagnostics, chip-based systems, micro- and nanofabrication technologies, and deep sequencing, among others.

The Partnership for Dengue Control (PDC) wishes to cast a wider net to facilitate and expedite the development of the most promising technologies that are urgently needed, not only for pressing research questions, but also to be rolled out in affected countries. Drawing from cutting-edge technologies that have been developed for diseases such as HIV, malaria and tuberculosis, the PDC convened a stakeholder´s workshop to identify what needs to be considered to guarantee the market uptake of imminent technological innovations for flaviviral diagnosis in the near and long-term future.

The overall objectives of the workshop were to review novel technologies, to identify which platform/test characteristics should be prioritized, how to meet regulatory requirements, and how to move from innovation to market.

This document is the report of the meeting. It includes a summary of all the presentations, comments, and strategies that resulted from the workshop.

PDC wishes to thank all the participants for contributing to such an engaging and positive experience. Special thanks go to the Bill & Melinda Gates Foundation, bioMérieux, Takeda and Sanofi Pasteur for funding the workshop.

Agenda

DAY 1 - WHERE ARE WE NOW?

1.30pm – 3pm	Session 1 Chairs: Annelies Wilder-Smith, Duane Gubler • Setting the objectives of the meeting – Annelies Wilder-Smith, PDC • What are the necessary characteristics of diagnostic assays depending on the intended use – Duane Gubler, GDAC • Review of Zika standard assays (CDC MAC, IgG and PRNT) – Aravinda de Silva for Amy Lambert, CDC • Landscape analysis of the current status of Flavivirus diagnostics (Dengue and Zika) – Maurine Murtagh, The Murtagh Group
3.30pm – 5pm	Session 2 Chairs: Francis Moussy, Amy Lambert • Learning from successful novel technologies that made it to the market – Mark Miller, BioMerieux • Demand forecasting and market considerations – Nagwa Hasanin, UNICEF • Learning from past emergency authorizations – Elliot Cowan, Partners in Diagnostics
Networking session	
6.00pm – 7.00pm	Evening Chair: May Chu • Talk: Zika and Dengue diagnostics from a strategic investor's perspective – Julie Schäfer, BARDA

DAY 2 - WHERE DO WE GO? TECHNOLOGICAL INNOVATIONS IN FLAVIVIRUS DIAGNOSTICS

8.30am – 12.30pm	Session 3: Detecting acute infection Chairs: Maurine Murtagh, Arlene Chua • Imminent technological innovations • Cutting edge innovations for the intermediate and long-term future • Discussion
8.30am – 10.00am	• Near-care molecular testing for Dengue, Zika and related pathogens – Jesse Waggoner, Emory University • Development of molecular tests for management of febrile illnesses and diagnosis of viral haemorrhagic fever – Kevin Arien, ITM • Tetracore T-COR8 diagnostic system for the detection of Dengue and other arboviruses using real-time-rtPCR at the point-of-care – Bill Nelson, Tetracore • Triplex PCR ZIKV/DENV/CHIKV assay and simple to operate real-time portable device – Craig Mosman, Kirkland Biosciences
10.30am – 12.30pm	• Molecular test to detect dengue, Zika & Chikungunya and run on the QuRapID LV platform – Emily Adams, LSTM & David Edge, BioGene • NS1 antigen capture assays for the specific detection of different Dengue virus serotypes and other arboviruses – Katharina Roeltgen, Swiss TPH • Application of a parsimonious targeted-enrichment methodology for full-genome sequencing of viral genomes directly from patient samples – October Sessions, Duke-NUS • Multiplex Diagnosis kit for Zika, Dengue, Chikungunya and Japanese Encephalitis Viruses using Printed Array Strip based on Single-stranded Tag Hybridization method – Takahiro Haruyama, AVSS • Portable, high-surety detection of Zika virus directly in mosquitoes and humans – John Carrano, Parratus • Discussion

2pm – 5.45pm	Session 4: Detecting past infections Chairs: Eva Harris, Aravinda de Silva
2pm – 3.40pm	Dx antigen discovery and imminent technological innovations • New discoveries about the molecular specificity of the human antibody response to Dengue and Zika viruses – Aravinda de Silva, UNC • NS1 blockade-of-binding ELISA distinguishes between Dengue and Zika virus antibodies – Davide Corti, Humabs BioMed SA • In-country development and evaluation of new molecular and serological methods for Zika diagnosis and surveillance and their applications – Eva Harris, University of California, Berkeley • Structure based design of novel Zika virus antigens for serodiagnosis – Prem Lakshmanane, UNC-CH, North Carolina
4.00pm – 5.00pm	Cutting Edge Innovations For The Intermediate And Long-Term Future • VIDAS Zika and Dengue: Preliminary results on an automated immunoAssay platform – Nathalie Renard, Biomerieux • VIRO-TRACK – A rapid, quantitative, and accurate dengue/zika diagnostics platform for point of care use in endemic regions – Filippo Giacomo Bosco, BluSense Diagnostics • A nanoscale plasmonic-gold platform for specific diagnosis of Zika and differentiation from other Flavivirus infections – Benjamin A. Pinsky, Stanford, CA
5.00pm – 5.45pm	Discussion

DAY 3 - HOW DO WE MAKE IT HAPPEN? FROM IDEA TO MARKET

8.30am – 10am	Session 5: Preparing for the inevitable: open technology platforms for rapid outbreak response Chairs: Bill Rodriguez, Bernadette Murgue • WHO call for open technology platforms to accelerate test development – Bernadette Murgue, WHO Blueprint • Diagnostics preparedness platform – Thomas Ullrich, Alere • Learning from influenza POCT – Julie Schäfer, BARDA • Genetic analysis tool kit for rapid outbreak response – Michael Baffi, Thermo Fisher
10.30am – 12.30pm	Session 6: Critical steps to ensure quality products Chairs: Rosanna Peeling, Piero Olliaro • Critical steps to ensure quality products – Bill Rodriguez • Lessons learnt on bringing diagnostics to the market in low and middle income countries – Cassandra Kelly, FIND • Two new WHO IVD initiatives: SAGE-IVD and EDL – Francis Moussy, WHO Blueprint • Regional regulatory harmonization working parties – Rosanna Peeling, LSHTM • Streamlining from bench-to-bedside – Piero Olliaro, WHO TDR • Panel Discussion
1.30pm – 3:30pm	Session 7: Identifying PDC's roles to support innovative diagnostics Chairs: May Chu, Julie Schäfer • Panel 1: Networks and alliances – Dhamari Naidoo, WHO; – Maria Guzman, RELDA Cuba; – Amadou Sall, Institute Pasteur Senegal; – Francois Xavier Babin, Merieux; – Annelies Wilder-Smith and In-Kyu Yoon, GDAC • Panel 2: Accelerating from ideas to market – Jennifer Fluder, USAID; – Rosanna Peeling, LSHTM; – Thomas Ullrich, Alere • Panel 3: Views from stakeholders and partners

	– Sumi Paranjape, Vulcan; – Nagwa Hasanin, UNICEF; – Julie Schäfer, BARDA • Concluding discussion: research agenda and roadmap for the next 12 months
3.30pm – 4pm	Summary and Recommendations – May Chu, Rosanna Peeling and Annelies Wilder-Smith

Executive Summary

In May 2017, the Partnership for Dengue Control (PDC) and the Mérieux Foundation, with the support of the Bill & Melinda Gates Foundation, bioMérieux, Takeda and Sanofi Pasteur, organised a workshop with key stakeholders and opinion leaders to address critical issues related to the diagnosis of Zika, Dengue and other arboviruses.

The workshop was attended by approximately 80 people from academia, industry, NGO´s, UN agencies (including WHO), investor organizations, policy makers and regulatory bodies (please refer to Appendix I for a complete list of participants). The event was driven by the critical need for more sensitive, specific and accurate diagnostic tests to support more effective surveillance and prevention and to halt the increased frequency and magnitude of epidemics and the higher incidence of severe disease outcomes caused by Zika, Dengue and other arboviral infections.

The purpose of the workshop was to identify the gaps in the diagnosis of these viral infections, to review the new technological innovations that may be available to fill those gaps and to promote and develop mechanisms for accelerating the tool development to impact pathway. Specifically, the workshop was organized around three questions:

1) **Where are we now?** - What is the current status of Zika and Dengue diagnostics?
2) **Where do we go?** - What technological innovations might be available in the near, intermediate and long-term future?
3) **How do we make it happen?** - What is needed to make these technologies available from concept to market?

Key insights that emerged from the workshop:

1) Where are we now?

- Diagnostic methods to detect DENV and ZIKV infections are still mainly laboratory-based and require further clinical evaluations;
- Few validated assays are available on the market to differentiate ZIKV from other flavivirus infections;
- Diagnostics that can be used at or near the point-of-patient care (POC) to detect and discriminate between the different flaviviral infections are needed.

2) Where do we go?

- Towards specific molecular, serological, antigen-based and combination assays to identify and differentiate co-circulating flaviviral infections at or near POC, as well as for surveillance, in the intermediate and long-term future.

3) How do we make it happen?

- Identify and address challenges: uncoordinated efforts, lack of funding mechanisms, limited access to well characterized clinical samples, lack of definition of evaluation methods and sites, lack of regulatory harmonization, market opacity, low guarantee of return on investment (ROI), slow and fragmented pathway to implementation.
- Offer solutions: establish early partnerships and promote information sharing, facilitate access to samples and standardized protocols, promote regulatory harmonization, define priorities and build diagnostic preparedness strategies.

Question 1: Where are we now?

Dengue virus (DENV 1-4), Zika virus (ZIKV) and chikungunya virus (CHIKV) are mosquito-borne viruses that co-circulate in tropical urban centers around the globe. Patients infected with DENV, ZIKV and CHIKV can display a similar set of initial clinical symptoms. As a result, clinical manifestations and relevant epidemiologic exposure are not reliable etiologic indicators of infection. Virus-specific diagnostic assays are required for the accurate diagnosis of arboviral infections.

Since DENV was first isolated in 1943 and 1944 by the groups of Susumu Hotta and Albert Sabin, a number of diagnostic assays have been developed (Figure 1).

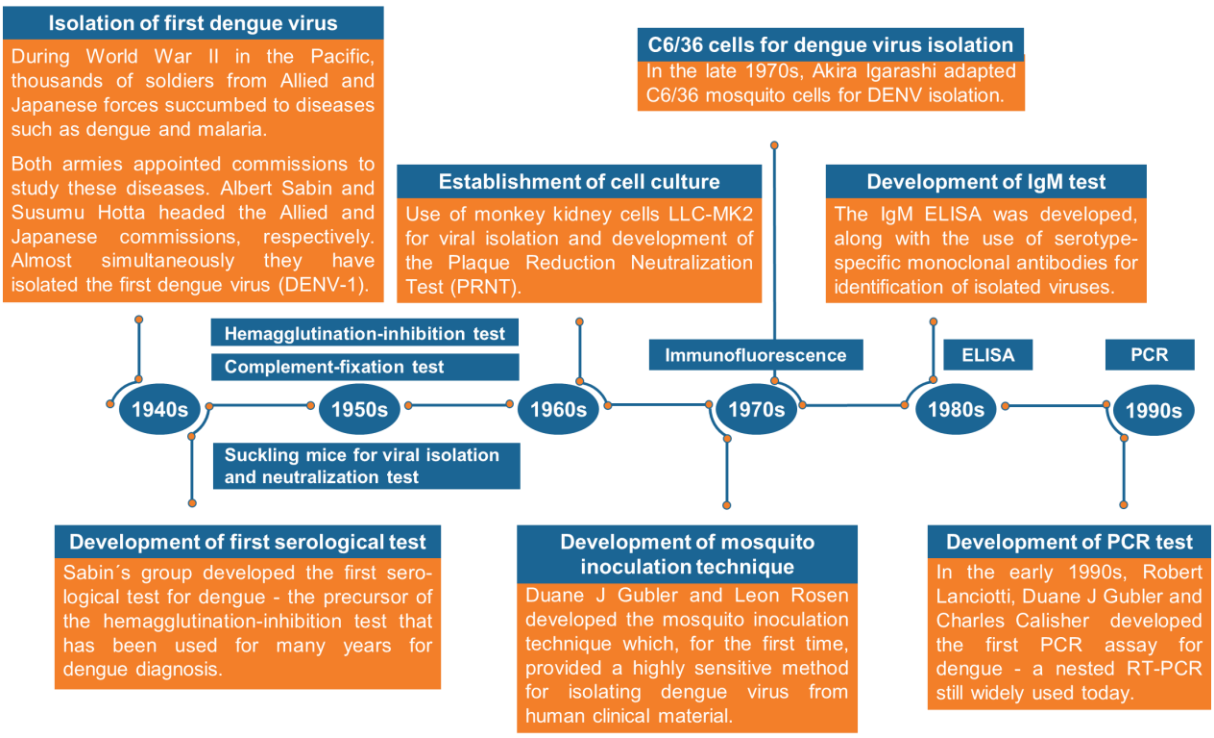

Fig 1. Historical timeline of Dengue diagnostics, 1940s-1990s (Duane Gubler´s opening remarks)

Since the 1990´s, not many innovative technologies have been developed for the detection of Dengue and other arboviral infections. For the last 25 years, standard diagnostic approaches have mainly included Polymerase Chain Reaction (PCR) and immunoglobulin M (IgM) detection. The rapid and common spread of flaviviruses, along with the emergence of more frequent and severe epidemics, has triggered the need for highly specific assays to identify and discriminate between the different viral species.

