Chapter 14
Arbovirus Detection in Vectors

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Abstract Detection of arthropod-borne (arbo)viruses is a fundamental element of mosquito surveillance programmes. Moreover, recent advances in modifying mosquitoes for managing arbovirus vector populations rely on sensitive arbovirus detection methods, which are applied at various stages of development, evaluation and production of modified mosquitoes. An increasingly wide range of mosquito trapping, sampling and testing options are available. Although virus culture will remain important for isolating viruses for research and reference purposes, the widespread use and application of real-time reverse transcription polymerase chain reaction (RT-PCR) offers rapid and cost-effective workflows for detecting arbovirus nucleic acid. Advances in next-generation sequencing (NGS) techniques and bioinformatics approaches have also enabled increasingly rapid, accurate and inexpensive arbovirus genome sequencing that can be employed following virus detection using conventional methods or used independently as a stand-alone platform. Unbiased NGS is also a powerful tool for arbovirus discovery and metagenomics. Continued advances in arbovirus detection methods and approaches are expected to provide ever more sophisticated tools for controlling and responding to the threat of pathogenic arboviruses.

Keywords Mosquito traps · Arbovirus detection · Laboratory techniques · Next-generation sequencing

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B. K. Tyagi (ed.), *Genetically Modified and other Innovative Vector Control Technologies*, https://doi.org/10.1007/978-981-16-2964-8_14

14.1 Introduction

As advances are made in the development and application of modified mosquito vectors of human pathogens, the ability to accurately detect target pathogens is critical to assess the effectiveness of genetic or biological modifications to inform field trials and their longer-term implementation. Ensuring laboratory-reared mosquito colonies are pathogen free is important to ensure the integrity of research results. Similarly, arbovirus testing of initiating colonies for modified mosquito production and subsequent screening of derived mass-reared colonies intended for field release is an important element of quality control and biosafety. Detection of vector-borne pathogens also underpins mosquito surveillance programmes for public health purposes: estimation of infection rates allows an assessment of arbovirus transmission risk, while early detection facilitates focussed control efforts. This chapter will focus on mosquito trapping methods and laboratory diagnostic techniques used to detect the major arthropod-borne (arbo)viruses of global public health concern, namely, the flaviviruses dengue virus (DENV), Zika virus (ZIKV), Japanese encephalitis virus (JEV) and West Nile virus (WNV) and the alphavirus chikungunya virus (CHIKV). Each of these viruses has demonstrated an alarming propensity to spread and emerge into new geographic areas and cause disease outbreaks in human and animal populations.

DENV is found throughout the tropics with transmission occurring in over 100 countries and approximately four billion people at risk (Brady et al. 2012). An estimated 394 million infections occurred in 2010, with the majority (~70%) occurring in Asia (Bhatt et al. 2013). First described in Uganda, ZIKV has now spread to over 80 countries in Africa, Asia, the Pacific and the Americas. ZIKV has more recently emerged as an important cause of neurological disease (e.g. congenital Zika and Guillain-Barre syndrome) following major outbreaks in Latin America (Gulland 2016), which were preceded by large outbreaks in the Pacific (Duffy et al. 2009; Musso et al. 2018). JEV is the most important cause of human viral encephalitis in Southeast Asia. Approximately 68,000 human cases occur annually in JE-endemic areas, with high rates of associated morbidity (~30–50%) and mortality (20–30%) (Campbell et al. 2011). WNV is one of the most widely distributed flaviviruses and has been found in Africa, the Middle East, Europe, Asia, Australasia and North America. It has caused large and unexpected outbreaks involving high incidences of neurological infection in humans in Europe and the United States (Hayes and Gubler 2006; Sambri et al. 2013), as well as outbreaks of equine neurological disease and avian mortality events (Castillo-Olivares and Wood 2004; Frost et al. 2012). The alphavirus CHIKV is a major cause of debilitating arthralgia and has been the cause of explosive epidemics. CHIKV has a similar distribution to DENV and can now be found across the tropics. In the past 15 years, CHIKV has spread to the Indian Ocean, the Caribbean and Central and South America; autochthonous transmission has also occurred in Europe and the United States (reviewed in Weaver and Forrester 2015).

These viruses share common mosquito vectors: *Aedes aegypti* and *Ae. albopictus* are major vectors for DENV, ZIKV and CHIKV, while *Culex* species mosquitoes are the main vectors for JEV and WNV. Despite this, major vectors for each virus can vary between regions. For example, *Culex tritaeniorhynchus* is the main vector for JEV in much of Southeast Asia; however, at the edge of its southeastern range in Australasia, the main vector is *Cx. annulirostris* (Ritchie et al. 1997). Hence, understanding the breadth of arbovirus vectors through surveillance is important for focussing control efforts.

Traditional methods of vector surveillance comprise of mosquitoes being collected, identified, pooled by species or other taxonomic grouping, and sent to the laboratory where they are tested for virus infection status using one or more techniques (see below). This approach may be applied for early detection of arboviruses of medical or veterinary importance, determination of virus infection rates, identification of vectors and vector abundance. Depending on resources, surveillance activities may be undertaken year-round in areas endemic for arboviruses or on a seasonal basis in epidemic regions. In areas with low-level mosquito infections or when trapping is undertaken early in the transmission season, efforts should be directed towards performing targeted surveillance at 'hotspots' where a high likelihood of arbovirus presence is suspected; as vector populations increase later in the season, the number of sampling sites should be expanded for broader monitoring.

14.2 Mosquito Traps for Arbovirus Surveillance

A large variety of mosquito traps have been developed for commercial and research and surveillance purposes. Mosquito traps use attractants that are based on olfactory, visual and sometimes temperature and sound signals that mosquitoes are able to sense with their antennae, compound eyes or palpi (Takken and Kline 1989; Daniel 2006).

Commercial traps mostly intended, e.g. for private usage, are usually designed to attract and kill as many mosquitoes and other nuisance insects as possible. They are available in many shape, size and price categories (Mosquito World n.d.). High-end models combine different attractants, mostly UV light, CO_2 gas either produced by burning propane or emanated from cylinders, other olfactory baits (e.g. octenol chips) and heat. Many traps use a fan or otherwise generated suction to aspirate mosquitoes once they have come close to the trap (Ritchie et al. 2007).

Traps used in research studies and for vector surveillance are often intended to target mosquito species more selectively and tend to be designed to allow for less destructive sampling, so that the collected mosquitoes can be studied further. In addition, collections involving very many traps, or in studies in remote locations, ease of use and extended operating duration without maintenance are desirable trap features. As such, traps used in research or for surveillance often use more specific cues or attractants believed to lure specific mosquito species or sub-populations (e.g. gravid, male etc.).

Widely used Biogents (BG) Sentinel Traps, for example, rarely use a light source but attract females of many *Aedes* and *Culex* mosquito species simply by colour contrast of a white lid with a black intake. Once mosquitoes have come close to the intake opening, suction from a fan aspirates them into a catch bag. These traps can further be baited with CO_2 or specific lures to attract other mosquito species as well (Maciel-de-Freitas et al. 2006; Williams et al. 2006).

CDC miniature light traps, widely used for research and surveillance purposes, use incandescent white, coloured LED or UV light as a main attractant. They, too, are equipped with an intake fan and can be set up with CO_2 cylinders (Mathenge et al. 2004).

