

Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses

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ABSTRACT

Through the Advisory Committee on Dengue and other Flavivirus Vaccines, the World Health Organization (WHO) has had a long-standing commitment to facilitate and to guide research and development of vaccines for medically important flaviviruses. Recently, the Paediatric Dengue Vaccine Initiative (PDVI) was formed to accelerate the development, testing, and introduction of dengue (DEN) vaccines worldwide, partnering with WHO in this important public health effort. There are now a variety of DEN vaccines in various stages of the developmental pipeline. In an attempt to make inter-laboratory information more directly comparable, WHO with the support of PDVI initiated a program to coordinate the procedures used for the plaque-reduction neutralization test (PRNT). The PRNT is the most common assay used to measure neutralizing antibody. The presence of antibody is believed to be most relevant means of determining protective anti-DEN virus (DENV) immunity. While other neutralizing antibody assays are being considered for use in large-scale vaccine field trials, the PRNT is still considered to be the laboratory standard against which other neutralizing antibody assays should be compared. The need for PRNT coordination has been identified at several consultations between the WHO and PDVI. A more complete version of these guidelines is available on the WHO website: <http://www.who.int/immunization/documents/date/en/index.html>.

INTRODUCTION

THE DENGUE VIRUSES (DENVs) ARE MEMBERS OF THE FAMILY FLAVIVIRIDAE, genus *Flavivirus*, are mosquito-borne, and represent a major public health problem throughout the tropical world. The DENVs are a set of four different serotypes of viruses (DENV 1–4). Each of these DENVs is similar to one another, but serologically distinct enough that infection with one serotype will not protect against infection with another serotype. For this and other reasons to be discussed later, an effective

DENV vaccine should induce an immune response against the four serotypes simultaneously. Although now somewhat dated, the review book on DEN compiled by Gubler and Kuno still serves as an excellent source for information on DEN (16).

Additionally, there are no laboratory animal models that reliably mimic clinical human dengue disease. The lack of a suitable animal model makes it difficult to assess protective capacities of vaccine candidates and correlates of protection *in vivo*. In the absence of correlates, the protective capacity of any vaccine candidate will be

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finally defined as its ability to protect humans from DENV infection. Laboratory studies with DENVs and other flaviviruses have indicated, however, that protection of small animals from virus infection is best correlated to levels of virus-neutralizing antibodies (1,24,25,27). Similar studies with DENV in sub-human primate models have confirmed these observations (2,17,41,53). Sub-human primate studies have been used to identify and down-select candidate dengue vaccines with levels of dengue-neutralizing antibody (as measured by the PRNT), serving as the correlate of vaccine immunogenicity and reduced viremia following challenge with virulent wild-type virus used as a measure of vaccine efficacy. While the correlation of the presence of virus-neutralizing antibody to protection from infection is not absolute, the studies suggest that the PRNT is the best and most widely accepted approach to measuring virus-neutralizing and protective antibodies. Newer assays measuring virus-neutralizing antibodies are being developed and will be briefly discussed later.

All flaviviruses are simple positive-sense, single-stranded, RNA viruses, approximately 55 nm in diameter. The genome is approximately 11,000 nucleotides long, with 5' capped and 3'-end usually not polyadenylated. The genome encodes 10 proteins in a single open reading frame (Fig. 1). There are three structural proteins encoded in the 5'-one quarter of the viral genome: the capsid (C) protein forms the nucleocapsid shell protecting the viral genome, and the premembrane (prM), and envelope (E) proteins, both virion surface proteins embedded in the virion envelope. Seven non-structural (NS) proteins are encoded in the 3'-two thirds of the viral genome: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Each of the NS proteins has specific viral functions, but for the purpose of these guidelines, will not be discussed in further detail.

FLAVIVIRUS IMMUNOCHEMISTRY

Antibody-mediated virus neutralization is defined as the interaction of virus and antibody resulting in inactivation of virus such that it is no longer able to infect and replicate in cell cultures or animals.

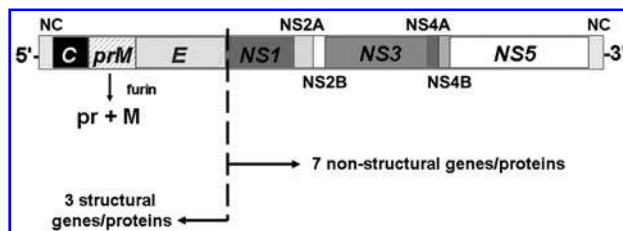


FIG. 1. Organization of the flaviviral genome.

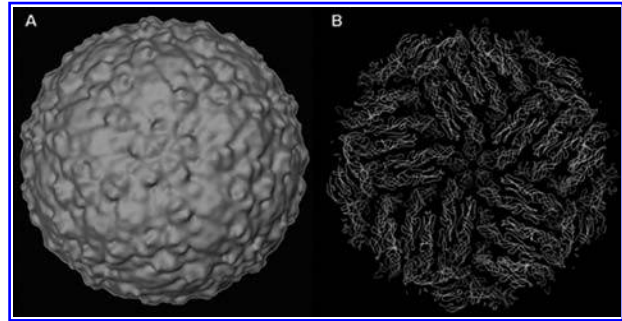


FIG. 2. Structure of the flavivirion. Cryoelectron microscopic structure of the West Nile virus. (A) Surface shaded view of the West Nile virion looking down the fivefold axis. (B) C-alpha trace showing the fitted E protein heterodimers looking down the fivefold axis. Courtesy of Buerbel Kaufmann and Richard Kuhn, Purdue University.