Assay selection depends both on the timing of sample collection and the purpose of testing (Fig. 2). In the acute phase of infection, assays that detect the virus directly by viral isolation, viral RNA or viral antigen perform best. In the convalescent phase of infection, serological assays that detect the host antibody response (IgM, IgG and IgA antibodies) can be used, though paired acute and convalescent samples are needed to distinguish current from past infections (Fig. 2). The dynamics of the antibody

response is different in primary and secondary infections, and this needs to be considered when designing serological diagnostic tests for flavivirus infections.

The Centers for Disease Control and Prevention (CDC) is currently using “in-house” developed reverse transcriptase PCR (RT-PCR) for the detection of acute ZIKV infection, along with IgM and Plaque Reduction Neutralization Test (PRNT) assays for the detection of current and past ZIKV infections. The use and limitations of the different assays was presented by Amy Lambert and will be discussed in the next section.

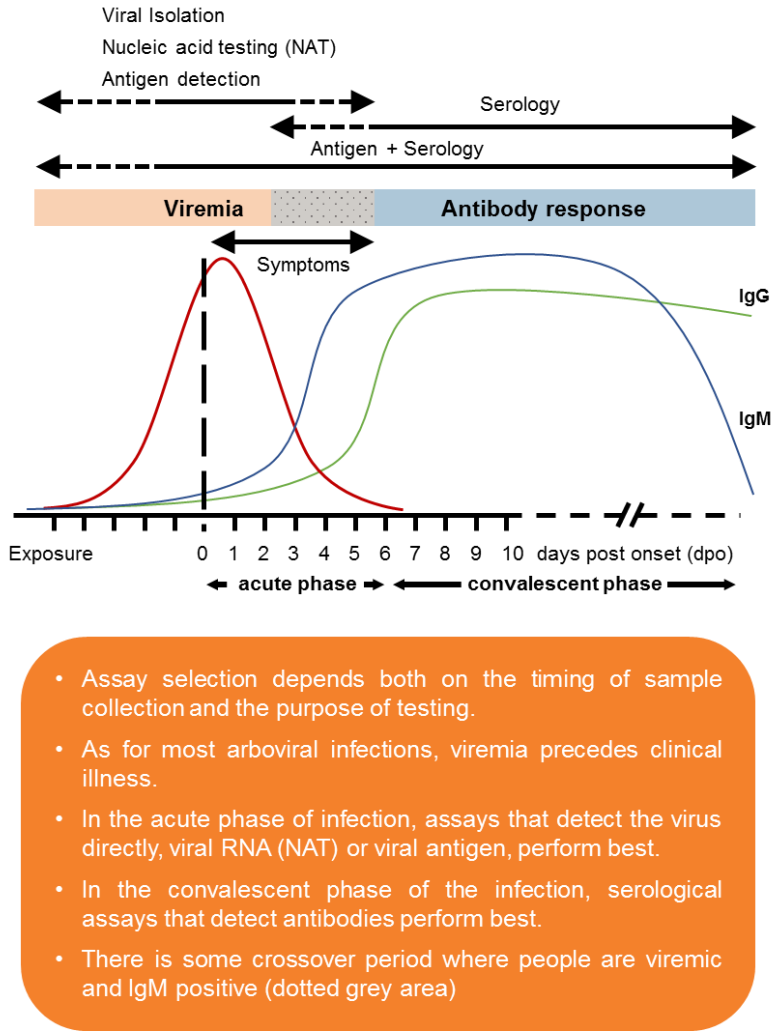

Fig 2. Infection dynamics and diagnostic features of flavivirus infections

Nucleic-acid testing (NAT) by RT-PCR is highly specific, but heterogeneity in sensitivity has been reported from different laboratories both within and outside the CDC. This wide range in sensitivities results from virus strain, prior flavivrus immunity, sample type, platform differences and intra-laboratory variation. Similar observations can be found in the literature for Dengue RT-PCR assays (Appendix III, Table 1).

In contrast, the major challenge of serological assays is the extensive cross-reactivity between antibody responses to ZIKV and other flavivirus infections. Serology is further complicated by original antigenic sin, according to which the dominant immune

response is directed towards the primary infecting serotype following a secondary infection with a heterologous serotype. As a result, specific serodiagnosis in co-endemic regions is difficult and currently limited by the capabilities of the local laboratory.
Figure 3 provides a general description of the main diagnostic tests in use for the detection of flaviviral infections, along with their respective applications, advantages and limitations. Although viral isolation and nucleic-acid testing (NAT) are very specific, their complexity and high cost makes decentralization at POC challenging, particularly in low-resource settings. Isothermal amplification NAT assays that are faster, cheaper and less complex have the potential to overcome some of the issues. These assays can be used in lower infrastructure levels at or near POC. Still, most of the available NAT assays, as well as Enzyme-Linked Immunosorbent Assays (ELISA) for antibody detection, require some level of laboratory infrastructure and diagnostic competence that might not be widely available in low-resource settings. Rapid diagnostic tests (RDTs) that detect viral antigen (NS1) and viral antibodies at the point-of-care are of use in resource-limited settings to improve access to diagnosis of acute and past flaviviral infections, but have severe limitations in accuracy.

The detailed diagnostic landscape for the detection of DENV and ZIKV infections was presented by Maurine Murtagh and is summarised in Appendix III of this report (publication in preparation by Murtagh *et al*). In summary, although RT-PCR and ELISA tests have been developed, few validated commercially-available assays of any type are available for ZIKV diagnosis. Diagnostics for DENV infection, however, are more readily available and have improved over the last decade. For example, combination RDT assays that detect both antigen (NS1) as well as IgM/IgG antibodies for DENV are applicable throughout the entire temporal spectrum of the infection and may hold promise as future POC applications. Nonetheless, the majority of the assays available for detection of DENV and ZIKV infections require sophisticated laboratory facilities. Specific limitations associated with each of the available assays are illustrated in Figure 3. Importantly, the clear majority of available assays were not adequately and independently validated using clinical specimens and consistent methods. In fact, the lack of standardized protocols and the difficulty in accessing standardized and well-characterized biobanks of clinical specimens was pointed out as a major limitation by all industry representatives who attended the workshop.

In order to respond to the emerging global threat of DENV, ZIKV and CHIKV epidemics, quality-assured diagnostics are required at all levels of care and settings. As pointed out by Mark Miller, the Chief Medical Officer of bioMérieux:

> "*Diagnostic GLOBAL biopreparedness means preparing for the next important pathogen(s), wherever they might be – from highly developed countries to remote areas in emerging countries, and everything in between.*"

Diagnostic assays that can accurately detect and distinguish co-circulating flaviviral infections and predict severe disease outcomes at or near POC are of particular need. In the next two sections of the report, we summarize newly developed technological innovations along with strategies on how to make them available into the market.

Clinical Diagnosis

Clinical + Epidemiological data

- **Application**
  Not applicable on their own.
- **Limitations**
  Viral infections such as Zika, dengue and chikungunya share a similar geographical distribution, cause non-specific symptoms, and are also often asymptomatic. As a result, clinical manifestations and relevant epidemiologic exposure are not reliable indicators of etiologic diagnosis on their own.

Assays to detect current infections

Viral isolation:

- **Application**
  Detection of active infection - interpretation of positive result: confirmatory; allows identification of viral species and DENV serotypes.
- **Limitations**
  Lab-based; requires acute sample; takes more than one week to complete; expensive; laborious and impractical in point-of-care and resource-limited settings.
- **Advantages**
  Most specific and conclusive diagnosis; only method that allows the detection and isolation of living virus.

Nucleic acid testing (NAT):

- **Application**
  Detection of active infection - interpretation of positive result: confirmatory*;
  Allows identification of viral species and dengue virus serotypes.
- **Limitations**
  Mainly lab-based; requires acute sample; complex; requires power supply; expensive; potential false positives.
- **Advantages**
  Highly accurate if done correctly; faster and less laborious than viral isolation; multiplex and near point-of-care testing possible.
- **Landscape**
  Appendix III Table 1: Dengue virus (DENV1-4) NAT assays
  Appendix III Table 2: Zika virus (ZIKV) NAT assays
  Appendix III Table 3: Multiplex (DENV, ZIKV, CHIKV) NAT assays

Antigen detection (NS1):

- **Application**
  Detection of active infection - interpretation of positive result: confirmatory.
- **Limitations**
  Less accurate than viral isolation and NAT; requires acute sample; potential false positives.
- **Advantages**
  Diagnostic window up to day 8 post-onset of DENV infection with less sensitivity; ELISA (high throughput) or RDT; can be used at or near the point-of-care; easy to perform; less expensive.
- **Landscape**
  Appendix III Table 4: DENV NS1 antigen detection assays
  (ZIKV NS1 antigen assays were not available at the time of publication of the landscape)

Assays to detect past infections

Serology:

- **Application**
  - IgM/IgG (paired serum specimens):
  Detection of active infection upon seroconversion (negative IgM/IgG in an acute specimen followed by a positive IgM/IgG in a convalescent specimen) or a ≥4-fold rise in IgG or total antibody titer - interpretation of positive result: confirmatory.
  - IgM (single serum specimen):
  Detection of recent past infection - interpretation of positive result: probable;
  Useful for surveillance.
  - IgG (single serum specimen):
  Detection of past infection. Classification of primary or secondary infections (IgG avidity, IgM/IgG ratio, titer)
  - Plaque reduction neutralization test (PRNT):
  Detection of past infection;
  Identification of viral species and infecting DENV serotypes in primary infection;
  Useful for research and vaccine studies.
- **Limitations**
  - IgM/IgG (single/paired serum specimens):
  Limited specificity due to high cross-reactivity among flaviviruses (particularly challenging in febrile patients living in or visiting co-endemic regions);
  Limited sensitivity during acute phase of infection;
  Testing single serum specimens is not confirmatory of current infection;
  Confirmatory results can only be obtained after a second visit; convalescent samples are difficult to obtain.
  Variation among laboratories;
  - Plaque reduction neutralization test (PRNT):
  Time and labor intensive with variation among laboratories;
  Not always able to resolve cross-reactivity (particularly in secondary infections).
- **Advantages**
  - IgM/IgG (single/paired serum specimens):
  Can be used as ELISA (high throughput) or as RDT at or near the point-of-care; easy to perform; affordable.
  - Plaque reduction neutralization test (PRNT):
  Higher specificity than IgM/IgG assays;
  Possible identification of infecting viral species and DENV serotypes during the convalescent phase;
  Indicates the level of protection against an infecting virus.
- **Landscape**
  Appendix III Table 5: DENV serological assays
  Appendix III Table 6: ZIKV serological assays

Assays to detect current and past infections (combination assays)

Antigen (NS1) & IgM/IgG combination assays:

- **Application**
  Detection of active infection - interpretation of positive result: confirmatory.
- **Limitations**
  Less accurate than viral isolation and NAT during acute phase.
  Variation among laboratories;
- **Advantages**
  Entire temporal spectrum following infection (applicable in acute and convalescent samples); RDT; can be used at or near the point-of-care; easy to perform; less expensive; can use whole blood.
- **Landscape**
  Appendix III Table 7: DENV NS1 & IgM/IgG assays
  (ZIKV NS1 antigen & IgM/IgG combination assays were not available at the time of publication of the landscape)

*not always true (RNA copy number is not an accurate measure of infectious virus – detectable RNA persists for longer periods than infectious virus)

Fig 3. Diagnostic methods to detect flaviviral infections

Question 2: Where do we go?

Different companies and research groups were invited to present a new generation of diagnostic technologies that will be available in the near- and long-term future to detect 1) current flaviviral infections; 2) past flaviviral infections and 3) both current and past flaviviral infections.

Oral presentations were selected from an open call for abstracts to reflect innovative solutions within each of the above categories (Appendix II includes a list of selected abstracts). In this section, we present each of the technologies in detail and discuss their potential to change the paradigm of flaviviral diagnosis.

1. Technological innovations to detect **current infections**

1.1 Nucleic acid testing (NAT) assays

Various companies and research groups are developing random-access, “sample in, answer out” multiplex real-time RT-PCR assays for the simultaneous detection of ZIKV, DENV, and CHIKV infections from a single specimen (Table 1). Many of the technologies resulted from academia-industry collaborations, through which academia-developed PCR amplification methods were adapted to existing industry platforms. This strategy illustrates the utility of “open-platforms” that can easily incorporate newly-developed molecular amplification methods to suit a particular emergency or present medical need. A detailed description of each of these technologies can be found in Table 1. These platforms offer simpler and faster alternatives to traditional PCR methods and can be used to detect viral species as well as serotypes at or near POC in less than two hours. One of their disadvantages, however, is the relatively high price of the instruments and cartridges, which can make them unaffordable in many resource-limited settings. Initial validation with clinical samples has taken place, but further multicentre evaluations with well-characterized clinical samples are required to confirm the initial results.

A portable multiplex isothermal platform is being developed to identify the infecting viral species at POC in humans (saliva) and mosquitoes. A fluorescent-based microfluidic platform that is powered and operated by a connected smartphone whose camera functions as a spectrometer was presented. The system applies Loop-mediated isothermal amplification (LAMP) and oligo strand displacement technologies to amplify target sequences at a constant temperature (thermal cycling not required). Heating is achieved by a “hand warmer”-like chemistry without the need for a power supply. The patented technology uses a rolling sampling mechanism to remove the maximum amount of sample from a sampling swab, optimizing the sensitivity of the assay. This is a simple and portable “sample-in, answer-out” device that can work in settings without electricity or highly trained users. Scalability can be achieved in-house, with reproducible 3D-printing technology and patented injection moulds. Time to result is about 1h, and preliminary analytical performance has been evaluated with spiked viral RNA samples. Clinical and fieldtesting is planned in the near-future.