Some traps developed for research and surveillance purposes, such as Passive Box Traps, focus on collecting nucleic acid samples deposited by mosquitoes, e.g. for arbovirus detection. In Passive Box Traps, nucleic acids are deposited by the mosquitoes while sitting on a (honey-baited) filter paper card. The card can then be removed and subjected to molecular analyses (Ritchie et al. 2013).

Gravid or oviposition traps are designed to trap especially gravid female mosquitoes by resembling a container ovipositioning site. Gravid mosquitoes are older and have blood-fed and are thus more likely to carry arboviruses (Liew et al. 2019; Alemayehu et al. 2018). These traps are usually black containers filled with, e.g. hay-infused water and a geometry allowing for entry, but not exit of the mosquitoes.

Promising new trap developments include, for example, the Male Aedes Sound Trap (MAST), which uses a small speaker assembly to imitate the wingbeat frequency of female mosquitoes, thereby attracting males in the vicinity. These traps can operate for weeks without maintenance, making them an attractive alternative for research and surveillance in remote locations (Staunton et al. 2020, 2021).

Trapping efficiency using any of the aforementioned methods is low, in particular for some anophelines, such as *An. farauti*. Alternatives for adult mosquito collection include the widely used human landing catches (Mathenge et al. 2004), barrier screen (or vertical barrier) methods (Keven et al. 2019), animal- or human-baited double net or tent traps (Govella et al. 2009; Tangena et al. 2015), sweep net collections and resting collections using, for example, manually operated aspirators, such as Prokopacks (Vazquez-Prokopec et al. 2009). In particular human landing catches may be viewed as ethically controversial, as humans are exposed directly to potentially infectious mosquito bites. However, it is believed that normally, exposure in human landing catch collections is equal or less than that normally experienced by the collectors (Gimnig et al. 2013). In addition, studies are usually expected to provide malaria prophylaxis and treatment free of charge to collectors.

14.3 Mosquito Sampling

Processing pooled samples of mosquitoes comes with inherent limitations. Testing individual caught mosquitoes offers a very precise means of determining infection rate. However, mosquito populations often have very low carriage rates, and, to increase the probability of detection, large numbers of mosquitoes are usually required. Under such circumstances, individual mosquito testing may be impractical. Gu and Novak (2004) showed that detection of low levels of mosquito infections requires large samples (>1600 individuals) for a high probability of detection. The authors recommended an intensified sampling strategy at sites where potential vector mosquitoes are abundant, or in areas with a history of arbovirus circulation. Such an approach is cost-effective and increases the probability of detection, which is advantageous if the primary objective of the surveillance activity is early detection.

Estimation of mosquito infection rates is important for determining risk of transmission. In this regard, it has been shown that in situations where mosquito infection rates are high (e.g. peak season) and there may be more than one infected mosquito per pool, the use of variable size pools provides more accurate estimates of infection rates than constant size pooling (Gu and Novak (2004).

The size of mosquito pools for testing can vary in size, but typically pool sizes of 25–100 mosquitoes have been used (e.g. Johansen et al. 2000; Kauffman et al. 2003). However, arboviruses can be detected in larger pools of mosquitoes. Using real-time RT-PCR, a single WNV-infected mosquito could be reliably detected in a pool of 500, and virus isolation and commercial antigen capture assays could detect virus in pools of 200 mosquitoes (Sutherland and Nasci 2007). Tang et al. (2020) showed the detection of CHIKV, WNV, ZIKV and Usutu virus could be achieved in pools comprising up to 1600 mosquitoes using real-time RT-PCR. Molecular testing of pools of 100–1000 mosquitoes is now routinely undertaken by some surveillance programmes for early detection of arboviruses.

Following collection, mosquitoes are typically sorted (e.g. male/female, blood-fed), speciated and pooled, ideally on cold tables to minimise degradation. To preserve samples, freezing whole traps soon after collection or pools following sorting is recommended. Nucleic acid preservative solutions can also be used for molecular testing applications. Mosquito pools are typically homogenised using sterile glass beads or ball bearings in PBS or virus transport medium solutions. Clarified homogenates are then used as inoculum for selected culture systems or for direct testing for antigen or viral RNA.

14.4 Conventional Methods of Arbovirus Detection

Effective vector surveillance requires rapid and accurate methods to identify trapped insect samples and screen them for pathogenic arboviruses. Detection and identification of arboviruses also facilitate research into patterns of virus activity and

movement, by enabling genetic analysis of geographically and temporally distinct isolates and strains. Laboratory testing of mosquitoes involves direct detection of virus from samples or following virus isolation.

14.4.1 Virus Isolation

Historically, arbovirus isolation involved intracerebral inoculation of suckling mice, guinea pigs or hamsters or inoculation of embryonated chicken eggs via the chorio-allantoic or allantoic membranes or the yolk sac (Beaty et al. 1995). Furthermore, the use of animals for scientific purposes normally requires institutional animal ethics approval to ensure animal welfare standards are followed and adhered to. With the establishment of cell lines, virus isolation in cell culture became the gold standard for arbovirus detection from pools of mosquitoes. Several mosquito cell lines have been established that are susceptible to arboviruses (Walker et al. 2014). Notably the C6/36 clone from *Ae. albopictus* is susceptible to a wide range of arboviruses (Singh 1967), in part due to having a dysfunctional innate antiviral RNA interference response (Brackney et al. 2010). A range of mammalian cell lines have also been used to isolate arboviruses, including African green monkey kidney (Vero), rhesus monkey kidney (LLC-MK2), baby hamster kidney (BHK), rabbit kidney (RK-13) and pig kidney (PS) cells. The PS cell clone PSEK has also been used widely for isolation of arboviruses; however, this should be used with caution since it is known to harbour contaminating pestivirus. Alphaviruses and flaviviruses do not normally cause cytopathic effect (CPE) in mosquito cells such as C6/36, and therefore further culture in vertebrate cells in which CPE occurs and/or detection of viral antigen by immunoassay or viral RNA by RT-PCR is required to confirm virus isolation.

Arboviruses can also be isolated using direct inoculation of laboratory colonies of susceptible mosquitoes or mosquito larvae (Rosen and Gubler 1974; Gajanana et al. 1995; Alera et al. 2015). Susceptible mosquito species such as *Ae. albopictus* or *Toxorhynchites splendens* are typically inoculated intracerebrally or intrathoracically with homogenate samples from pools of trapped mosquitoes. For biosafety reasons, male *Aedes* mosquitoes are used as they do not ingest blood; neither male nor female *Toxorhynchites* species ingest blood. Since arbovirus infection does not overtly affect the inoculated mosquitoes, as for propagation in mosquito cell lines, virus detection is undertaken by immunodetection or RT-PCR. A simple method to test inoculated mosquitoes is to perform an indirect immunofluorescence test using a virus-specific antibody on head smears (Gajanana et al. 1995).

Virus isolation techniques are costly, laborious and time-consuming and require specialised laboratories with highly trained personnel. Virus isolation also relies on the presence of infectious virus, which can be degraded in mishandled samples or when cold chain from the point of collection to the laboratory cannot be maintained. Despite these challenges, the ability to isolate and cultivate arbovirus is essential to provide reference isolates for biological, antigenic and pathogenic characterisation, for generating diagnostic reagents and for diagnostic test and vaccine development.