Antibody-mediated virus neutralization is defined as the interaction of virus and antibody resulting in inactivation of virus such that it is no longer able to infect and replicate in cell cultures or animals. Virus-neutralizing antibody is the primary protective antibody type elicited by flaviviral vaccines that result in protection from disease. The flaviviral E glycoprotein is a class II fusion protein and is responsible for viral attachment to host-cell receptors, and virus-mediated cell membrane fusion (49). As such, it is the most important viral protein with regard to viral infectivity, and therefore elicits essentially all virus-specific neutralizing antibody. It is primarily anti-E glycoprotein antibody that is measured in the current PRNT. Other non-E glycoprotein-specific antibodies (e.g., anti-NS1 antibody) can demonstrate virus protective effects in vivo in small animal models; however, these effects are not mediated by virion-antibody interactions (6,8,9,46,57).

Great progress in understanding the structure and function of the flaviviral E glycoprotein has recently been made (33,35,37,54,55). The E glycoprotein exists as 90 "head-to-tail" homodimers on the virion surface (Fig. 2). The E glycoprotein monomer can be divided into three structural domains: DI, DII, and DIII (Fig. 3). DII (also known as the dimerization domain) is a long finger-like structure that contains the hydrophobic membrane-fusion sequence at its tip. In the homodimer, the fusion tip is protected during replication by a combination of DIII of the associated monomer, E protein glycosylation, and the prM protein (40). DIII has been shown with DENV to be involved in virus attachment to Vero cells in culture (11). These binding characteristics have been confirmed using expressed DIII (7). The DI contains the E glycoprotein molecular hinge. As a class II fusion protein, the E glycoprotein can undergo an acid-catalyzed oligomeric reorganization into a fusogenic homotrimer (3,36,48,56). It is believed that this

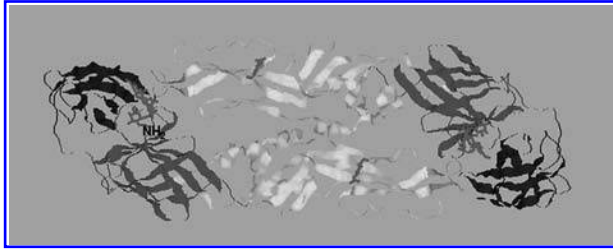


FIG. 3. Structure of the E glycoprotein homodimer.

event occurs in the endosome, allowing the viral nucleocapsid to escape into the cytoplasm and initiate RNA and protein synthesis. Antibodies to both DII and DIII have been shown to neutralize virus. Anti-DIII antibodies tend to be powerful neutralizing antibodies, and are more virus type-specific. Anti-DII antibodies are more virus cross-reactive, and while they can neutralize virus infectivity, they are usually less potent than anti-DIII antibodies (10). Two mechanisms of flaviviral neutralization (blocking attachment of virus to cells, and blocking the virus fusion process) have been identified (11,13). It is not known at this time how many antibody molecules are needed to neutralize the infectivity of a single virion. However, there is evidence that for some monoclonal antibodies it is not necessary for antibodies to bind to all 180 copies of the E protein to mediate neutralization (15,39).

DENV IMMUNOLOGY

The adaptive, protective immune response to DENV infection is driven by the presence or absence of virus-neutralizing antibodies and T-cell responses involved in helping antibody synthesis. Upon infection DENV elicits IgM, IgG, and IgA antibody responses. The IgM response begins early, frequently before onset of symptoms. IgM is usually detectable in serologic assays by 7–8 d after onset of symptoms (44). IgA antibodies are also detectable and have half-lives similar to those of IgM (14). IgG antibodies are detectable soon after infection and are maintained for years (19). Infection with any given DENV serotype results in immunity to that particular serotype; however, there is no long-term protection (>6 mo) against infection with any of the other three DEN serotype viruses (45).

Re-infection of individuals with a distinct second or third serotype of DENV may result in dengue hemorrhagic fever (DHF), or a more severe infection resulting in dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS). There have been a number of hypotheses presented that might explain the more severe

manifestation of disease following secondary DENV infections. Detailed discussions on these mechanisms are beyond the scope of this paper. Regardless of the hypothesized mechanism, pre-existing DENV-reactive antibody appears to be one factor involved in mediating DHF/DSS (5,18,20–23,28–30,32). The antibodies that are most likely involved are those that cross-react with, but do not neutralize, multiple serotypes of DENV. Non-neutralized DENV-antibody complexes can be ushered into DENV-susceptible cells via surface expressed Fc receptors (4,34,42,47). This phenomenon is known as antibody-dependent enhancement (ADE) of DENV replication.