Table 1. Near point-of-care multiplex real-time RT-PCR platforms to detect current infections

Availability	Assay	Company/ Group	Target/s	Platform/s	Clinical performance	Other assay characteristics	Regulatory approval	Comments	Abstract
In the pipeline	Multiplex Real-time RT-PCR (ZCD Xpert)	Waggoner JJ et al	Pan-DENV ZIKV CHIKV	GeneXpert (Cepheid)	Awaiting validation in clinical samples.	„Sample in, answer out“; Random access 3-plex assay; Connected; Time to result: 90 min; LOD: 100 copies/mL*; Requires electricity/battery; Cost: instrument: ~30.000$; Cost cartridge: ~10$	No	Validated academia developed assay (ZCD assay) adapted to be used in “open cartridges” on GeneXpert.	Appendix II, Abstract #1
	Multiplex Real-time RT-PCR	Arien K et al	DENV-1, DENV-2, DENV-3, DENV-4, CHIKV, ZIKV, YFV Pan-flavivirus *Plasmodium*	Idylla (Biocartis)	Awaiting validation in clinical samples.	„Sample in, answer out“; Up to 30 targets/cartridge; Sample/s: whole blood, serum, plasma, urine, semen and CSF; Connected; Time to result: 100 min; Requires electricity; Cost instrument: ~30.000€ Cost cartridge: ~30€	No	Different extraction protocols need to be developed to extract different sample types. Cartridges compatible with NGS.	Appendix II, Abstract #2
	Multiplex Real-time RT-PCR	Nelson B (Tetracore) Mosman C (Kirkland)	DENV ZIKV CHIKV	T-COR8™/ C2T sampling device	Awaiting validation in clinical samples.	„Sample in, answer out“; Up to 32 targets/run (4 channels x 8 wells); Sample/s: oral fluids, urine, whole blood; Connected; Battery for up to eight hours; No pipetting required; Cost instrument: ~28.000$	No	The C2T collect and sampling device is designed to require no pipetting.	Appendix II, Abstract #11 Abstract #13
	Multiplex Real-time RT-PCR	Adams E (LSTM) Edge D (BioGene)	DENV-1 DENV-2 DENV-3 DENV-4 ZIKV CHIKV	QuRapID LV	Awaiting validation in clinical samples.	„Sample in, answer out“; 6-plex assay; Sample/s: whole blood; Random access; Time to result: 35min Requires electricity	No	Uses of a freeze and thawing method to release nucleic acids for amplification	Appendix II, Abstract #12

LOD Limit of Detection; NGS Next Generation Sequencing; CSF Cerebrospinal fluid *preliminary data

A simple method to detect PCR products on a paper chromatography-like strip was presented (Appendix II, Abstract #16). The STH (Single-strand Tag Hybridization)-PAS (Printed Array-Strip) method can detect down to 1copy/mL of amplified viral RNA. The assay allows inexpensive, simple and sensitive detection of viral RNA amplicons, but it still requires prior viral RNA extraction and amplification steps. The different assay kit combinations include primers, developer and strips for the detection of DENV (serotypes 1-4), ZIKV, CHIKV, and Japanese encephalitis virus PCR products. This is a patented technology.

A Parsimonious Targeted-Enrichment Methodology to simultaneously capture complete genome sequences of DENV1-4, CHIKV and ZIKV – the PTEM-DCZ assay (Appendix II, Abstract #9) was presented. This methodology is superior to traditional deep sequencing methods as it allows full-coverage genome sequencing with high sequencing depth, directly from clinical samples. The method is also able to capture genomic diversity. This is a robust and highly sensitive method with the potential to be scaled-up and applied to analyse transmission chains and the detailed molecular epidemiology of outbreaks. The method was successfully applied to two large outbreak cohorts as a proof-of-principle (submitted for publication). The methodology can also be used to identify and discriminate the different viral species and serotypes in acute samples, and was shown to identify ZIKV and DENV co-infections. The methodology is currently limited to academic, research and central laboratories. The entire process is complex, expensive and takes about 7 days to complete. The aim is to bring this technology into the market and use robotics and microfluidic strategies to compress time-to-results and reduce costs. Further development is hindered by a lack of supporting funding mechanisms.

1.2 Antigen detection assays

Commercially-available antigen-based assays to detect ZIKV infections were not available at the time of publication of the ZIKV diagnostic landscape (Figure 3). Antigen-specific monoclonal antibodies (mAbs) that can bind specific viral antigens with high affinities are required to develop such assays. A cell-based method that yielded highly specific panels of mAbs against the non-structural protein 1 (NS1) proteins of different DENV serotypes and ZIKV was presented (Appendix II, abstract #6). The antibodies are able to distinguish between the different DENV serotypes and between DENV and ZIKV viruses. A sandwich ELISA was developed as a proof-of-principle, and initial results on the analytical sentitivity and specificity look promising. Other groups are working towards the development of antigen capture ELISAs that use similar ZIKV- and DENV-specific anti-NS1 mAbs. Validation with clinical specimens is ongoing. These assays have the potential to be converted into a lateral-flow rapid test format. Lack of funding is limiting further development.

2. Technological innovations to detect **past infections**

Extensive cross-reactivity among co-circulating flaviviruses confounds the serological diagnosis of recent and past flaviviral infections. Current commercial antibody detection methods are not able to identify infecting viral species in individuals who have been

exposed to previous flaviviral infections. Highly specific antibody detection methods are required for surveillance, seroprevalence and vaccine studies. Therefore, the question remains: how can we achieve specific serologic diagnosis of past flaviviral infections?

A deeper understanding of the molecular specificity and kinetics of human antibody responses after primary and secondary flaviviral infections is needed to inform the design of specific antibody recognition tools (Appendix II, abstract #7). The levels and nature of cross-neutralizing antibodies change over time. Studies have shown that primary DENV infections induce type-specific neutralizing antibody responses, whereas secondary infections lead to increased neutralization capacity to a wide range of different DENV serotypes. An antibody depletion method was developed that allows distinguishing between type-specific and cross-reactive immune responses in late convalescent samples (>2 months post-infection). Such an approach can be used to identify the history of exposure to past infections and vaccination. Upon sequential DENV infections, cross-reactive neutralizing antibodies can be found against a variety of different serotypes (including the ones a patient has not been exposed in the past). In contrast, type-specific antibody responses are detected against the specific serotypes a patient has been exposed to. In late convalescent samples from DENV and ZIKV co-infections, little cross-neutralization occurs. Studies to better characterize the antibody response in early convalescent samples are needed, as cross-reactivity among different viral species is a common feature of early antibody responses. These observations highlight the need to consider the timing of sample collection to inform the design of specific serological assays.

Assay design also requires the identification of the antigenic signatures recognized by type-specific antibodies. Strong neutralizing human monoclonal antibodies are known to target quaternary structure epitopes only displayed at the surface of the viral particles (i.e., they typically bind across different surface proteins or protein dimers). Epitopes with high sequence homology among serotypes trigger cross-reactive antibody responses, whereas unique epitopes lead to serotype-specific antibody responses. This information was used to rationally design ZIKV and DENV envelope (E) protein and NS1 recombinant antigens for specific serological detection of recent and past infections (Appendix II, abstract #8). Selective mutagenesis to modify conserved regions between flaviviruses is being used to further increase specificity. Selection of the best performing antigens is ongoing and clinical evaluations are planned in the near future. The group is open to collaborate with industry to adapt their antigens to a POC diagnostic platform.

Given the cross-reactivity of current methods, there is a lack of reliable “gold standard diagnostics” to evaluate newly developed serological specific assays. Well-characterized biobanks within prospective cohort studies are useful to evaluate newly developed diagnostic assays. The strength of this approach relies on the ability to stratify patients by exposure, immune status and timing of sample collection. The NS1 blockade-of-binding (BOB) ELISA that distinguishes between DENV and ZIKV antibodies was developed and evaluated using this type of approach (Appendix II, abstract #14). The BOB ELISA is a competition-based ELISA in which serum antibodies are measured for their ability to block the binding of a human ZIKV NS1-specific mAb

to solid-phase ZIKV NS1. This approach was shown to be more specific than traditional ELISAs based on the total binding of antibodies to NS1. Initial clinical validation results have confirmed the high sensitivity and specificity of the assay (under revision for publication). The assay could easily be developed and commercialized into novel cheap and simple to use ELISA or RDTs and the company is interested in out-licensing their technology.

Rationally designed antigenic proteins can be combined with a sample pre-treatment step to maximize the specificity of ELISA assays to detect specific antibody responses to DENV and ZIKV infections in settings where the viruses are co-circulating. This strategy was adapted to an automated multiplex ELISA platform for the simultaneous detection of DENV infections (antigen and IgM/IgG detection) and past exposure to ZIKV infection (IgM and IgG detection). Initial clinical validation studies have taken place, but challenges associated with a lack of appropriate reference standard and well-characterized clinical samples remains.

The pGOLD Zika and Dengue virus serologic assay, a multiplex nanotechnology-based serological assay for measuring IgG, IgA and IgG avidity against both ZIKV and DENV infections was described (Appendix II, abstract #5). This published assay (1) uses Near-Infrared Fluorescence Enhanced (NIR-FE) imaging on a nanoscale Plasmonic Gold (pGOLD) microarray 12plex antigen platform for antibody detection on two different channels. Initial validation has shown that the assay can detect and distinguish IgG antibodies from co-infected patients as well as determine the timing of exposure to infection by measuring IgG avidity, particularly useful to monitor Zika exposure during pregnancy. Clinical validation with well-characterized samples is required for further development. Time to results is about two hours, and the assay is relatively expensive as it uses gold and a confocal microscope scanner to read the slides.

3. Technological innovations to detect both **current and past infections**

Technologies that detect both current and past infections by combining antigen and antibody detection approaches provide a wider spectrum of application following infection. A nanotechnology platform for the independent or simultaneous detection of ZIKV and DENV NS1 protein and IgM/IgG antibodies was presented. The technology uses a Blu-Ray-based reader, single-use microfluidic cartridges, and an opto-magnetic nanoparticle-based readout. The system is simple to use, portable and allows remote connectivity. Serum, plasma or whole blood samples can be used. Initial multicenter clinical validation looks promising but further studies are required to precisely define the accuracy of the different assay components and formats.

Question 3: How do we make it happen?

The previous section presented several promising diagnostic technologies that are currently in the pipeline and have the potential to address many of the existent gaps in the detection and differentiation of flaviviral infections. These technologies represent a small sample of all the product development efforts that are currently ongoing. While these efforts must be praised, it is equally important to look downstream and identify the issues around translating research into a product that is useful in the field and has impact. Previous R&D experience has demonstrated that there is a massive loss between the number of tests that are being developed and the ones that make it into the market. In fact, the vast majority of tests that are under development never reach the end-users. Participants were invited to identify challenges and present strategies on how to address them and guarantee that technologies can move from concept to market. Common themes are summarised in Table 2 and discussed in detail in the next section.

1) Market opacity, scale-up and costs

Major challenges exist with regard to product development associated with market forecasts, scale-up and costs. One of the challenges companies are facing is the development of appropriate business models with high margin return on investments (ROI). It is not clear who is going to pay for the tests (most affected countries are not able to afford costs) and what is the final cost of a given product. The final cost includes not only the price of the technology but also the hidden costs associated with bringing a product into the market and its adoption and maintenance in countries. Forecasting the market needs is also challenging. The development and scale-up of artemisinin-based antimalarial drugs was used as an example to illustrate the challenges of market predictions. In this example, predicted demand ended up being several fold lower than actual demand for the drugs. As a result, production capacity was not able to cope with product demand and drugs were not available to patients. Even when a good market forecast is available, unpredictable factors such as policy updates can affect the demand.

Market opacity is a challenge that affects all the different stakeholders ranging from investors and product developers to end-users. Nagwa Hasanin presented UNICEF´s work on market forecasting models to predict the demand for ZIKV diagnostics and mechanisms to ensure the distribution of the final products into the countries that need it. Funding agencies such as BARDA (Biomedical Advanced Research and Development Authority), USAID (United States Agency for International Development) and Vulcan are providing funds at all levels of the pathway from development to adoption. Investments are used to support product development (e.g., USAID ZIKV grand challenge award), as well as to develop tools to promote scale and sustainability to adoption. A global approach that involves early partnerships between/among the different stakeholders and all of the agencies that help shaping the market is needed.

2) "The Perfect is Enemy of the Good"

This quote was constantly mentioned by different participants throughout the workshop. The fact is that there is no perfect test. How can we define how good is good enough? Traditionally, Target Product Profiles (TPPs) have been used to define the desired technical and operational characteristics of test platforms. However, requirements that are set at too stringent levels can mean that no tests meet the desired characteristics. For example, TPPs have often set very stringent sensitivity and specificity requirements that are not achievable. This contributes to the valley of death of technology development. There are tradeoffs to be considered; even a less sensitive test might be beneficial to target populations. When defining accuracy targets, it is also important to consider the application of the test as well as the setting in which it is going to be applied, including the prevalence of disease (i.e. positive and negative predictive values). An approach that weighs risk and benefits within the different applications is more appropriate not only to define accuracy targets, but also to guide regulatory approval and adoption. Piero Olliaro and Bill Rodriguez presented examples of how this type of approach was successfully implemented to approve the use of HIV self-tests and malaria RDTs. In the case of Ebola, it also became clear that a less accurate test can have a higher impact at the heart of an outbreak, i.e., when there is a higher chance that a subject with a positive test is truly infected (higher PPV). PDC could play a role in advocating for this type of approach and establishing models that weigh risks and benefits to define accuracy targets within the different intended uses of a technology.