Virus whole genome sequencing is also more efficient and reliable using high-titred virus cultures. Although culture methods remain important in mosquito surveillance activities, molecular methods are increasingly being used for rapid detection of arboviruses in mosquito collections.

14.4.2 Antigen Detection by Immunodetection

Immunodetection methods are commonly employed for detection and identification of arboviruses isolated from or using mosquitoes. These techniques employ species- or group-specific monoclonal antibodies and include antigen capture ELISA to detect arbovirus particles in field-collected mosquito pools, including commercially available tests (Burkhalter et al. 2006; Gajanana et al. 1995; Konishi and Takahashi 1985; Sutherland and Nasci 2007; Kumari et al. 2011), fixed cell enzyme immunoassay following virus propagation (Broom et al. 1998; Johansen et al. 2000; Zhang et al. 1984) and direct or indirect immunofluorescence assay of infected mosquito impression smears from bioassays (Alera et al. 2015; Gajanana et al. 1995). These methods are relatively rapid and inexpensive to perform and, in the case of the ELISA method, can be adapted for high-throughput testing. However, as mentioned above, for virus isolation in cell culture or mosquito bioassay, the preceding steps are laborious and time-consuming.

14.4.3 Polymerase Chain Reaction (PCR)

Molecular testing by reverse transcription polymerase chain reaction (RT-PCR) has become the most commonly employed tool in diagnostic and research laboratories for detecting arbovirus genetic material in mosquito specimens or following culture. Numerous assays have been reported, including both conventional and real-time tests; specific examples are shown in Table 14.1 for DENV, ZIKV, JEV, WNV and CHIKV.

Many of these assays were designed for detection of a particular virus lineage (s) or genotype(s). Therefore, test selection should consider the lineage/genotype of regionally circulating arboviruses. For instance, the WNV real-time PCR reported by Pyke et al. (2004) was developed for the specific detection of WNV-KUNV (lineage 1b), found only in the Australasian region, where other WNV lineages are exotic and alternative assays are required for their detection. For surveillance studies of several target viruses, multiplex or generic assays can be used. These assays are useful to support syndromic surveillance studies targeting viruses that cause similar disease (e.g. febrile or neurological). Flavivirus or alphavirus generic assays can also be employed; however, these can be less sensitive, particularly in the conventional formats. Generic assays—when coupled with sequencing—can be useful for

Table 14.1 RT-PCR assays used to detect dengue, Zika, Japanese encephalitis, West Nile and chikungunya viruses in mosquitoes

Virus	Format	Genome target(s)	References
Dengue virus 1 to 4	Conventional	E gene	Balingit et al. (2020)
		Capsid/prM genes	Johnson et al. (2005)
	Real time	NS5 (DENV-1) E gene (DENV-2) prM/M gene (DENV-3) prM/M-E genes (DENV-4)	Balingit et al. (2020), Johnson et al. (2005)
		NS5	Hue et al. (2011)
Zika	Conventional	E gene	Faye et al. (2008)
		NS5 gene	Balm et al. (2012)
	Real time	NS5	Faye et al. (2013)
		M-E genes E gene	Lanciotti et al. (2008)
		NS1 gene E genes	Pyke et al. (2014)
Japanese encephalitis	Real time	NS5–3′UTR	Pyke et al. (2004)
West Nile	Conventional	E gene	Johnson et al. (2001)
	Real time	E gene 3′UTR	Lanciotti et al. (2000)
		NS5-3′UTR	Pyke et al. (2004)
Chikungunya	Conventional	nsp1 gene	Hasebe et al. (2002)
	Real time	E1 gene	van den Hurk et al. (2010)
		nsp1 gene nsp4 gene	Lanciotti et al. (2007)
Flavivirus generic	Conventional	NS5-3′UTR	Pierre et al. (1994)
		E gene	Gaunt and Gould (2005)
		NS5 gene	Scaramozzino et al. (2001)
	Real time	NS5 gene	Moureau et al. (2007)
		NS5	Patel et al. (2013)
Alphavirus generic	Conventional	nsP1 gene	Pfeffer et al. (1997)
		nsP4 gene	Sanchez-Seco et al. (2001)
		nsP4 gene	Grywna et al. (2010)
	Real time	nsP4 gene	Giry et al. (2017)
Multiplex	Real time	5′-UTR (DENV) nsp1 gene (CHIKV) E gene (ZIKV)	Santiago et al. (2018)
		3′UTR (DENV) NS5 gene (CHIKV) E gene (ZIKV)	Mansuy et al. (2018)
		NS5 (YFV, JEV, WNV, SLEV, DENV-1 to -4)	Chao et al. (2007)
		NS2A (WNV, JEV)	Barros et al. (2013)

inexpensive and rapid identification of virus isolates from mosquito samples when other available tests have failed.

Other formats of molecular testing have also been reported for arbovirus laboratory diagnosis. RT-LAMP assays have been published for the detection of JEV (Liu et al. 2012; Parida et al. 2006), WNV (Parida et al. 2004), ZIKV (Silva et al. 2019), DENV (Lopez-Jimena et al. 2018) and CHIKV (Parida et al. 2007). Multiplex RT-LAMP assays have also been reported for detecting combinations of DENV, ZIKV, CHIKV, JEV or WNV (Li et al. 2011; Yaren et al. 2017). LAMP offers comparable performance to real-time RT-PCR, in a simple and convenient assay format without the need for sophisticated equipment or highly trained personnel. At the other end of the technology spectrum, RT-PCR-based microsphere array assays have been reported for the multiplex detection of medically important flaviviruses and alphaviruses from mosquitoes (Foord et al. 2014; Glushakova et al. 2019). Although these assays require specialised equipment and trained staff to run the assays, they can offer high-throughput multiplexed testing for arbovirus surveillance activities.

Selection of a molecular test should also consider prior validation using infected mosquitoes. In-house test verification is recommended to ensure the assay is fit for purpose in the laboratory where it will be applied. This may be undertaken using mosquito samples that are known to be infected from prior testing or using mosquito pools spiked with a known quantity of the target virus. This is to ensure that variation in laboratory conditions and equipment, extraction methods and reaction chemistries do not affect the sensitivity or performance of the selected assay.

14.5 Next-Generation Sequencing Methods for Arbovirus Surveillance

Conventional diagnostic test methods typically target certain pathogens based on likelihood and risk; however, this approach fails to detect novel or unexpected arboviruses when present in mosquito samples. Next-generation sequencing (NGS) describes a DNA or RNA sequencing technology which has revolutionised genomic research (Behjati and Tarpey 2013) and has been applied to vector-borne disease surveillance for the identification of both known and previously unknown arboviruses. Although several NGS platforms are available, they all perform sequencing of millions of small fragments of nucleotides in parallel. Appropriate bioinformatics are then used to remove host sequences and assemble target sequence fragments either as de novo synthesis or mapped to known genomes. Total RNA sequencing enables non-targeted, high-throughput detection and characterisation of viruses in a sample, such as mosquitoes.

Combined with metabarcoding, NGS can allow the rapid identification of large numbers of mosquitoes with simultaneous screening for pathogens (Batovska et al.

2018). Based on the sensitivity of the technique, this method also can quantify the number of mosquitoes in a trap. In Australia, viral metagenomics has been used for the identification of multiple arboviruses, including novel rhabdoviruses, bunyaviruses (Quan et al. 2011; Coffey et al. 2014; Briese et al. 2016), ephemeroviruses (Blasdell et al. 2014) and mesoniviruses (Warrilow et al. 2014) from field collected mosquitoes.

By combining unbiased sequencing, rapid data analysis and comprehensive reference databases, metagenomics can be applied for hypothesis-free, universal pathogen detection, providing a promising approach to improved surveillance of arboviruses. Several studies have used NGS approaches to detect viruses in individual mosquitoes or pools of mosquitoes using various technologies. There are currently several NGS platforms available for use, including Illumina (Chandler et al. 2015), Ion Torrent (Hall-Mendelin et al. 2013) and Oxford Nanopore (Batovska et al. 2017), each with its own benefits and limitations. The laboratory workflow is also determined by the technology used, and this can limit feasibility of usage. It is important that the surveillance laboratory should clearly define the intended use, range of pathogens and reporting workflow as this will determine choice of data analysis. Since bioinformatics forms a key aspect of the workflow, it is important to validate the bioinformatics pipeline along with laboratory techniques. The bioinformatics analyses chosen to process the NGS reads (e.g., Flygare et al. 2016; Andrusch et al. 2018; Oulas et al. 2015; Naccache et al. 2014) can also affect sensitivity and specificity. A common method used to detect viruses in a sample is by mapping reads back to viral reference sequences. However, when dealing with short reads, this can lead to false-positive results if a virus is present with partial sequence homology to a virus of interest. Recent advances in bioinformatics can overcome this by de novo assembly of reads to produce longer contiguous sequences (contigs) (Schlaberg et al. 2017).

There are numerous benefits of using NGS for surveillance of mosquito-borne pathogens. Advances in the NGS technology in recent years have allowed for detection of all viruses in mosquito samples in a cost-effective and unbiased manner. This methodology can be used to detect both known and unknown viruses and bacteria. Due to the untargeted approach, the method allows for accurate detection of mosquito species as well and can serve as an early warning system for invasive mosquitoes (Batovska et al. 2018). With appropriate protocols, it can be very sensitive, detecting low quantities of pathogen nucleic acid. Multiplexing with barcoding can help in higher throughput of sample processing (Batovska et al. 2018). NGS data analysis with sufficient read depth and coverage can also inform about mutation and variants circulating or evolving in the environment. This not only can help in performing molecular epidemiology (Ko et al. 2020; Bialosuknia et al. 2019; Maan et al. 2015; Johnson et al. 2012) and identifying source and sinks of pathogens in mosquito populations but also can help in formulating appropriate strategy against vector and pathogen spread.

The major drawback of use of NGS in surveillance is the infrastructure cost of setting up the instruments. The high costs, long sequencing times and slow, unwieldy data analysis tools have made it impractical for wider use of these

methods. With advances in instrumentation and sequencing chemistries, the costs for sequencing are dropping and the amount of data being generated is increasing; however, the current bottleneck is the bioinformatics pipeline for large data analysis and appropriate interpretation.

Appropriate sample preparation determines the analytical sensitivity and specificity of the assay, which is important in assessing the transmission risk and temporal changes in virus abundance. Samples can be a single mosquito, pool of mosquitoes or honey-baited FTA cards (Birnberg et al. 2020). The quality of the generated data depends on the stability of RNA in the sample; hence appropriate storage conditions of samples, such as in RNAlater, are necessary. Sensitivity of detection can be increased artificially by enriching for arbovirus using size filtration (Sadeghi et al. 2018), PEG precipitation or sequence-independent amplification (Xiao et al. 2018). While this does increase the number of viral sequences, enrichment can also introduce bias (Conceição-Neto et al. 2015). An alternate way to increase the number of viral sequences is by depleting the mosquito RNA, generally by targeting highly abundant ribosomal RNA (rRNA). A variety of rRNA depletion kits are available; however, these are not specific to mosquitoes, and so custom probes based on mosquito rRNA sequences need to be generated (Fauver et al. 2019).

There are also bioinformatics tools and approaches to increase the specificity of pathogen sequence detection, such as performing de novo assembly, where short reads are assembled into longer contigs, and then comparing these contigs to a database containing viral reference sequences. This approach can improve specificity because longer fragments are taxonomically classified with greater accuracy (McHardy et al. 2007).

A recent study (Batovska et al. 2019) in a lab setting showed that NGS was highly specific in identification of Ross River virus or Umatilla virus in mosquito pools spiked with these viruses, recovering whole genome information and detecting 19 other viruses. However, the method was not as sensitive as RT-qPCR or RT-ddPCR.

NGS generates several million to billion short-read sequences of the DNA and RNA isolated from a sample. In contrast to traditional Sanger sequencing, with read lengths of 500–900 base pairs (bp), short reads of NGS range in size from 75 to 300 bp depending on the application and sequencing chemistry. Newer NGS technologies such as those from PacBio (Rhoads and Au 2015), Nanopore and 10× Genomics (Singh et al. 2019) enable longer read sequences of more than 10 kb.

Until recently, NGS was restricted to the laboratory due to the size of the sequencers available. MinION by Oxford Nanopore Technologies provides a powerful tool for in-field surveillance, allowing non-targeted (unbiased) detection of viruses in a sample within a few hours. The MinION is a relatively low-cost, handheld sequencer. Although it's sequencing accuracy is considerably lower than Illumina MiSeq and HiSeq, with an error rate of approximately 5–10% (Tyson et al. 2018) for the recent MinION R9 chemistry with 2D (double-strand) reads, compared to <0.1% for the Illumina sequencers (Houldcroft et al. 2017), MinION makes it up by producing long reads (up to 233 kb) in real time (Jansen et al. 2017) and achieving >99% accuracy post-data analysis (Wang et al. 2015). It has recently

been demonstrated that the MinION can be used for metagenomic arbovirus detection from infected mosquitoes (Batovska et al. 2017), so it could be used during arbovirus outbreaks. Even with its limitations, it is expected that MinION will play a significant role in making sequencing available in real time, helping appropriate public health response.

Xenosurveillance is a novel surveillance technique that leverages and extends mosquito surveillance activities to the detection of non-vectored pathogens using PCR- or NGS-based methods. With mosquitoes feeding on a variety of vertebrate hosts, they also have potential to act as samplers for circulating viruses present in host blood. This can offer an alternative to direct sampling of hosts (such as sentinel animals), with mosquitoes acting as 'syringes'. Other than acting as surveillance for arboviruses (Yang et al. 2015), it can also serve as surveillance for non-vector-borne pathogens (Grubaugh et al. 2015).

These emerging technologies have also translated into trap development as well with development of the next generation of mosquito traps. Other than previously mentioned use of NGS on honey-baited FTA cards to detect circulating viruses in mosquito traps, technology companies such as Microsoft are developing mosquito traps ('Microsoft Premonition' n.d.) which have in situ NGS technology. The idea behind this is using drone technology to position these traps at select locations and using baits to attract female mosquitoes, which are photographed followed by whole genome sequencing to generate pathogen profiles. An alternative to this which has also been proposed includes in situ technology to detect specific arboviruses using lateral flow technology. Although these traps are not used widely, they are currently being trialled in various locations for feasibility studies.