3) Assay optimization and clinical validation challenges

Lack of appropriate and standardized diagnostic evaluation protocols along with limited access to well-characterized clinical samples delay the process of test optimization, clinical validation and product adoption – another challenge that was frequently pointed out throughout the workshop. Both industry and public-sector developers alike have stressed that further development efforts are often stuck at the stage of clinical validation. Clinical validation of multiplex assays or newly developed assays with superior performance are particularly challenging. As many of the newly developed technologies fall into these categories, well-defined strategies and protocols along with engagement with regulatory agencies is recommended.

PDC can add value by 1) contributing to the development of standardized protocols across platforms and 2) establishing mechanisms to support access to field studies and well-characterized samples.

In terms of strategy, May Chu proposed the development of a reference panel that could be available for standardization between/among different technologies. Julie Schaefer highlighted lessons learned from influenza response and presented how leveraging efforts with clinical trials for drugs or vaccines can be beneficial to both parties. Rosanna Peeling highlighted the potential of open networks of quality-assured laboratories with access to well-characterized specimens that allow developers to validate their tests "in-country". This type of approach facilitates country involvement

at the early stages of product development and helps minimize distrust issues when countries need to decide which technologies to adopt. Several laboratory networks from different geographical regions are committed to contribute to this type of initiative as well as to the strengthening of national laboratory capacities. Networks presented at the workshop include the Global Laboratories Alliance for the Diagnosis of High Threat Pathogens from WHO (GLAD HP), the Institute Pasteur International Network, the network of Dengue laboratories in the American region (Relda/PAHO), and the West African Network of Clinical Laboratories. PDC can play a role in coordinating these efforts.

4) Siloed stakeholders and uncoordinated efforts

In fact, siloed stakeholders and uncoordinated efforts are two of the critical issues in product development. Early partnerships and alignment of goals among stakeholders are important to ensure that efforts and resources are used efficiently and lead to results. Alliances such as the PDC and the Global Dengue & Aedes-transmitted diseases Consortium (GDAC), established to promote the development and implementation of approaches to prevent and control the spread of DENV and other flaviviruses, can provide the environment to break silos among different actors and promote the establishment of early partnerships. Annelies Wilder-Smith presented how GDAC is currently promoting communication between developers and target-country National Regulatory Agencies (NRAs). This allows countries and regulators to understand what to expect from future diagnostics for flavivirus and to be prepared in terms of policy. These efforts can accelerate regulatory approval and the adoption process (discussed in the section below). The PDC workshop is also a good example of a platform that brings stakeholders together to discuss and define common strategies.

5) Challenges with regulatory approval

Data from clinical validation studies is required to obtain regulatory approval and inform policy decisions in countries. Regulation is essential to ensure the safety, quality and effectiveness of diagnostic tests. Rosanna Peeling has shown that over 50% of countries do not regulate *in vitro* diagnostics (IVDs). The regulatory landscape for IVDs is highly variable, and regulatory approval mechanisms vary from country to country. As a result, regulatory approval and programme uptake processes are slow, costly and not transparent. Regulatory harmonization between national regulatory agencies and information sharing among the different interest groups (industry, regulators, researchers, laboratories, health systems and patients) is required.

6) How to respond to public health emergencies

Traditional diagnostic development to address emerging infectious diseases is slow and only triggered when public health emergencies of international concern are declared. The lack of appropriate diagnostic tools and the inadequacy of R&D models for emergency preparedness have led the global health community to establish a

variety of mechanisms to finance and accelerate the rapid development and deployment of diagnostics during public health emergencies. The WHO R&D Blueprint for Actions to Prevent Epidemics was presented as one of these initiatives. In order to be prepared to respond to outbreaks in a timely manner, the WHO has opened a call for open platform technologies to improve research and development preparedness against global health emergencies. In the field of diagnostics, an integrated open platform diagnostic system and country capacity building strategy has gained the support of the WHO Blueprint. Similar strategies were developed by other industry, academia and government stakeholders. A representative from industry presented a toolkit that integrates pathogen detection workflows, epidemiological sequencing and vector surveillance strategies (Appendix II, abstract #15). Emergency regulatory approval mechanisms under the US FDA Emergency Use Authorization (EUA) and the Emergency Use Assessment and Listing Procedure (EUAL) from WHO can be put in place to ensure rapid adoption of diagnostics during emergency situations.

7) How to ensure sustainable capacity in-country.

In addition, to the needs and applications discussed above, sustained capacity in-country is needed to respond in the intermediate and long-term to arboviral (and other) infections. Higher-cost commercial kits are unlikely to solve this issue at a national level in many resource-constrained countries. Therefore, approaches to make available key reagents, protocols, and quality control standards (e.g., proficiency panels, etc.) to national reference laboratories and other such public sector entities are necessary.

Table 2. Challenges in product development and strategies to accelerate the pathway to adoption

Challenges	Strategies in use	Limitations of current strategies	What is required and how can PDC or other consortia add value?
Market opacity, scale up and costs	UNICEF presented market forecasting models to predict the demand for ZIKV diagnostics and ensure the distribution of the final products into the countries that need it. Funding agencies such as USAID, BARDA and Vulcan are providing funds along the pathway from development to adoption. Reimbursement can be provided by 3rd-party payors such as Global Fund	UNICEF focus relies on African countries. Lack of strategies to coordinate efforts and promote long-term sustainability. 3rd-party payment is complex, does not solve distribution issues and leads to low profit margins from companies	Contribute to a global partnership approach that includes all the agencies that help shaping the market.
There is no perfect test. **How to define the margin of error that can be accepted?**	Target Product Profiles (TPPs) are used to define the desired technical and operational characteristics of a test. Very stringent sensitivity and specificity requirements are often set.	TPP accuracy levels are often not met by newly developed technologies. As a result, many tests that could benefit the populations in need are lost to the valley of death.	Advocate for an approach that weights risks and benefits to inform decision making.
Clinical validation takes time and resources. **Multiplex platforms multiply the challenges.**	Current strategies rely either on collaborations between developers and research groups or on the purchase of clinical samples.	Heterogeneities of protocols and validation strategies. There is no guarantee on the quality of clinical samples or appropriateness of protocols.	Contribute to the harmonization of protocols; Facilitate access to quality assured samples and field laboratory networks; Develop reference panel for standardization between different technologies.
Siloed stakeholders and uncoordinated efforts	Different stakeholders employ independent strategies to ensure diagnostic preparedness and product adoption.	Efforts and resources are not used efficiently to lead to results	Provide the environment for the establishment of early-partnerships between stakeholders.
Regulatory approval challenges	The Food and Drug Administration (FDA) in the United States regulates IVDs. Individual countries and regions have their own policy and legal frameworks for medical devices.	Regulatory approval is region-specific, non-transparent, variable, complex, slow and costly.	Regulatory transparency and harmonization is required. Strategies to add value include: - promote better information sharing between the different interest groups - establish regulatory networks and common strategies - organise workshops to inform and train

Challenges	Strategies in use	Limitations of current strategies	What is required and how can PDC or other consortia add value?
How to respond to public health emergencies?	The product development to adoption pathway often starts too late (i.e. when a public health emergency is declared) to cause impact.	A reactive approach is slow, hard to coordinate and delays implementation of medical countermeasures to control the outbreak.	Move from being reactive to being proactive. Outbreak response is by nature reactive – how to do it? Define priorities and establish diagnostic preparedness platforms: - WHO R&D Blueprint - Integrated diagnostic preparedness and capacity building platforms - WHO list of essential diagnostics - Emergency regulatory approval mechanisms
How to ensure sustainable capacity in-country	Current strategies rely on individual parterships, often between academic laboratories and NGOs or Ministries of Health in-country.	Difficult to scale out this approach to multiple countries due to lack of available reagents, funding, and quality control standards.	Establishment of: - networks of interested parties including both assay developers and country stakeholders and users, as well as international agencies, such as WHO and PDC - mechanisms for appropriate transfer of technology in a more stream-lined fashion - proficiency panels and a mechanism to distribute and regulate quality of “in-house” assays appropriate for national scale-up and sustainable implementation.

Action Plan & Major Milestones

In general, lessons from HIV, malaria and TB experiences have taught us that global attention drives impact. We are currently experiencing widespread global attention on flaviviruses, in particular, ZIKV, but this can rapidly change as new emergencies arise in the international agenda. It is important to take advantage of this momentum and align the goals and efforts of different stakeholders and make sure that the necessary diagnostic tools reach those who need them. PDC and its parent organization, the Global Dengue and Aedes-Transmitted Diseases Consortium (GDAC), can provide the right environment to achieve this; we are confident that this workshop was a step in that direction.

Through this workshop, key stakeholders from industry, academia, regulatory bodies, investor organizations, WHO and the affected countries could sit together and define common strategies to address major challenges. Collaborations between the different stakeholders were also reinforced. Academia-developed products such as specific monoclonal antibodies, purified immunoreactive antigens, primers and probes, as well as early technologies can be successfully adapted to industry-developed platforms. On the other hand, developers were able to contact representatives from academia and laboratory networks to further validate their technologies. Country representatives are now aware of the upcoming technologies, and they have all profited from learning about the recent developments from the WHO, regulatory agencies and investors. We heard from USAID regarding efforts to accelerate innovations from ideas to market, BARDA to identify and lend support to companies that have tests that need to qualify for FDA clearance, qualifying clinical laboratories that can provide the critical, locale-appropriate evaluation of promising diagnostic tests, and philanthropic organizations making efforts to lend their technologies and support to connect systems for better response.

In summary, this workshop has demonstrated how PDC can add value by providing the right platform for early partnerships to occur. In fact, the establishment of earlypartnerships was one of the major identified actions required to drive the field further. PDC is also committed to coordinate some of the specific efforts that are needed. Biobanking, a critical resource that most workshop participants indicated again and again as the greatest challenge in evaluating, harmonizing and standardizing promising technologies, was identified as a priority. In response to this call for better access to well characterized samples, PDC is committed to:

a. Create a governance system for access to reference samples
b. Create an internal reference sample and a small proficiency panel for initial test evaluation
c. Establish a PDC consortium of validated laboratories for field evaluation of promising technologies
d. Organize PDC workshops and training as appropriate to meet the needs

Workshop participants agreed that they will support PDC taking steps forward to meet the challenges. A show of hands from the audience indicated that many will stay engaged and help with realizing the steps that need to be taken. The goal is to support innovative technologies to find their way to adoption from their current stage of product development.

Appendix I - Participants list

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Appendix II - Selected abstracts

S/N	Name	Company/University
#1	Jesse Waggoner	Emory University School of Medicine
#2	Kevin Arien (Lieselotte Cnops)	Institute of Tropical Medicine, Antwerp (ITM)
#3	Kevin Arien	Institute of Tropical Medicine, Antwerp (ITM)
#4	Nathalie Renard	bioMérieux
#5	Benjamin Pinsky	Stanford University School of Medicine
#6	Katharina Roeltgen	Swiss Tropical and Public Health Institute (Swiss TPH)
#7	Aravinda de Silva	University of North Carolina, Chapel Hill (UNC)
#8	Prem Lakshmanane	University of North Carolina, Chapel Hill (UNC)
#9	October Sessions	Duke-NUS Medical School
#10	Filippo Bosco	BluSense Diagnostics
#11	Bill Nelson	Tetracore Inc.
#12	Emily Adams	Liverpool School of Tropical Medicine
#13	Craig Mosman	Kirkland Biosciences
#14	Davide Corti	Humabs BioMed SA
#15	Michael Baffi	Thermo Fisher Scientific
#16	Takahiro Haruyama	AVSS

#1 Near-Care Molecular Testing for Dengue, Zika and Related Pathogens

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Abstract
The ZCD assay is a multiplex molecular test for the detection and differentiation of Zika virus (ZIKV), chikungunya virus (CHIKV), and Dengue virus (DENV) in clinical samples. Our aim is to adapt this for use in "open cartridges" on the GeneXpert platform (Cepheid). The ZCD assay proved more sensitive for ZIKV and CHIKV than reference, monoplex comparators, and it demonstrated similar sensitivity to the original DENV assay upon which it was based. Importantly, additional tests for related pathogens can be incorporated into the ZCD assay with little impact on performance. These include assays for malaria, leptospirosis, yellow fever virus, Mayaro virus, o'nyong-nyong virus, West Nile virus, and Rift Valley fever virus. This feature allows testing to be tailored to local epidemiology or adapted to respond to emerging pathogens. Optimization of the ZCD assay on the GeneXpert platform will allow for testing near the point of patient care, and the utilization of open cartridges will maintain the flexibility of our molecular testing protocols. The GeneXpert assay will be intended for use in clinics and acute-care settings to provide a confirmed diagnosis during a patient's medical evaluation. The assay may also provide information on arbovirus surveillance as data from the GeneXpert can be monitored remotely in real-time. The ZCD assay itself has been validated and published. We have preliminary data that the DENV component of the ZCD performs well in the GeneXpert, with an estimated lower limit of detection ~10-fold lower than the ZCD assay performed with the published protocol. This device may be ready for sale in three years, and we expect this to be useful for surveillance, clinical practice, and monitoring of vector populations. Barriers to getting this device to market include well-characterized specimens for assay validation and funding to study the impact of this assay in clinical practice.

#2 Development of a multiplex pan-arboviral molecular diagnostic (ARMADA) test for the clinical management of febrile illnesses

Investigators: Lieselotte Cnops, Birgit De Smet, Johan Michiels, Marjan Van Esbroeck, Kevin K. Ariën

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Abstract

Arboviruses are spreading rapidly and globally, and cause sporadic and unpredictable outbreaks. Examples are the recent invasion of Chikungunya virus (CHIKV) followed by Zika virus (ZIKV) into the America's, a region where before 2013, Dengue virus (DENV) and Yellow Fever virus (YFV) were the most important circulating arboviruses. Due to similarities in clinical presentation and overlapping geographical distribution, broad and simultaneous screening of different arboviruses and co-circulating pathogens (e.g. *Plasmodium*) is required to identify the origin of disease in febrile patients living in or visiting these regions.