14.6 Conclusion

In recent years there have been numerous advances in the methods and approaches used for arbovirus detection. Together with tried and tested conventional methods, there is an array of options that can be applied to different situations and conditions, as well as budgets and resources. While most of these methods have been developed for and applied to arbovirus surveillance programs, they also have a place in the laboratories of researchers working on modified mosquitoes and the companies and factories that rear the millions of modified mosquitoes required for field release. In the future, molecular diagnostic methods such as real-time RT-PCR will likely remain a workhorse platform for arbovirus laboratories; however, it is expected that advancements in NGS technologies will continue, providing increasingly inexpensive, accessible and sensitive platforms that can be applied to arbovirus surveillance and mosquito testing in the field and laboratory.

References

Alemayehu D, Reyes T, Haas-Stapleton EJ (2018) Field evaluation of a redesigned oviposition trap to monitor gravid invasive *Aedes* mosquitoes in a suburban environment. J Am Mosq Control Assoc 34(1):67–69

Alera MT, Hermann L, Tac-An IA, Klungthong C, Rutvisuttinunt W, Manasatienkij W, Villa D, Thaisomboonsuk B, Velasco JM, Chinnawirotpisan P, Lago CB, Roque VG Jr, Macareo LR, Srikiatkhachorn A, Fernandez S, Yoon IK (2015) Zika virus infection, Philippines, 2012. Emerg Infect Dis 21:722–724

Andrusch A, Dabrowski PW, Klenner J, Tausch SH, Kohl C, Osman AA, Renard BY, Nitsche A (2018) PAIPline: pathogen identification in metagenomic and clinical next generation sequencing samples. Bioinformatics 34(17):i715–i721

Balingit JC, Carvajal TM, Saito-Obata M, Gamboa M, Nicolasora AD, Sy AK, Oshitani H, Watanabe K (2020) Surveillance of dengue virus in individual Aedes aegypti mosquitoes collected concurrently with suspected human cases in Tarlac City, Philippines. Parasit Vectors 13(1):594

Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW (2012) A diagnostic polymerase chain reaction assay for Zika virus. J Med Virol 84(9):1501–1505

Barros SC, Ramos F, Zé-Zé L, Alves MJ, Fagulha T, Duarte M, Henriques M, Luís T, Fevereiro M (2013) Simultaneous detection of West Nile and Japanese encephalitis virus RNA by duplex TaqMan RT-PCR. J Virol Methods 193(2):554–557

Batovska J, Lynch SE, Rodoni BC, Sawbridge TI, Cogan NO (2017) Metagenomic arbovirus detection using MinION nanopore sequencing. J Virol Methods 249:79–84

Batovska J, Lynch SE, Cogan NOI, Brown K, Darbro JM, Kho EA, Blacket MJ (2018) Effective mosquito and arbovirus surveillance using metabarcoding. Mol Ecol Resour 18(1):32–40

Batovska J, Mee PT, Lynch SE, Sawbridge TI, Rodoni BC (2019) Sensitivity and specificity of metatranscriptomics as an arbovirus surveillance tool. Sci Rep 9(1):19398

Beaty BJ, Calisher CH, Shope RE (1995) Arboviruses. In: Lennette EH, Lennette DA, Lennette ET (eds) Diagnostic procedures for viral, rickettsial and chlamydial infections, 7th edn. American Public Health Association, Washington, DC, pp 189–212

Behjati S, Tarpey PS (2013) What is next generation sequencing? Arch Dis Child Educ Pract Ed 98 (6):236–238

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI (2013) The global distribution and burden of dengue. Nature 496:504–507

Bialosuknia SM, Tan Y, Zink SD, Koetzner CA, Maffei JG, Halpin RA, Muller E, Novatny M, Shilts M, Fedorova NB, Amedeo P, Das SR, Pickett B, Kramer LD, Ciota AT (2019) Evolutionary dynamics and molecular epidemiology of West Nile virus in New York state: 1999–2015. Virus Evol 5(2):vez020

Birnberg L, Temmam S, Aranda C, Correa-Fiz F, Talavera S, Bigot T, Eloit M, Busquets N (2020) Viromics on honey-baited FTA cards as a new tool for the detection of circulating viruses in mosquitoes. Viruses 12(3):274

Blasdell KR, Widen SG, Diviney SM, Firth C, Wood TG, Guzman H, Holmes EC, Tesh RB, Vasilakis N, Walker PJ (2014) Koolpinyah and Yata viruses: two newly recognised ephemeroviruses from tropical regions of Australia and Africa. Vet Microbiol 174 (3–4):547–553

Brackney DE, Scott JC, Sagawa F, Woodward JE, Miller NA, Schilkey FD, Mudge J, Wilusz J, Olson KE, Blair CD, Ebel GD (2010) C6/36 *Aedes albopictus* cells have a dysfunctional antiviral RNA interference response. PLoS Negl Trop Dis 4(10):e856

Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, Moyes CL, Farlow AW, Scott TW, Hay SI (2012) Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis 6(8):e1760

Briese T, Williams DT, Kapoor V, Diviney SM, Certoma A, Wang J, Johansen CA, Chowdhary R, Mackenzie JS, Lipkin WI (2016) Analysis of arbovirus isolates from Australia identifies novel bunyaviruses including a Mapputta group virus from Western Australia that links Gan Gan and Maprik viruses. PLoS One 11(10):e0164868

Broom AK, Hall RA, Johansen CA, Oliveira N, Howard MA, Lindsay MD, Kay BH, Mackenzie JS (1998) Identification of Australian arboviruses in inoculated cell cultures using monoclonal antibodies in ELISA. Pathology 30(3):286–288

Burkhalter KL, Lindsay R, Anderson R, Dibernardo A, Fong W, Nasci RS (2006) Evaluation of commercial assays for detecting West Nile virus antigen. J Am Mosq Control Assoc 22 (1):64–69

Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, Marfin AA, Solomon T, Tsai TF, Tsu VD, Ginsburg AS (2011) Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ 89(10):766–774, 774A-774E

Castillo-Olivares J, Wood J (2004) West Nile virus infection of horses. Vet Res 35(4):467–483

Chandler JA, Liu RM, Bennett SN (2015) RNA shotgun metagenomic sequencing of Northern California (USA) mosquitoes uncovers viruses, bacteria, and fungi. Front Microbiol 6:185

Chao DY, Davis BS, Chang GJ (2007) Development of multiplex real-time reverse transcriptase PCR assays for detecting eight medically important flaviviruses in mosquitoes. J Clin Microbiol 45(2):584–589

Coffey LL, Page BL, Greninger AL, Herring BL, Russell RC, Doggett SL, Haniotis J, Wang C, Deng X, Delwart EL (2014) Enhanced arbovirus surveillance with deep sequencing: identification of novel rhabdoviruses and bunyaviruses in Australian mosquitoes. Virology 448:146–158

Conceição-Neto N, Zeller M, Lefrère H, De Bruyn P, Beller L, Deboutte W, Yinda CK, Lavigne R, Maes P, Van Ranst M, Heylen E, Matthijnssens J (2015) Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. Sci Rep 5:16532

Daniel LK (2006) Traps and trapping techniques for adult mosquito control. J Am Mosq Control Assoc 22(3):490–496

Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, Guillaumot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB (2009) Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 360(24):2536–2543

Fauver JR, Akter S, Morales AIO, Black WC IV, Rodriguez AD, Stenglein MD, Ebel GD, Weger-Lucarelli J (2019) A reverse-transcription/RNase H based protocol for depletion of mosquito ribosomal RNA facilitates viral intrahost evolution analysis, transcriptomics and pathogen discovery. Virology 528:181–197