Arbovirus diagnosis during the acute phase of disease relies mostly on molecular RNA-detection technologies, requiring skilled laboratory personnel, technical infrastructure and often sequential testing with different virus-specific single RT-PCRs. The goal of our project is to develop a multiplex assay that allows simultaneous detection of arboviruses and *Plasmodium* in one simple test on an automated molecular diagnostics platform (Idylla, Biocartis). This sample-to-result molecular platform is easy-to-use, requires minimal hands-on time, has a turnaround time of 100 minutes, and can combine a maximum of 30 different targets in one assay cartridge.

We are currently developing a prototype syndromic test, combining the detection of DENV-1, DENV-2, DENV-3, DENV-4, CHIKV, ZIKV, YFV and pan-flavivirus RT-PCRs in combination with *Plasmodium* detection. The assay includes sample and extraction controls and can be performed on whole blood samples, serum, plasma, urine, semen and cerebrospinal fluid. Preliminary testing demonstrated good performance of the single RT-PCRs when combined on the Idylla cartridge and no cross-reaction among arboviruses. Further validation is ongoing to determine analytical sensitivity of the multiplex assay in comparison to the single RT-PCRs and to evaluate the diagnostic sensitivity on a large panel of clinical samples. We are searching for additional funding for this validation, and if successful, we expect a regulatory-approved test on the market within 3-5 years.

#3 Development of a multiplex molecular test for the diagnosis of viral hemorrhagic fever

Investigators: Lieselotte Cnops, Birgit De Smet, Leo Heyndrickx, Sandra Coppens, Marjan Van Esbroeck, Kevin K. Ariën

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Abstract

Viral hemorrhagic fever is a severe illness that may be caused by a number of viruses, including Lassa fever virus (LASV), Crimean-Congo hemorrhagic fever virus (CCHFV), Rift Valley Fever virus (RVFV), Ebola virus (EBOV), Marburg virus (MARV) and yellow fever virus (YFV). With the EBOV outbreak in Western Africa (2013–2016), it became clear that easy-to-use assays for rapid diagnosis of these high consequence pathogens were urgently needed. We report here on the Idylla™ Ebola Virus Triage Test of Biocartis (Mechelen, Belgium), developed in close collaboration with the Antwerp Institute of Tropical Medicine and Janssen Diagnostics. This fully automated sample-to-result molecular diagnostic test analyses a 200µL whole blood sample within 100 minutes and detects both EBOV and Sudan virus (SUDV) (Cnops et al., 2016). The assay requires minimal sample handling and received Emergency Use Authorization from the US FDA. We are currently developing a multiplex assay on the same platform that is able to detect and differentiate between multiple hemorrhagic fever viruses. Viruses targeted in the prototype test are EBOV, SUDV and Bundibugyo virus (BDBV), LASV, YFV, CCHFV in combination with *Plasmodium* and sample and extraction controls. A pan-filovirus RT-PCR and pan-phlebovirus RT-PCR is also under development to port on the Idylla platform, and to allow even broad screening for other viruses, like Marburg virus and RVFV. Optimization and validation of the assay is ongoing but hampered by the limited access to clinical samples and reference strains. We are searching for additional funding, and we aim to have a regulatory-approved test on the market within 3-5 years. The added value of such a test is that is easily deployable in mobile labs to provide a rapid diagnostic response at the moment that a new hemorrhagic fever virus outbreak takes place. Further assets include the ease of use, safety and standardization.

#4 VIDAS Zika and Dengue: Preliminary results on an Automated ImmunoAssay platform

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Abstract

With 50 years of experience in the field of *in vitro* diagnostics, bioMerieux has contributed to public health with innovative and increasingly sophisticated tools. Our Immunoassay R&D department is currently developing prototypes for use on the instruments of the VIDAS® family. VIDAS is an automated immunoassay testing system. Based on the Enzyme Linked Fluorescent Assay technology, it provides single-use reagents for routine testing in clinical laboratories worldwide.

VIDAS Zika and Dengue prototypes are intended for use as an aid in the diagnosis of these infections. They are meant to identify and discriminate between Zika (ZIKV) and Dengue (DENV) virus, with specific intended uses. VIDAS Dengue assays are designed for detection of acute infection, while Zika ones focus on the management of virus infection during pregnancy.

One of the biggest challenges is developing specific serodiagnostic assays. Antibodies against ZIKV, DENV, and other flavivirus are highly cross-reactive. Current serological assays display issues to distinguish previous infections or vaccination with other flavivirus. Access to well-characterized samples with no sign of prior infection with other flavivirus is thus crucial for assay development and is our main technical issue.

To fulfill these needs for specific serodiagnostic assays, we applied innovative approaches based on the analysis of regions that display dissimilarity across flaviviruses and are highly immunogenic. Protein regions conserved within ZIKV or DENV and variable among other flaviviruses were identified. Such fragments were produced as recombinant proteins conserved among ZIKV lineage or all DENV serotypes respectively. They were used as antigen for the development of our much-needed specific Zika or Dengue serodiagnostic prototypes. In line with the intended use; we focused on Ag/IgM detection and eventually IgG follow-up for Dengue prototypes, and IgM and IgG detection for Zika prototypes. Preliminary results are very encouraging with regards to specificity and sensitivity.

#5 A Nanoscale Plasmonic-Gold Platform for Specific Diagnosis of Zika and Differentiation from other Flavivirus Infections

Investigators

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Abstract

The pGOLD Zika virus and Dengue virus serologic assay (Nirmidas Biotech Inc., Palo Alto, CA) is a multiplex serologic test for the detection and differentiation of Zika virus (ZIKV) and Dengue virus (DENV) infection in human serum samples. This assay utilizes a novel nanotechnology based plasmonic gold microarray platform with ~ 100-fold near infrared (NIR) fluorescence enhancement. This enables multiplexed, simultaneous detection of serum IgG/IgM/IgA and IgG avidity against a panel of mosquito-borne viral antigens down to 1 femtomolar level over > 6 logs of dynamic range. Our results indicate that the combined pGOLD ZIKV/DENV serology test allows for the rapid, sensitive, and specific serological diagnosis of ZIKV infection, even in patients previously infected with DENV. Our aim is to further utilize the multiplexing capabilities of this platform to include antigens from other mosquito-borne viruses including other flaviviruses, such as West Nile virus and yellow fever virus, and alphaviruses, such as chikungunya virus and Mayaro virus. Importantly, this flexibility of the pGOLD platform allows antibody testing to be tailored to the local epidemiology and rapidly adapted to respond to emerging pathogens. The pGOLD ZIKV/DENV test is intended for use in clinical laboratories to detect and differentiate ZIKV from DENV infection, and to estimate the timing of exposure. The pGOLD methodology has been published and a manuscript describing the pGOLD ZIKV/DENV assay has been provisionally accepted. This device may be ready for sale in one year or less, and we anticipate submission to the FDA for emergency use authorization. We expect this test to be useful for clinical practice, particularly for pregnant women, as well as for epidemiologic surveillance. The major barrier to getting this device to market is the availability of well-characterized serum specimens for assay validation.

#6 NS1 antigen capture assays for the specific detection of different Dengue virus serotypes and other arboviruses

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Abstract

Challenges for diagnosis of Dengue and other arboviral infections have been exacerbated by the recent emergence and upsurge of chikungunya and Zika virus in dengue-endemic countries. In addition to the differentiation between these virus infections, the identification of the infecting Dengue serotype will gain importance for prognosis in individual patients due to the increasing co-circulation of multiple Dengue serotypes in endemic areas.

Our project aims at the development of highly specific, inexpensive antigen capture assays as point-of-care tests to detect acute Dengue, chikungunya and Zika virus infections as well as to identify the infecting Dengue serotype. Pre-requisite for the development of the envisaged assays are tandems of antigen-specific monoclonal antibodies (mAbs) that have the ability of binding specifically and with high affinity to their target antigen and that do not compete for antigen binding sites.

For that purpose, we have established a novel strategy to rapidly generate mAbs against native antigens by immunization of mice with transfected mammalian cells expressing the target antigens in multiple displays on their cell surface (1, 2). By using this cell-based approach, we have produced panels of mAbs against the NS1 proteins expressed by different Dengue virus serotypes and by Zika virus.

Based on the obtained sets of mAbs with diverse fine specificity, we are currently designing sandwich ELISAs to distinguish between the four different Dengue virus serotypes and between Dengue and Zika virus. Pilot testing of the sensitivity and specificity of such assays has been performed with Dengue and Zika virus material. Moreover, the potential to develop subtype specific assays for epidemiological studies is currently being assessed. The described assay development strategy has great potential to yield field-compatible lateral flow rapid tests. While assays could become available within the next 3-5 years, the main barrier retarding the progress of our project is limited funding.

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Dreyer A.M, Beauchamp J, Matile H, Pluschke G. An efficient system to generate monoclonal antibodies against membrane-associated proteins by immunisation with antigen-expressing mammalian cells. BMC Biotechnol. 2010;10:87

#7 A High-Throughput Focus Reduction Antibody Neutralization Test For Detecting Remote Zika Virus Infections

Investigators: Matthew H. Collins, Eileen McGowan, Ramesh Jadi, Ellen Young, Cesar A. Lopez, Ralph S. Baric, Helen M. Lazear, Aravinda M. de Silva

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Abstract

Cross-reactive flavivirus antibodies (Abs) elicited by prior Dengue virus (DENV) infection may favorably or unfavorably impact clinical manifestations of Zika virus (ZIKV) infection and confound serologic diagnosis of ZIKV. Investigators have become weary of using Ab neutralization assays for tracking ZIKV infections because of recent studies reporting antibody cross-neutralization between DENVs and ZIKV. As levels of cross-neutralizing Abs are known to change over time, we asked whether cross-neutralization of ZIKV is preserved in late DENV convalescence (> 2 months post-infection). Additionally, we depleted DENV-reactive Ab from sera to assess ZIKV-specific neutralization responses. Despite extensive cross-reactivity in IgG binding, ZIKV neutralization was not observed among people exposed to primary DENV infections. We observed low frequency (23%) and low levels of ZIKV cross-neutralizing Abs in people exposed to repeat DENV infections. DENV-immune individuals who experienced ZIKV as a secondary flavivirus infection had distinct populations of Abs that neutralized DENVs and ZIKV. These data suggest most people infected by DENV infections do not develop durable and high levels of ZIKV cross-neutralizing Abs. Our results also indicate that people infected with ZIKV develop *de novo* ZIKV-specific neutralizing Ab responses irrespective of prior DENV immunity. We have developed a high-throughput, semi-automated focus reduction neutralization test for quantitative measurement of DENV and ZIKV neutralizing Abs in human specimens. While not intended as a routine point of care test, this assay has much potential for detecting past infections in high risk populations such as pregnant women, routine ZIKV surveillance, and for supporting vaccine trials.

#8 Structure based design of novel Zika virus antigens for serodiagnosis

Investigators: Prem Lakshmanane[1], Matthew Collins[1], Stephen Graham[1], Alice Liou[1], James Brackbill[2], Stefan Metz[1], Michael J. Miley[2], and Aravinda M. de Silva[1]

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Abstract

The major challenge for serodiagnosis of Zika virus (ZIKV) infection is extensive serological cross-reactivity between ZIKV and other flaviviruses, particularly in areas with high levels of DENV transmission. We are addressing this problem by rationally designing ZIKV and DENV envelope (E) protein and non-structural protein 1 (NS1) recombinant antigens for serological detection of recent and past infections. Fortified with high resolution structural information and the location of human antibody epitopes, we have designed and expressed three recombinant substructures of ZIKV E and NS1 proteins likely to display ZIKV type-specific antibody epitopes. These candidate recombinant antigens are quite sensitive and detected 7/9 reference ZIKV-immune sera in ELISA format. We are now using protein-engineering approaches to redesign the surface of candidate antigens to increase sensitivity. We also evaluate these antigens for specificity and observed just 17% cross-reactivity in a panel of 23 primary and secondary DENV-immune serum samples. We are selectively mutating regions conserved between flaviviruses to increase specificity. Studies are in progress to evaluate the best antigens with large reference panels and to evaluate their performance in ZIKV endemic field sites in Nicaragua and Colombia. By August 2017, we hope to identify the best combination of antigens for accurate detection of recent and past ZIKV infections. Before the end of 2017, we expect to partner with a commercial diagnostic company to develop a multiplex diagnostic test that is affordable, and suitable for use at point of care.

#9 Application of a parsimonious targeted-enrichment methodology for full-genome sequencing of viral genomes directly from patient samples

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Abstract

The frequency of epidemics caused by Dengue viruses 1-4, Zika virus and Chikungunya viruses have been on an upward trend in recent years driven primarily by uncontrolled urbanization, motility of human populations and geographical spread of their shared vectors, Aedes aegypti and Aedes albopictus. Infection with these viruses present with similar clinical manifestations making them challenging to clinically diagnose; this is especially difficult in regions of the world hyperendemic for these viruses. In the present study, we present a Parsimonious Targeted-Enrichment Methodology for the simultaneous acquisition of complete Dengue 1-4, Chikungunya and Zika viral genomes (PTEM-DCZ) directly from a clinical sample. This methodology is robust in its ability to capture diverse sequences, sensitive and can be easily scaled for large-scale epidemiological studies. We have applied this methodology to two large cohorts: a febrile study based in Colombo, Sri Lanka taken during the largest Dengue epidemic in the country's history and another taken during the 2016 outbreak of Zika virus in Singapore. Results from these studies indicate that we were able to capture an average 97.04% genome coverage at an average of 60,816-fold depth coverage. Using this methodology, we have also identified one DENV-3/ZIKV co-infected patient where we recovered full genomes for both viruses.