Faye O, Faye O, Dupressoir A, Weidmann M, Ndiaye M, Alpha Sall A (2008) One-step RT-PCR for detection of Zika virus. J Clin Virol 43(1):96–101

Faye O, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA (2013) Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol J 10:311

Flygare S, Simmon K, Miller C, Qiao Y, Kennedy B, Di Sera T, Graf EH, Tardif KD, Kapusta A, Rynearson S, Stockmann C, Queen K, Tong S, Voelkerding KV, Blaschke A, Byington CL, Jain S, Pavia A, Ampofo K, Eilbeck K, Marth G, Yandell M, Schlaberg R (2016) Taxonomer: an interactive metagenomics analysis portal for universal pathogen detection and host mRNA expression profiling. Genome Biol 17(1):111

Foord AJ, Boyd V, White JR, Williams DT, Colling A, Heine HG (2014) Flavivirus detection and differentiation by a microsphere array assay. J Virol Methods 203:65–72

Frost MJ, Zhang J, Edmonds JH, Prow NA, Gu X, Davis R, Hornitzky C, Arzey KE, Finlaison D, Hick P, Read A, Hobson-Peters J, May FJ, Doggett SL, Haniotis J, Russell RC, Hall RA, Khromykh AA, Kirkland PD (2012) Characterization of virulent West Nile virus Kunjin strain, Australia, 2011. Emerg Infect Dis 18(5):792–800

Gajanana A, Rajendran R, Thenmozhi V, Philip Samuel P, Tsai TF, Reuben R (1995) Comparative evaluation of bioassay and ELISA for detection of Japanese encephalitis virus in field collected mosquitoes. Southeast Asian J Trop Med Public Health 26:91–97

Gaunt MW, Gould EA (2005) Rapid subgroup identification of the flaviviruses using degenerate primer E-gene RT-PCR and site specific restriction enzyme analysis. J Virol Methods 128:113–127

Gimnig JE, Walker ED, Otieno P, Kosgei J, Olang G, Ombok M, Williamson J, Marwanga D, Abong'o D, Desai M, Kariuki S, Hamel MJ, Lobo NF, Vulule J, Bayoh MN (2013) Incidence of malaria among mosquito collectors conducting human landing catches in western Kenya. Am J Trop Med Hyg 88(2):301–308

Giry C, Roquebert B, Li-Pat-Yuen G, Gasque P, Jaffar-Bandjee MC (2017) Improved detection of genus-specific alphavirus using a generic TaqMan® assay. BMC Microbiol 17(1):164

Glushakova LG, Alto BW, Kim MS, Hutter D, Bradley A, Bradley KM, Burkett-Cadena ND, Benner SA (2019) Multiplexed kit based on Luminex technology and achievements in synthetic biology discriminates Zika, chikungunya, and dengue viruses in mosquitoes. BMC Infect Dis 19 (1):418

Govella NJ, Chaki PP, Geissbuhler Y, Kannady K, Okumu F, Charlwood JD, Anderson RA, Killeen GF (2009) A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex. Malar J 8:157

Grubaugh ND, Sharma S, Krajacich BJ, Fakoli LS III, Bolay FK, Diclaro JW II, Johnson WE, Ebel GD, Foy BD, Brackney DE (2015) Xenosurveillance: a novel mosquito-based approach for examining the human-pathogen landscape. PLoS Negl Trop Dis 9(3):e0003628

Grywna K, Kupfer B, Panning M, Drexler JF, Emmerich P, Drosten C, Kummerer BM (2010) Detection of all species of the genus alphavirus by reverse transcription-PCR with diagnostic sensitivity. J Clin Microbiol 48(9):3386–3387

Gu W, Novak RJ (2004) Short report: Detection probability of arbovirus infection in mosquito populations. Am J Trop Med Hyg 71(5):636–638

Gulland A (2016) Zika virus is a global public health emergency, declares WHO. BMJ 352:i657

Hall-Mendelin S, Allcock R, Kresoje N, van den Hurk AF, Warrilow D (2013) Detection of arboviruses and other micro-organisms in experimentally infected mosquitoes using massively parallel sequencing. PLoS One 8(2):e58026

Hasebe F, Parquet MC, Pandey BD, Mathenge EG, Morita K, Balasubramaniam V, Saat Z, Yusop A, Sinniah M, Natkunam S, Igarashi A (2002) Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. J Med Virol 67(3):370–374

Hayes EB, Gubler DJ (2006) West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. Annu Rev Med 57:181–194

Houldcroft CJ, Beale MA, Breuer J (2017) Clinical and biological insights from viral genome sequencing. Nat Rev Microbiol 15(3):183–192

Hue KD, Tuan TV, Thi HT, Bich CT, Anh HH, Wills BA, Simmons CP (2011) Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma. J Virol Methods 177(2):168–173

Jansen HJ, Liem M, Jong-Raadsen SA, Dufour S, Weltzien FA, Swinkels W, Koelewijn A, Palstra AP, Pelster B, Spaink HP, Thillart GEVD, Dirks RP, Henkel CV (2017) Rapid *de novo* assembly of the European eel genome from nanopore sequencing reads. Sci Rep 7(1):7213

Johansen CA, van den Hurk AF, Ritchie SA, Zborowski P, Nisbet DJ, Paru R, Bockarie MJ, Macdonald J, Drew AC, Khromykh TI, Mackenzie JS (2000) Isolation of Japanese encephalitis virus from mosquitoes (Diptera: Culicidae) collected in the Western Province of Papua New Guinea, 1997–1998. Am J Trop Med Hyg 62(5):631–638

Johnson DJ, Ostlund EN, Pedersen DD, Schmitt BJ (2001) Detection of North American West Nile virus in animal tissue by a reverse transcription-nested polymerase chain reaction assay. Emerg Infect Dis 7(4):739–741

Johnson BW, Russell BJ, Lanciotti RS (2005) Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol 43(10):4977–4983

Johnson N, Voller K, Phipps LP, Mansfield K, Fooks AR (2012) Rapid molecular detection methods for arboviruses of livestock of importance to northern Europe. J Biomed Biotechnol 2012:719402

Kauffman EB, Jones SA, Dupuis AP II, Ngo KA, Bernard KA, Kramer LD (2003) Virus detection protocols for West Nile virus in vertebrate and mosquito specimens. J Clin Microbiol 41 (8):3661–3667

Keven JB, Katusele M, Vinit R, Koimbu G, Vincent N, Thomsen EK, Karl S, Reimer LJ, Walker ED (2019) Species abundance, composition, and nocturnal activity of female *Anopheles* (Diptera: Culicidae) in malaria-endemic villages of Papua New Guinea: assessment with barrier screen sampling. Malar J 18(1):96

Ko HY, Salem GM, Chang GJ, Chao DY (2020) Application of next-generation sequencing to reveal how evolutionary dynamics of viral population shape dengue epidemiology. Front Microbiol 11:1371

Konishi E, Takahashi J (1985) Detection of chikungunya virus antigen in *Aedes albopictus* mosquitoes by enzyme-linked immunosorbent assay. J Virol Methods 12(3–4):279–285

Kumari R, Kumar K, Chauhan LS (2011) First dengue virus detection in *Aedes albopictus* from Delhi, India: its breeding ecology and role in dengue transmission. Trop Med Int Health 16 (8):949–954

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT (2000) Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol 38(11):4066–4071

Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, Campbell GL (2007) Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis 13(5):764–767

Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR (2008) Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 14(8):1232–1239

Li S, Fang M, Zhou B, Ni H, Shen Q, Zhang H, Han Y, Yin J, Chang W, Xu G, Cao G (2011) Simultaneous detection and differentiation of dengue virus serotypes 1–4, Japanese encephalitis virus, and West Nile virus by a combined reverse-transcription loop-mediated isothermal amplification assay. Virol J 8:360

Liew JWK, Selvarajoo S, Tan W, Ahmad Zaki R, Vythilingam I (2019) Gravid oviposition sticky trap and dengue non-structural 1 antigen test for early surveillance of dengue in multi-storey dwellings: study protocol of a cluster randomized controlled trial. Infect Dis Poverty 8(1):71

Liu H, Liu ZJ, Jing J, Ren JQ, Liu YY, Guo HH, Fan M, Lu HJ, Jin NY (2012) Reverse transcription loop-mediated isothermal amplification for rapid detection of Japanese encephalitis virus in swine and mosquitoes. Vector Borne Zoonotic Dis 12(12):1042–1052

Lopez-Jimena B, Bekaert M, Bakheit M, Frischmann S, Patel P, Simon-Loriere E, Lambrechts L, Duong V, Dussart P, Harold G, Fall C, Faye O, Sall AA, Weidmann M (2018) Development and validation of four one-step real-time RT-LAMP assays for specific detection of each dengue virus serotype. PLoS Negl Trop Dis 12(5):e0006381

Maan S, Maan NS, Belaganahalli MN, Rao PP, Singh KP, Hemadri D, Putty K, Kumar A, Batra K, Krishnajyothi Y, Chandel BS, Reddy GH, Nomikou K, Reddy YN, Attoui H, Hegde NR, Mertens PP (2015) Full-genome sequencing as a basis for molecular epidemiology studies of bluetongue virus in India. PLoS One 10(6):e0131257

Maciel-de-Freitas R, Eiras AE, Lourenco-de-Oliveira R (2006) Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: *Culicidae*). Mem Inst Oswaldo Cruz 101(3):321–325

Mansuy JM, Lhomme S, Cazabat M, Pasquier C, Martin-Blondel G, Izopet J (2018) Detection of Zika, dengue and chikungunya viruses using single-reaction multiplex real-time RT-PCR. Diagn Microbiol Infect Dis 92(4):284–287

Mathenge EM, Omweri GO, Irungu LW, Ndegwa PN, Walczak E, Smith TA, Killeen GF, Knols BG (2004) Comparative field evaluation of the Mbita trap, the Centers for Disease Control light trap, and the human landing catch for sampling of malaria vectors in western Kenya. Am J Trop Med Hyg 70(1):33–37

McHardy AC, Martín HG, Tsirigos A, Hugenholtz P, Rigoutsos I (2007) Accurate phylogenetic classification of variable-length DNA fragments. Nat Methods 4(1):63–72

Microsoft Premonition (n.d.) Microsoft Research (blog). https://www.microsoft.com/en-us/research/project/project-premonition/. Accessed 29 Jan 2021

Mosquito World (n.d.) Mosquito trap reviews 2021. http://www.mosquitoworld.net/mosquito-control/trap-reviews/. Accessed 29 Jan 2021

Moureau G, Temmam S, Gonzalez JP, Charrel RN, Grard G, de Lamballerie X (2007) A real-time RT-PCR method for the universal detection and identification of flaviviruses. Vector Borne Zoonotic Dis 7(4):467–477

Musso D, Bossin H, Mallet HP, Besnard M, Broult J, Baudouin L, Levi JE, Sabino EC, Ghawche F, Lanteri MC, Baud D (2018) Zika virus in French Polynesia 2013–14: anatomy of a completed outbreak. Lancet Infect Dis 18(5):e172–e182

Naccache SN, Federman S, Veeraraghavan N, Zaharia M, Lee D, Samayoa E, Bouquet J, Greninger AL, Luk KC, Enge B, Wadford DA, Messenger SL, Genrich GL, Pellegrino K, Grard G, Leroy E, Schneider BS, Fair JN, Martínez MA, Isa P, Crump JA, DeRisi JL, Sittler T, Hackett J Jr, Miller S, Chiu CY (2014) A cloud-compatible bioinformatics pipeline for ultrarapid pathogen identification from next-generation sequencing of clinical samples. Genome Res 24 (7):1180–1192

Oulas A, Pavloudi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas G, Arvanitidis C, Iliopoulos I (2015) Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinform Biol Insights 9:75–88

Parida M, Posadas G, Inoue S, Hasebe F, Morita K (2004) Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of West Nile virus. J Clin Microbiol 42 (1):257–263

Parida MM, Santhosh SR, Dash PK, Tripathi NK, Saxena P, Ambuj S, Sahni AK, Lakshmana Rao PV, Morita K (2006) Development and evaluation of reverse transcription-loop-mediated isothermal amplification assay for rapid and real-time detection of Japanese encephalitis virus. J Clin Microbiol 44(11):4172–4178

Parida MM, Santhosh SR, Dash PK, Tripathi NK, Lakshmi V, Mamidi N, Shrivastva A, Gupta N, Saxena P, Babu JP, Rao PV, Morita K (2007) Rapid and real-time detection of chikungunya virus by reverse transcription loop-mediated isothermal amplification assay. J Clin Microbiol 45 (2):351–357

Patel P, Landt O, Kaiser M, Faye O, Koppe T, Lass U, Sall AA, Niedrig M (2013) Development of one-step quantitative reverse transcription PCR for the rapid detection of flaviviruses. Virol J 10:58

Pfeffer M, Proebster B, Kinney RM, Kaaden OR (1997) Genus-specific detection of alphaviruses by a semi-nested reverse transcription-polymerase chain reaction. Am J Trop Med Hyg 57 (6):709–718

Pierre V, Drouet MT, Deubel V (1994) Identification of mosquito-borne flavivirus sequences using universal primers and reverse transcription/polymerase chain reaction. Res Virol 145:93–104

Pyke AT, Smith IL, van den Hurk AF, Northill JA, Chuan TF, Westacott AJ, Smith GA (2004) Detection of Australasian Flavivirus encephalitic viruses using rapid fluorogenic TaqMan RT-PCR assays. J Virol Methods 117(2):161–167

Pyke AT, Daly MT, Cameron JN, Moore PR, Taylor CT, Hewitson GR, Humphreys JL, Gair R (2014) Imported zika virus infection from the Cook Islands into Australia, 2014. PLoS Curr 6: ecurrents.outbreaks.4635a54dbffba2156fb2fd76dc49f65e

Quan PL, Williams DT, Johansen CA, Jain K, Petrosov A, Diviney SM, Tashmukhamedova A, Hutchison SK, Tesh RB, Mackenzie JS, Briese T, Lipkin WI (2011) Genetic characterization of K13965, a strain of Oak Vale virus from Western Australia. Virus Res 160(1–2):206–213

Rhoads A, Au KF (2015) PacBio sequencing and its applications. Genomics Proteomics Bioinformatics 13(5):278–289

Ritchie SA, Phillips D, Broom A, Mackenzie J, Poidinger M, van den Hurk A (1997) Isolation of Japanese encephalitis virus from *Culex annulirostris* in Australia. Am J Trop Med Hyg 56 (1):80–84