In terms of development, we are now in discussion with two potential industrial partners to convert PTEM-DCZ into a regulatory-approved diagnostic. We envision that this methodology would be of particular use in discriminating these clinically similar diseases and, given it's ability to produce whole genome information, for detailed molecular epidemiological interrogation of outbreaks. The current limitation, which we are now trying to address, to the conversion of this methodology into a commercial application is a supporting funding mechanism.

#10 VIRO-TRACK - A Rapid, quantitative, and accurate dengue/zika diagnostics platform for point of care use in endemic regions

Investigators: Filippo Giacomo Bosco[a], Marco Donolato[a], Shamala Devi Sekaran[b]

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BluSense Diagnostics ApS

Abstract

BluSense Diagnostics is developing an innovative blood testing point-of-care technology fitting WHO/UNICEF's Target Product Profile for flaviviruses diagnostics. The technology is based on a reader (BluBox), and single-use cartridges (Viro-Track), specifically designed for targeting different viruses. The Viro-Track cartridges have been designed to be the first quantitative rapid tests able to accurately diagnose different stages of Dengue, zika and chikungunya infections, using less than 30 uL of blood. Main applications are clinical diagnostics in decentralized settings in endemic regions, and on-field seroprevalence studies where antibody titers need to be quantified over a population sample widely scattered on the territory.

The key technological innovation is based on the immune-magnetic agglutination format and opto-magnetic readout. Superparamagnetic nanoparticles are coated with capturing probes (antibodies for antigen, e-proteins/NS1 for serology) forming sandwich agglutination in presence of the target analyte. The nanoclusters are optically measured and quantified using components from the Blu-Ray industry. The single-use plastic cartridges are based on centrifugal microfluidics, which allow multiplexing up to 5 different analytes within the same test, without the need of any user's sample preparation step. Three different cartridges are under development: ACUTE (DENV-NS1), COMBO (DENV-NS1/IgG/IgM), and DIFFERENTIAL (DENV-IgM/ZIKV-IgM/CHIKV-IgM). The products will hold features as:

- Quantitative differential antigen/antibody measurement
- Laboratory-grade testing (LoD=0.1ng/mL) and short sample-to-answer (<10 min)
- Multi-plexed cartridges for addressing intrinsic cross-reactivity
- Sample versatility (capillary blood, venous blood, and serum/plasma)
- Minimally-trained operators for empowering local facilities with laboratory capacity
- Remote-connectivity for real-time monitoring of performances/failures and case reporting

We characterized the performances of the assay and the clinical sensitivity/specificity on more than 200 samples from South America and Malaysia. Results are comparable to the one obtained using SD NS1 ELISA kit (above 95%). We expect ACUTE to be certified and commercially available by end of 2017, while COMBO and DIFFERENTIAL are expected to be certified in 2018. We arm at BluBox production cost of less than $500.

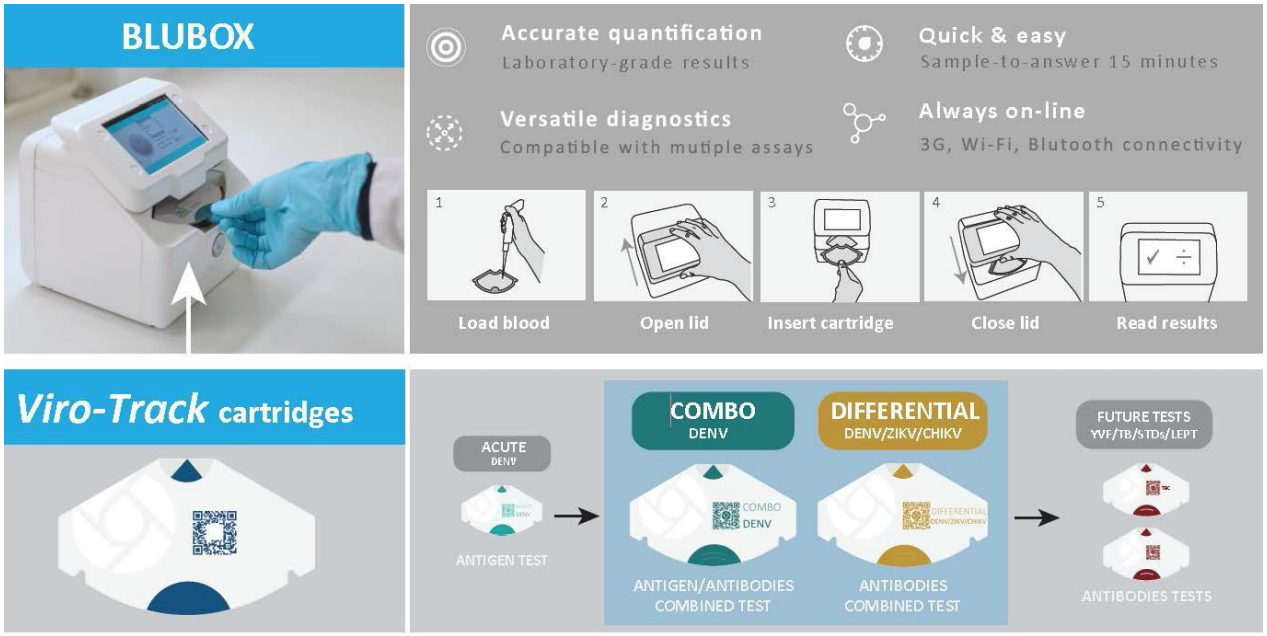

Figure 1: BluBox reader (durable), running the single-use Viro-Track cartridges (consumables). After development of a test cartridge (ACUTE), BluSense will release COMBO and DIFFERENTIAL, paving the way towards development of future disease control tests.

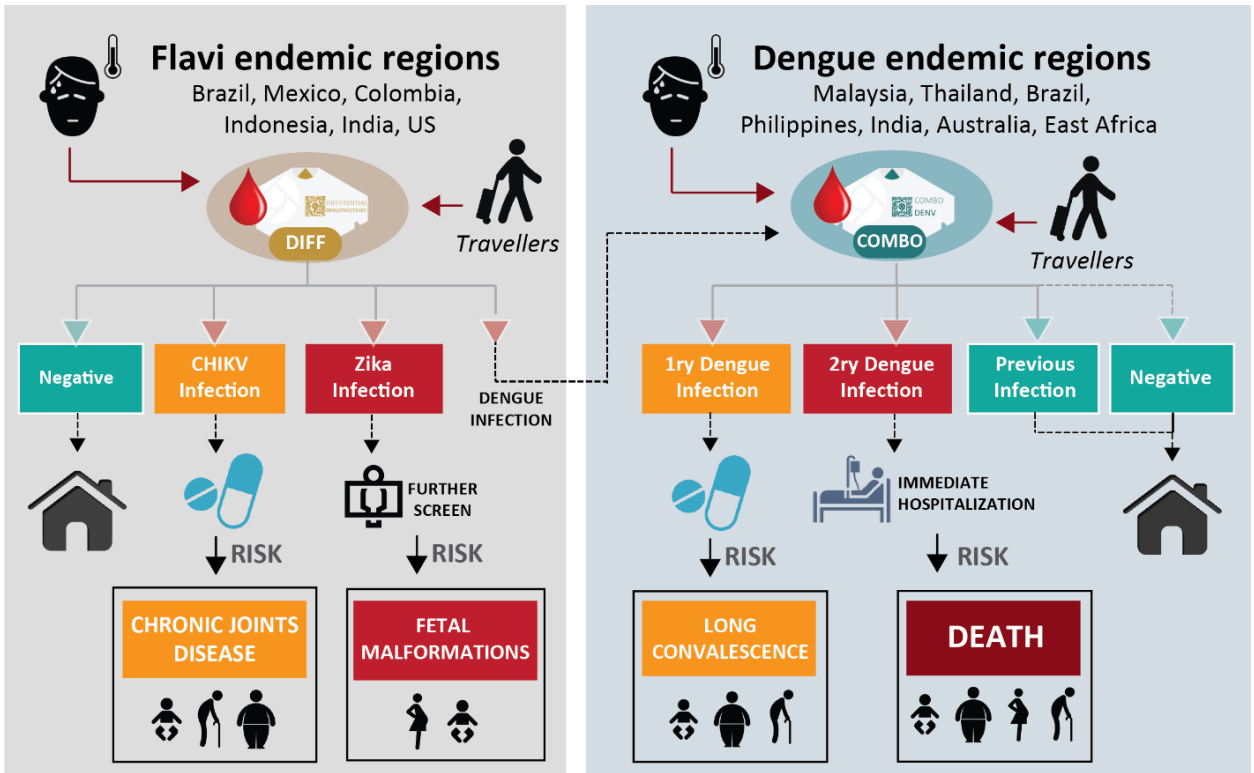

Figure 2: Three main diseases, all transmitted by Aedes Aegypti, cocirculate in endemic regions. While the three viruses cause similar symptomatic reactions to infected people, the health risks and the groups at highest risk, are different.

#11 Tetracore T-COR8 Diagnostic System for the Detection of Dengue and Other Arboviruses Using Real-Time-rtPCR at the Point-of-Care

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Abstract

The T-COR 8™ (portable real-time PCR) and C2T (collect to test cartridge and sampling device) are an integrated system for the direct (no extraction) detection of Dengue, chikungunya, and zika viruses in a Tetracore's triplex (DCZ) assay (room temperature stable dry reagents in the cartridge). The T-COR 8 can be used in the field because the sample-to-answer workflow (oral fluids, urine, or finger stick blood) was designed for a user with minimal training (no pipetting). Using the sampling device, the user swabs the patient's mouth, or dips the swab in urine, or collects blood from a finger stick, or serum with a capillary; the swab or capillary is then placed back into its housing, the snap-valve is cracked, the bulb squeezed, the reaction buffer flows through the sample. The sample in buffer, is dispensed, 4 – 15 drops, into the C2Tcartridge hydrating the assay. The instrument set up is supplied from a bar code on the C2T cartridge. The T-COR8 has eight independently accessible wells capable of reading four dye channels each. This allows for the simultaneous detection of up to 32 unique targets per run. An integrated lithium ion battery allows 32 – 64 tests per charge. The T-COR 8 can be connected to any WiFi network and supports remote access. The system has been evaluated in a laboratory setting using serum samples sent in to a central/reference laboratory (Mexico, Brazil and Grenada) for comparison between RNA extraction verses direct sample preparation using C2T sample processing device. The instrument will have an IVD-CE mark in Q1 2017. In East Africa, the T-COR 8 has undergone field evaluations for the diagnosis of animal viruses directly using Tetracore's simplified workflow compared to extracted results.

#12

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Abstract

LSTM and BioGene are collaborating to develop a molecular test capable of detecting Dengue, Zika and chikungunya and run on the QuRapID LV. The assay is designed to target acute fever for detection of recent infection at the point of care for clinical use.
LSTM have designed primer and probes sets for each of the 4 Dengue serotypes, Zika and chikungunya virus. They are designed for use on the QuRapID LV platform which is a simplified molecular diagnostic and runs directly from whole blood. It incorporates a rapid RNA extraction based on freeze/thaw cycling as part of a closed tube real-time PCR. The system combines rapid temperature transitions and real-time optics based on laser spectrophotometery to perform detection of viral RNA from crude samples in the presence of whole blood. This allows for a higher degree of multiplexing than the optical detection systems utilised by standard qPCR systems, making a 6-plex assay possible.
BioGene are currently developing the QuRapID LV platform and expect to pilot test in Q3 of 2017 with LSTM. The test, together with the platform is expected to be regulatory approved and for sale by Q2 2019 only if BioGene are successful in obtaining UK DFID follow on funding later this year.
We will present data on the single-plex assays and the 6-plex assay for the QuRapID LV, the limit of detection on culture and clinical sample collections from Guatemala in 2015 and 2016.
Our main challenge to bring the diagnostic to market is funding for field validation/IVD certification and generating sufficient volume in terms of tests to provide economies of scale.

#13 Triplex PCR ZIKV/DENV/CHIKV Assay and simple to operate real-time portable device

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Abstract

The T-Core 8 portable real-time PCR system has been developed for point of care testing and surveillance of disease. It is capable of identifying acute viremia or for detection of past and recent infection of Zika, Chikungunya, and Dengue. By use of RFID technology on sample collection devices and cartridges, the device may be operated by virtually anyone who has sufficient training to collect a sample. The device was developed by Tetracore, Inc., a US biotechnology company, and is being marketed and sold in locations outside of the US by Kirkland Biosciences LLC.

The device has eight wells. Each operate independent of the other wells. The device is capable of operating on an included battery for up to eight hours, so that it may either be taken into the field without a power source, or used in a laboratory or clinic plugged into power. It has remote communications in order to send results or to permit monitoring of testing in real time.

The device has been used for detection of biological threats, in some veterinary settings, and in limited situations for detection of other human diseases. The device has not been submitted for regulatory approval in the US, although it is CE marked, and manufactured under ISO medical device standards. The triplex assay has been validated in internal testing, and tested in approved laboratories in Brazil and the Caribbean. It has not been submitted for regulatory approval.

Kirkland Biosciences has been asked to test the device and assays in clinical trials working with two established laboratories and a hospital. The purpose of the trials would be to demonstrate the benefit of a portable device, the sensitivity and specificity of the assay, and how remote communications benefit countries in detecting and controlling the spread of disease.

Kirkland Biosciences is seeking funding to validate the system and assay in the field. It expects to seek regulatory approval in the next 24 months.