Ritchie SA, van den Hurk AF, Zborowski P, Kerlin TJ, Banks D, Walker JA, Lee JM, Montgomery BL, Smith GA, Pyke AT, Smith IL (2007) Operational trials of remote mosquito trap systems for Japanese encephalitis virus surveillance in the Torres Strait, Australia. Vector Borne Zoonotic Dis 7(4):497–506

Ritchie SA, Cortis G, Paton C, Townsend M, Shroyer D, Zborowski P, Hall-Mendelin S, Van Den Hurk AF (2013) A simple non-powered passive trap for the collection of mosquitoes for arbovirus surveillance. J Med Entomol 50(1):185–194

Rosen L, Gubler D (1974) The use of mosquitoes to detect and propagate dengue viruses. Am J Trop Med Hyg 23(6):1153–1160

Sadeghi M, Altan E, Deng X, Barker CM, Fang Y, Coffey LL, Delwart E (2018) Virome of >12 thousand *Culex* mosquitoes from throughout California. Virology 523:74–88

Sambri V, Capobianchi M, Charrel R, Fyodorova M, Gaibani P, Gould E, Niedrig M, Papa A, Pierro A, Rossini G, Varani S, Vocale C, Landini MP (2013) West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention. Clin Microbiol Infect 19 (8):699–704

Sanchez-Seco MP, Rosario D, Quiroz E, Guzman G, Tenorio A (2001) A generic nested-RT-PCR followed by sequencing for detection and identification of members of the alphavirus genus. J Virol Methods 95(1–2):153–161

Santiago GA, Vázquez J, Courtney S, Matías KY, Andersen LE, Colón C, Butler AE, Roulo R, Bowzard J, Villanueva JM, Muñoz-Jordan JL (2018) Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses. Nat Commun 9(1):1391

Scaramozzino N, Crance J-M, Jouan A, DeBriel DA, Stoll F, Garin D (2001) comparison of universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J Clin Microbiol 39(5):1922–1927

Schlaberg R, Chiu CY, Miller S, Procop GW, Weinstock G, Professional Practice Committee and Committee on Laboratory Practices of the American Society for Microbiology; Microbiology Resource Committee of the College of American Pathologists (2017) Validation of metagenomic next-generation sequencing tests for universal pathogen detection. Arch Pathol Lab Med 141(6):776–786

Silva SJRD, Paiva MHS, Guedes DRD, Krokovsky L, Melo FL, Silva MALD, Silva AD, Ayres CFJ, Pena LJ (2019) Development and validation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of ZIKV in mosquito samples from Brazil. Sci Rep 9(1):4494

Singh KRP (1967) Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (*L.*). Curr Sci 36(19):506–508

Singh M, Al-Eryani G, Carswell S, Ferguson JM, Blackburn J, Barton K, Roden D, Luciani F, Giang Phan T, Junankar S, Jackson K, Goodnow CC, Smith MA, Swarbrick A (2019) High-throughput targeted long-read single cell sequencing reveals the clonal and transcriptional landscape of lymphocytes. Nat Commun 10(1):3120

Staunton KM, Rohde BB, Townsend M, Liu J, Desnoyer M, Howell P, Amos B, Crawford J, Snoad N, Ritchie SA (2020) Investigating male *Aedes aegypti* (Diptera: Culicidae) attraction to different oviposition containers using various configurations of the sound gravid *Aedes* trap. J Med Entomol 57(3):957–961

Staunton KM, Crawford JE, Liu J, Townsend M, Han Y, Desnoyer M, Howell P, Xiang W, Burkot TR, Snoad N, Ritchie SA (2021) A low-powered and highly selective trap for male *Aedes* (Diptera: Culicidae) surveillance: the male *Aedes* sound trap. J Med Entomol 58(1):408–415

Sutherland GL, Nasci RS (2007) Detection of West Nile virus in large pools of mosquitoes. J Am Mosq Control Assoc 23(4):389–395

Takken W, Kline DL (1989) Carbon dioxide and 1-octen-3-ol as mosquito attractants. J Am Mosq Control Assoc 5(3):311–316

Tang Z, Yamada H, Kraupa C, Canic S, Busquets N, Talavera S, Jiolle D, Vreysen MJB, Bouyer J, Abd-Alla AMM (2020) High sensitivity of one-step real-time reverse transcription quantitative PCR to detect low virus titers in large mosquito pools. Parasit Vectors 13(1):460

Tangena JA, Thammavong P, Hiscox A, Lindsay SW, Brey PT (2015) The human-baited double net trap: an alternative to human landing catches for collecting outdoor biting mosquitoes in Lao PDR. PLoS One 10(9):e0138735

Tyson JR, O'Neil NJ, Jain M, Olsen HE, Hieter P, Snutch TP (2018) MinION-based long-read sequencing and assembly extends the *Caenorhabditis elegans* reference genome. Genome Res 28(2):266–274

van den Hurk AF, Hall-Mendelin S, Pyke AT, Smith GA, Mackenzie JS (2010) Vector competence of Australian mosquitoes for chikungunya virus. Vector Borne Zoonotic Dis 10(5):489–495

Vazquez-Prokopec GM, Galvin WA, Kelly R, Kitron U (2009) A new, cost-effective, battery-powered aspirator for adult mosquito collections. J Med Entomol 46(6):1256–1259

Walker T, Jeffries CL, Mansfield KL, Johnson N (2014) Mosquito cell lines: history, isolation, availability and application to assess the threat of arboviral transmission in the United Kingdom. Parasit Vectors 7(1):382

Wang J, Moore NE, Deng YM, Eccles DA, Hall RJ (2015) MinION nanopore sequencing of an influenza genome. Front Microbiol 6:766

Warrilow D, Watterson D, Hall RA, Davis SS, Weir R, Kurucz N, Whelan P, Allcock R, Hall-Mendelin S, O'Brien CA, Hobson-Peters J (2014) A new species of mesonivirus from the Northern Territory, Australia. PLoS One 9(3):e91103

Weaver SC, Forrester NL (2015) Chikungunya: evolutionary history and recent epidemic spread. Antivir Res 120:32–39

Williams CR, Long SA, Russell RC, Ritchie SA (2006) Field efficacy of the BG-sentinel compared with CDC backpack aspirators and CO_2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. J Am Mosq Control Assoc 22(2):296–300

Xiao P, Li C, Zhang Y, Han J, Guo X, Xie L, Tian M, Li Y, Wang M, Liu H, Ren J, Zhou H, Lu H, Jin N (2018) Metagenomic sequencing from mosquitoes in China reveals a variety of insect and human viruses. Front Cell Infect Microbiol 8:364

Yang Y, Garver LS, Bingham KM, Hang J, Jochim RC, Davidson SA, Richardson JH, Jarman RG (2015) Feasibility of using the mosquito blood meal for rapid and efficient human and animal virus surveillance and discovery. Am J Trop Med Hyg 93(6):1377–1382

Yaren O, Alto BW, Gangodkar PV, Ranade SR, Patil KN, Bradley KM, Yang Z, Phadke N, Benner SA (2017) Point of sampling detection of Zika virus within a multiplexed kit capable of detecting dengue and chikungunya. BMC Infect Dis 17(1):293

Zhang YU, Tian ZW, Ge JQ, Wang YI (1984) A simplified method for the identification of arboviruses: virus detection using enzyme immunoassay on cultured cells. J Virol Methods 9:45–51