#14 NS1 Blockade-of-Binding ELISA distinguishes between Dengue and Zika virus antibodies

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Abstract

Background. Zika virus (ZIKV) is a mosquito-borne flavivirus that emerged recently as a global health threat, causing a dramatic pandemic in the Americas. ZIKV infection is mostly asymptomatic or mild, but is also linked to congenital birth defects and Guillain-Barré syndrome in adults. The high level of cross-reactivity among flaviviruses and their co-circulation has complicated use of serological assays to detect clinical and subclinical ZIKV infections, accentuating the need for a highly specific serological assay.

Methods. We previously identified a human ZIKV nonstructural protein 1 (NS1)-specific monoclonal antibody (mAb ZKA35), which we used to develop an ELISA based on the ability of serum antibodies to block its binding to solid-phase ZIKV NS1. A large set of samples from ZIKV-positive patients and individuals exposed to other flavivirus infection or vaccination was used to establish the sensitivity and specificity of the NS1 blockade-of binding (BOB) assay.

Results. We analyzed samples from 158 ZIKV infections confirmed by RT-PCR from Nicaragua, Brazil, and Italy and found that, when samples were collected >10 days post-symptom onset, 91.7% yielded greater than 50% inhibition in the NS1 BOB assay. We also tested 227 samples from patients with primary or secondary Dengue virus (DENV) infections and found that in 90.0%, the assay scored negative. When the control group was extended to patients infected by other flaviviruses, such as West Nile virus or yellow fever virus (YFV), or by other viruses, as well as to YFV vaccinees or healthy donors (n=470), the specificity of the assay was 95.5%. We also analyzed the kinetics of the specific anti-ZIKV NS1 antibody response in previously DENV-immune and DENV-naïve ZIKV-confirmed infections and found that titers are maintained through the last time-point tested, up to 240 days post-infection. We are currently in the validation phase and expect that the assay will be widely available within one year; one possible challenge in its development might include distribution mechanisms.

Conclusions. The NS1 BOB assay can serve as a low-cost assay to decipher the complex serology of flaviviruses and specifically detect recent and past ZIKV infections for surveillance, sero-prevalence studies, and interventional trials.

#15 Genetic Analysis Tool Kit for Rapid Outbreak Response

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Abstract

For the global healthcare community, successful management of outbreaks requires tools and systems to rapidly identify emerging pathogens, minimize spread of disease and reduce the length of outbreaks. To achieve these goals, many technology vendors, academic institutes and government agencies have worked to develop targeted genetic analysis solutions. Supporting our public and private partners, our role in outbreak response has expanded over the past decade from genetic analysis platform provider to full pathogen detection workflows, epidemiological sequencing and vector surveillance applications.
Outbreak Response Solutions:
Target Specific Pathogen Detection: Real-time PCR based solutions have enabled highly specific, low complexity pathogen detection to ensure rapid response by the clinical community.
Sequencing Based Epidemiology: Critical for the Ebola outbreak in South Africa, NGS based solutions were deployed to track human-to-human transmission chains to minimize duration and magnitude of the outbreak
Vector Screening for Emergence: Support integrated environmental screening for pathogens of zoonotic origin, real-time single and multiplex PCR based solutions have been deployed during Zika outbreak.
Global Surveillance and Analytics: Proactive Response to emerging threats supported by regionally networked detection systems to support vector abatement programs and public health readiness.
Emerging Capabilities to Support Rapid Response:
Approved clinical and analytical validation material: For Zika, Ebola and other recent outbreak assay development programs, access to sufficient quality reference material and/or quantities of sufficient materials have contributed to challenges in the rapid development and validation of assays. Several approaches to support clinical sample access have been implemented: (1) Clinical validation panels, (2) International reference standards, and (3) Commercial vendors.
Cold chain independent deployment: Lyophilized solutions to ship complete sample ready kits for detection applications in healthcare treatment and laboratory testing settings. The kit solutions included both targeted Zika singleplex tests and multiplex tests to support differential diagnosis from other flavivirus (Zika, Dengue and Chikungunya).
Data analytics and sharing: Key innovation from Zika outbreak was the deployment of cloud enabled detection platforms for vector screening application allowing for real-time data aggregation and identification of local transmission risk.

Over each successive outbreak, we will continue to support our public and private partners with new innovations to promote early detection of emerging pathogens, minimize spread of disease and reduce the length of outbreaks.

#16 Multiplex Diagnosis kit for Zika, Dengue, Chikungunya and Japanese Encephalitis Viruses using Printed Array Strip based on Single-stranded Tag Hybridization method

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Abstract
Initial clinical symptoms of Zika, Dengue, Chikungunya and Japanese Encephalitis virus infections are similar. It is a challenging task to differentiate these infections at the early stage of infection using ELISA or Immunochromate (IC) kits. Furthermore, as these mosquito-borne virus diseases are most common in developing countries, cost of detection should be low yet effective. In this context, we have developed a new and highly sensitive diagnostic kit “Mosquito-borne virus detection (MBVD) kits” for these types of viruses using a proprietary technology based on STH (Single-stranded Tag Hybridization)-PAS (Printed Array Strip) method. The method is a cutting-edge technology targeting nucleic acid of each virus by hybridization. Results are read-out like paper chromatography, requiring no special training or special analyzer. MBVD4 is multiplex and specifically distinguish 4 viruses in single assay. It is also highly sensitive and detects as low as one copy of target viruses in either serum or plasma of infected patients. Moreover, like IC kit, results can be easily visualized by raw eyes and thus keep the cost performance low. Currently we are evaluating the kit in the Philippines and pilot testing by using clinical specimens is undergoing in Malaysia. We intended to market the kit in 2-3 years for both surveillance and point-of-care in clinics. The main barriers are funding to disseminate the know-how in affected nations and distribution mechanism.

Appendix III - Dengue and Zika diagnostic landscape (Appendix Tables 1-7)

Appendix Table 1 Dengue virus (DENV 1-4) NAT assays

Laboratory-based NAT assays

Availability	Assay	Company/ Group	Platform/s	Clinical performance≠	Other assay characteristics*	Regulatory approval	Comments	References
"In-house"	hemi-nested RT-PCR	Lanciotti et al	Gel electrophoresis (end-point PCR)	Highly sensitive	Singleplex or multiplex		Widely used.	**(2)**
	RT-PCR	Several	Thermal cycler (several)	Varies	Singleplex or multiplex		Performance heterogeneities between methods and labs.	**(3)**
On the market	Real-time RT-PCR	CDC, USA	Thermal cycler: ABI 7500 Fast (CDC); Rotor-Gene	Less sensitive than laboratory developed, in-house, assay	Internal controls included	FDA	Not approved for blood screening.	(4) **(5)**
	Real-time RT-PCR (Simplexa™)	Focus Diagnostics, USA	Thermal cycler: 3M integrated cycler (Focus)	Sensitivity∞: 93.2% [95% CI 89.3-97.1]			Sensitivities above 90% for all serotypes.	**(6)**
	Real-time RT-PCR (RealStar)	Altona Diagnostics, Germany	Thermal cycler: ABI Prism 7500	Sensitivity∞: 83.3% [95% CI 77.6-89.1]				**(6)**
	Real-time RT-PCR (Liferiver™)	Shanghai ZJ Bio-Tech Co, China	Thermal cycler: ABI Prism 7500	Poor sensitivity: ~40%				**(6)**
	Real-time RT-PCR (GenoSen's)	Genome Diagnostics Pvt, India	Thermal cycler: ABI Prism 7500	Sensitivity∞: 85.2% [95% CI 79.7-90.7]				**(6)**
	Real-time RT-PCR (abTES)	AITbiotech, Singapore	Thermal cycler: CFX96 (Bio-Rad)	Sensitivity∞: 97.4% [95% CI 92.7-99.5]			Specificity: 100% (only 8 neg. samples)	**(7)**
	Real-time RT-PCR (innuDETECT)	Analytik, Germany	Thermal cycler: CFX96 (Bio-Rad)	Sensitivity∞: 44.4% [95% CI 35.3-53.9]			Specificity: 100% (only 8 neg. samples)	**(7)**

Near point-of-care NAT assays

Availability	Assay	Company/ Group	Platform/s	Clinical performance≠	Other assay characteristics*	Regulatory approval	Comments	References
"In-house" /in the pipeline	Isothermal amplification (NASBA)	Wu SJ et al.	ECL reader	Sensitivity∞: 98.5% (66/67)	LOD: ≤ 25 PFU/ml		No thermal cycler required.	**(8)**
	Isothermal amplification (LAMP)	Parida M et al.	Photometer	Sensitivity∞: 100% Specificity: 93%	Time to result: ≤30 min		No thermal cycler required. Challenges with primer	**(9)**
	Isothermal amplification (TMA)	Muñoz-Jordan et al	Procleix system Tigris system	Potentially more sensitive than RT-	Single-tube amplification		No thermo cycler required. Not for serotyping.	**(10)**
	Isothermal amplification (RT-RPA)	Abd El Wahed A et al./Teoh et al	Twista from TwistDx (Cambridge, UK)	Sensitivity∞: 98% (Senegal) 72% (Thailand)	Time to result: ≤20min		No thermo cycler required.	**(11)** (12)

ECL Electrochemiluminescence; ≠Evaluation methods are not consistant among the different studies (displayed values refer to referenced study in bold in the last column of the table, clinical performances vary with location, reference method, timing of sample collection and methodology used);*other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, positive control; ∞refers to overall sensitivity. In general, evaluations in multicenter and prospective studies with standardized methods are lacking.

Appendix Table 2 Zika virus (ZIKV) NAT assays

Laboratory-based NAT assays

Availability	Assay	Target/s	Company/ Group	Platform/s	Clinical performance	Other assay characteristics*	Regulatory approval	Comments	References
"in house"	Real-time RT-PCR	prM and E	Lanciotti et al (CDC)		Evaluated on outbreak specimens (serum, plasma, saliva, urine, amniotic fluid). Assay has been used as reference.	2x one-step reactions targeting the prM and E genes; LOD: 100 copies (prM) and 25 copies (E gene); no cross-reactivity identified		Optimal diagnosis may require testing of multiple specimen types.	(13-17)
	RT-PCR	Several (prM,E,NS1, NS3,NS5)	Several	Several	Limited evidence available.				(17)
On the market	RealStar® Zika Virus RT-PCR Kit 1.0		Altona Diagnostics GmbH	Several	Sensitivity: 91% Specificity: 97%	Single-tube assay Analytical sensitivity: 0.61 copies/µl eluate; no cross-reactivity identified	CE-IVD EUA EUAL		(14)
	Genesig® Easy Kit		Primerdesign™ Ltd., UK	genesig® q16 qPCR instrument	Peer-reviewed evidence missing[§]	RUO			Company[#]
	Zika Virus – Single Check		Genekam Biotechnology AG, Germany	Several	Peer-reviewed evidence missing[§]	LOD of 6.53 genome equivalent; sample/s: blood, plasma, serum; analytical specificity: 99.9%	CE-IVD		Company[#]
	FTD Zika virus		Fast Track Diagnostics, Luxembourg	Several	Sensitivity: 86.7% Specificity: 100% Peer-reviewed evidence missing[§]	LOD: 735 copies/mL No cross-reactivity identified IC included	CE-IVD		Company[#]
	Zika Virus Real Time RT-PCR Kit	5`UTR	Liferiver/Shanghai ZJ Biotech Co., China	LightCycler 2.0 (Roche, Germany)	Peer-reviewed evidence missing[§]	Sample/s: serum or plasma; one-step real-time PCR assay	EUAL pipeline		Company[#]
	Abbott RealTime Zika		Abbott Molecular Inc., U.S.		Peer-reviewed evidence missing[§]		EUA		
	Zika Virus RNA Qualitative Real-Time RT-PCR		Focus Diagnostics Inc., U.S.		Peer-reviewed evidence missing[§]		EUA		
	Aptima® Zika Virus Assay (TMA assay)		Hologic Inc., U.S.	Automated Hologic's Panther® system	Peer-reviewed evidence missing[§].	LOD: 13.4 copies/mL; no cross-reactivity identified; sample/s: serum or plasma; IC included	EUA		Company[#]
	Zika Virus Real-time RT-PCR Test		Viracor-IBT Laboratories Inc., U.S.		Peer-reviewed evidence missing[§]	Qualitative detection; no cross-reactivity identified; sample/s: serum, plasma, or urine (collected alongside a patient-matched serum or plasma specimen); IC included	EUA		Company[#]

VERSANT® Zika RNA 1.0 Assay Kit	2 genomic regions of ZIKV	Siemens Healthcare Diagnostics Inc, U.S.	Several	Peer-reviewed evidence missing[§]	LOD: 721 copies/mL Qualitative detection; sample/s: serum, EDTA plasma, or urine (collected alongside a patient-matched serum or plasma specimen); IC included	EUA		Company[#]
xMAP® MultiFLEX™ Zika RNA Assay	6 distinct genomic regions of ZIKV	Luminex Corporation, U.S.	Luminex MAGPIX instrument; Luminex 200	Peer-reviewed evidence missing[§]	LOD: 687 copies/mL; Qualitative detection; sample/s: serum, plasma, or urine (collected alongside a patient-matched serum or plasma specimen); detection of 50-100 analytes per sample; IC included	EUA	Amplification of 6 genomic regions for improved detection of the genetic variation of ZIKV	Company[#]
Sentosa® SA ZIKV RT-PCR Test		Vela Diagnostics U.S., Inc., U.S.	Automated sentosa SX101; SA201 rRT-PCR cycler (ABI 7500 Fast Dx)	Peer-reviewed evidence missing[§]	LOD: 3 x 10^3 copies/mL Time to result: ~3h; No cross reactivity with 44 pathogens; Sample/s: human serum, EDTA plasma and urine.	EUA		Company[#]
Zika Virus Detection by RT-PCR		ARUP Laboratories, U.S.		Peer-reviewed evidence missing[§]		EUA		
Gene-RADAR® Zika Virus Test		Nanobiosym Diagnostics, Inc., U.S.		Peer-reviewed evidence missing[§]		EUA		

prM, precursor membrane; E, envelope; NS5, nonstructural protein 5; NS3, nonstructural protein 3; NS1, non-structural protein 1; RUO research use only; IC internal control; [§]no peer-reviewed studies available at the time of publication of the landscape *other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, positive control; [#]Information provided by the company. In general, there is a lack of peer-reviewed studies for the clinical validation of ZIKV molecular technologies. Further clinical validation is needed.

Appendix Table 3 Multiplex (DENV, ZIKV, CHIKV) NAT assays

Laboratory-based NAT assays

Availability	Assay	Company/ Group	Platform/s	Clinical performance	Other assay characteristics*	Regulatory approval	Comments	References
In the pipeline	ZCD assay	Waggoner et al	ABI7500 (Applied Biosystems)	Good agreement with another DENV and CHIKV RNA detection assay (0.907 and 0.662, respectively). Better performance than comparator ZIKV detection assay.	LOD (copies/mL): ZIKV: 7.8; CHIKV: 13.2; DENV-1: 11.7; DENV-2: 13.5; DENV-3: 4.1; DENV-4: 10.5; no cross-reactivity identified.			(18-20)
On the market	Trioplex Real-time RT-PCR Assay	Thermo Fisher Scientific, U.S	QuantStudio™ 5 (Applied Biosystems)	Peer-reviewed evidence missing[§].	Lyophilized, one-step real-time PCR assay; requires no cold-chain; preferred sample: serum	EUA	Testing must be conducted at qualified laboratories designated by the CDC	Company[#]
	AccuPower® Multiplex Real-Time RT-PCR Kit	Bioneer, Republic of Korean	ExiStation™ and Dual-HotStart™ technology (Bioneer)	Peer-reviewed evidence missing[§]	Master-mix components already aliquoted in each tube; ExiStation™ is a pipetting-free, semi-automated nucleic acid extraction and thermal cycler platform	EUAL CE-IVD		Company[#]
	VIASURE Real Time PCR Detection Kit	Certest Biotec, Spain	Several	Peer-reviewed evidence missing[§]	one-step real-time PCR assay, sample/s: serum, plasma, blood, urine LOD: ≥10 RNA copies/reaction no cross-reactivity identified			Company[#]
	FTD Zika/Dengue/Chik assay	Fast-Track Diagnostics, Malta	Several	Peer-reviewed evidence missing[§]	Sample/s: serum, plasma, urine	CE-IVD		Company[#]
	DiaPlexQ™ ZCD Virus Detection Kit	SolGent Co., Ltd., Republic of Korea	CFX96™ (Bio-Rad); 7500/7500 fast (Applied Biosystems).	Peer-reviewed evidence missing[§]	Sample/s: serum, whole blood; single-tube; Time to result: ~150min			Company[#]

*other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, positive control; [§]no peer-reviewed studies available at the time of publication of the landscape; [#]Information provided by the company. In general, there is a lack of peer-reviewed studies for the clinical validation of DENV, ZIKV and CHIKV multiplex molecular technologies. Further clinical validation is needed.

Appendix Table 4 DENV NS1 antigen detection assays

NS1 detection ELISA assays

Availability	Assay	Company/ Group	Platform/s	Clinical performance[≠]	Other assay characteristics*	Regulatory approval	Comments	References
On the market	Platelia™ Dengue NS1 Ag test	Bio-Rad, France	Plate reader	Sensitivity[∞] (meta-analysis): 74% [95%CI 63-82] Specificity (meta-analysis): 99% [95%CI 97-100]	Format: One-step sandwich ELISA (12x8 wells) Sample: 50µl of serum/plasma Time to result: 140 min Storage: 2-8°C		Performance heterogenieties by country, method and sample type.	(21-24) **(25)**
	Panbio® Dengue Early ELISA	Alere, USA	Plate reader	Sensitivity[∞] (meta-analysis): 66% [95%CI 61-71] Specificity (meta-analysis): 99% [95%CI 96-100]	Format: Capture ELISA (12x8 wells) Sample: 75µl of serum Time to result: 130 min Storage: 2-8°C		Performance heterogenieties by country, method and sample type.	(22-24) **(25)**
	SD Dengue NS1 Ag ELISA	Alere, USA	Plate reader	Sensitivity: 93.3% (112/120); Specificity: 98.9% (178/180)	Format: (12x8 wells) Storage: 2-8°C			**Company**
	DENV Detect NS1 ELISA	InBios International, USA	Plate reader	Sensitivity[∞]: 95.9% [95% CI 86.0-99.5] Specificity: ≥ 95%	Format: (12x8 wells) Sample: 50µl of serum Time to result: 111 min Storage: 2-8°C			**(26)**

NS1 detection Rapid Diagnostic Tests (RDTs)

Availability	Assay	Company/ Group	Platform/s	Clinical performance[≠]	Other assay characteristics*	Regulatory approval	Comments	References
On the market	NS1 Ag STRIP	Bio-Rad, France	Not required	Sensitivities between: 49.4% [95% CI 43.2-55.6] & 98.9% [95% CI 96.8-100] High specificity: ≥ 90%	Format: LF dipstick Sample: 50µl serum/plasma Time to result: 15-30 min Storage: 2-8°C		Performance heterogenieties by country, method and sample type.	**(27, 28)** (23)
	Panbio® Early Rapid NS1	Alere, USA	Not required	Sensitivities between: 58.6% [95% CI 48.2-68.4] & 69.2% [95% CI 62.8-75.6] High specificity: ≥ 92%	Format: LF dipstick Sample: 50µl serum Time to result:15 min Storage: 2-8°C		Performance heterogenieties by country, method and sample type.	**(27, 28)** (23)
	SD Bioline Dengue Duo	Alere, USA	Not required	Sensitivities between: 48.5% [95% CI 38.5-58.7] & 65.4% [95% CI 58.5-72.3] High specificity: ≥ 95%	Format: LF cassette Sample: 105µl serum/plasma/WB Time to result: 15-20 min Storage: RT		Performance heterogenieties by country, method and sample type.	**(27, 28)** (23)
	OnSite Dengue Ag Rapid Test	Ctkbiotech	Not required	Sensitivities: ~40% (acute samples; 1° infection) ~20% (convalescent samples; 2° infection)	Format: LF cassette Sample: 30-45µl plasma; 80-100µl WB Time to result: 20 min		Performance heterogenieties by sample type.	**(23)**
	Dengue NS1 Detect Rapid Test (1st generation)	InBios International, USA	Not required	Sensitivity: 76.5% (52/68) Specificity: 97.4% (1/38)	Format: LF dispstick Sample: 50µl serum Time to result: 30 min Storage: RT			**(26)**

LF lateral flow; WB whole blood; RT room temperature [≠]Evaluation methods are not consistant among the different studies (displayed values refer to referenced study in bold in the last column of the table; clinical performance varies with location, reference method, timing of sample collection and methodology used); *other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, singleplex or multiplex, positive control. In general, sensitivity of NS1-based tests is higher in acute than in convalescent samples and in primary than in secondary infections; sensitivity of ELISA tests is higher than RDTs.

Appendix Table 5 DENV serological assays

Availability	Assay	Antibody target	Company/ Group	Platform/s	Clinical performance≠	Other assay characteristics*	Regulatory approval	Comments	References
Antibody detection ELISA assays									
"in-house"/ on the market	MAC-ELISA	IgM	Several	Plate reader	Sensitivities between: 61.5% and 99.0% Specificities between: 84.4% and 98.0%	Time to result: 4h-72h Storage: 2-8°C 96 well format Sample type: serum	CE-IVD marked: - PATHOZYME® Dengue IgM Capture (Omega diagnostics) - Dengue Virus IgM Capture DxSelect™ (Focus Diagnostics)	Most widely used method; „in house"as well as more than 50 commercial kits; Lacks sensitivity and specificity to diagnose acute infection.	(23, 29, 30)
Antibody detection Rapid Diagnostic Tests (RDTs)									
On the market	IgM capture RDTs	IgM	Several	Not required	Sensitivities between: 20.5% and 98.0% Specificities between: 46.3% and 90.6%	Quick and easy-to-use lateral flow tests Sample type: serum, plasma or whole blood Time to result: 15-30 min Storage: 2-30°C (some assays)	CE-IVD marked: - IMMUNOQuick® Dengue Rapid Test (Biosynex, France) - *OnSite* Dengue IgG/IgM Combo (CTK Biotech, USA)	Well suited to low resource settings; Lacks sensitivity and specificity to diagnose acute infection.	(23, 28, 29, 31-34)

MAC-ELISA (IgM Antibody-Capture-ELISA); ≠Range of sensitivities and specificities accross the different referenced studies (heterogeneity in evaluation methodologies make comparisons among studies difficult; clinical performance varies with location, reference method, timing of sample collection and methodology used); *other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, positive control. In general sensitivity of ELISA tests is higher than RDTs.

Appendix Table 6 ZIKV serological assays

Antibody detection ELISA assays

Availability	Assay	Antibody target	Company/ Group	Platform/s	Clinical performance	Other assay characteristics*	Regulatory approval	Comments	References
on the market	MAC-ELISA	IgM	CDC, U.S.	Plate reader	Peer-reviewed evidence missing[§].	96-well format	EUA		
	ZIKV Detect™ IgM Capture	IgM	InBios International, Inc. U.S.	Plate reader	Peer-reviewed evidence missing[§]		EUA EUAL pipeline		
	LIAISON® XL Zika Capture	IgM	DiaSorin S.p.A., Italy	LIAISON® XL system	Peer-reviewed evidence missing[§]	180 tests/hour; time to result: 17 min. Remote access, conected	EUA		Company[#]
	NovaLisa® ZIKV IgM µ-capture	IgM	NovaTec Immunodiagnostica GmbH, Germany	Plate reader	Sensitivity: 100% Specificity: 98.2% Peer-reviewed evidence missing[§]		CE-IVD EUAL pipeline		Company[#]
	Anti-Zika Virus ELISA	IgM/IgG	EUROIMMUN, Germany	Plate reader	Specificity: 100% Peer-reviewed evidence missing[§].		CE-IVD (RUO) EUAL pipeline		Company[#]
	ZIKV IgM ELISA kit	IgM	DIA.PRO Diagnostic Bioprobes Srl, Italy	Plate reader	Peer-reviewed evidence missing[§]		EUAL pipeline		
	ZIKV IgG ELISA kit	IgG	DIA.PRO Diagnostic Bioprobes Srl, Italy	Plate reader	Peer-reviewed evidence missing[§]		EUAL pipeline		
	STANDARD E Zika IgM ELISA	IgM	SD Biosensor Inc., South Korea	Plate reader	Peer-reviewed evidence missing[§]		EUAL pipeline		

Antibody detection Rapid Diagnostic Tests (RDTs)

Availability	Assay	Antibody target/s	Company/ Group	Platform/s	Clinical performance[≠]	Other assay characteristics*	Regulatory approval	Comments	References
On the market	STANDARD Q Zika IgM/IgG	IgM/IgG	SD Biosensor Inc., South Korea	Not required	Sensitivity: 98.0% Specificity: 100%. Peer-reviewed evidence missing[§].	Sample/s: 10uL serum, plasma, whole blood; time to result: 15min; storage: RT	EUAL pipeline		Company[#]
	DPP® Zika IgM/IgG Assay	IgM/IgG	Chembio Diagnostic Systems Inc., U.S.	DPP micro reader	Good specificity reported Peer-reviewed evidence missing[§].	Sample/s: 10uL serum, plasma, whole blood; time to result: 20min; no cross-reactivity identified	CE-IVD EUAL pipeline Brazil's Health Regulatory Agency		Company[#]
	TELL ME FAST Zika Virus IgG/IgM	IgM/IgG	Biocan, Canada	Not required	Peer-reviewed evidence missing[§].	Sample/s: serum, plasma, whole blood Time to result: 10min	CE-IVD		Company[#]

MAC-ELISA (IgM Antibody-Capture-ELISA); RUO research use only; RT room temperature; [§]no peer-reviewed studies available at the time of publication of the landscap; *other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, positive control; [#]Information provided by company. In general, there is a lack of peer-reviewed studies for clinical validation. Further clinical validation is needed.

Table 7 DENV NS1 & IgM/IgG combination assays

NS1 and Antibody Detection Rapid Diagnostic Tests (RDTs)									
Availability	**Assay**	**Target/s**	**Company/ Group**	**Platform/s**	**Clinical performance≠**	**Other assay characteristics***	**Regulatory approval**	**Comments**	**References**
On the market	SD Dengue Duo RDT (NS1 with IgM/IgG)	NS1 and IgM/IgG	Alere, USA	Not required	Sensitivities between: 57.6% and 98.6% Specificities between: 86.8% and 98.9%	Time to result: 15-20min Storage: 1~30°C		Good sensitivity also using fingerstick whole blood.	(28, 35-41)

≠Range of sensitivities and specificities accross the different referenced studies (heterogeneity in evaluation methodologies make comparisons among studies difficult; clinical performance varies with location, reference method, timing of sample collection and methodology used) *other assay characteristics such as limit-of-detection (LOD), sample type, time to results, storage, integrated sample preparation, random access, connectivity, power supply, cost, positive control. Individual NS1 and IgM/IgG assays (RDT or ELISA) have also been used in combination – in general, the combination of the two assays is more sensitive than the respective individual assays.

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