The concept of ADE of DENV infections has been studied for a number of years, and remains a concern for vaccine developers. While the biological relevance of *in vitro* ADE remains ill-defined, our understanding of the antibody specificities that lead to protection or enhancement is improving. It is known that antibodies elicited by DIII of the E glycoprotein are more virus-type specific and neutralizing. Because of their high virus-neutralizing potential, these antibodies are highly protective in animal models of infection. On the other hand, antibodies elicited by either DI or DII are more cross-reactive among viruses, and demonstrate lower or no virus-neutralizing capacity. Recent evidence from West Nile virus (WNV) primary-infected humans, whose lymphocytes were used to prepare human monoclonal antibodies, indicates that the early antibody response may be directed towards DII (51). If the early antibody response to DENV is similar, then the primary humoral response will likely consist of cross-reactive, non-neutralizing antibodies. It is also possible that upon secondary infection with a different DENV serotype, there will be a rapid memory response that consists of DENV cross-reactive antibodies due to epitopes shared between DENV serotypes and the abundance of memory B cells specific for DI/DII cross-reactive epitopes. The theoretical possibility of ADE in post-vaccinal DENV infections resulting in DHF/DSS dictates that any vaccine candidate should best elicit only serotype-specific virus-neutralizing antibodies against all four serotypes, and therefore should be tetravalent, and include viruses from all four DENV serotypes. Because of this vaccine developers are creating tetravalent DENV vaccines (2,17,26,31,50).

THE PLAQUE-REDUCTION NEUTRALIZATION TEST

The PRNT measures the biological parameter of *in vitro* virus neutralization and is the most virus-specific serologic test among flaviviruses, and serotype-specific

test among DEN viruses, correlating well with serum levels of protection from viral infection. Newer tests measuring virus neutralization are being developed, but PRNT remains the laboratory standard against which these tests will need to be validated.

The virus PRNT assay was first described in the 1950s, and was later adapted to DENV (43). The basic design of the PRNT allows for virus-antibody interaction to occur in a test tube or microtiter plate, and then antibody effects on viral infectivity are measured by plating the mixture on virus-susceptible cells. The cells are overlaid with a semi-solid medium that restricts spread of progeny virus. Each virus that initiates a productive infection produces a localized area of infection (a plaque), that can be detected in a variety of ways. Plaques are counted and compared with the starting concentration of virus to determine the percentage reduction in total virus infectivity. In the PRNT, the serum specimen being tested is usually subjected to serial dilutions prior to mixing with a standardized amount of virus. The concentration of virus is held constant such that, when added to susceptible cells and overlaid with semi-solid medium, individual plaques can be discerned and counted. In this way, PRNT end-point titers can be calculated for each serum specimen at any selected percentage reduction of virus activity. A disadvantage of the PRNT is that it is labor intensive and therefore not readily amenable to high throughput, making it difficult to use for large-scale surveillance and vaccine trials.

TEST CONDITIONS FOR THE PLAQUE-REDUCTION NEUTRALIZATION TEST WITH DENGUE VIRUS

Cell lines and growth of viral stocks

DENVs grow in many different cell lines derived from both vertebrate and invertebrate sources. The cell line used for virus amplification needs to be coordinated between laboratories. Because DENV vaccines are targeted for humans, and because the processing of the prM protein is altered in C6/36 mosquito cell-grown DENV, a cell line of mammalian origin, such as the continuous African green monkey-derived Vero cells is recommended by the WHO to produce seed viruses and for use in the PRNT for DENV. Because different passages and clones of Vero cells may be phenotypically different, a WHO-certified cell line is available. To avoid the problems of cell-culture adaptation of virus, low-passage virus stock banks should be developed and employed for viral growth. While the number of virus passages deemed to be acceptable in the PRNT has never been experimentally determined, using viruses with no more than 5–10

cell-culture passages beyond the banking passage should be attainable if the master and working seed stock approach is used.

The conditions for virus amplification/preparation should be standardized with the use of an appropriate multiplicity of infection (around 10^{-2} to 10^{-3}). Virus should be harvested during the middle to end of the exponential phase of growth, to avoid high concentrations of inactivated particles in viral preparations. The supernatant from infected cell cultures should be clarified by low-speed centrifugation and stabilized with a cryo-protective agent (e.g., fetal calf serum >20%) before aliquoting and storage of virus at -70°C . Lyophilization is an alternative for long-term storage. Virus working banks should be appropriately qualified for sterility (absence of bacteria, fungi, and mycoplasma), potency (virus titration on the cell line used for PRNT), and if possible identity (using serotype-specific monoclonal antibodies).

Cell line for plaqueing viruses

DENVs will plaque in a variety of cell lines. Currently two mammalian cell lines are used most widely, Vero cells or rhesus monkey kidney-derived LLC-MK₂ cells. Each of these cell types has advantages; however, a specific derivation of Vero cells has been evaluated and certified by the WHO for production of live-attenuated vaccines and for use in the PRNT. Master and working cell banks should be prepared (as done for vaccine production). Doing so will limit the number of passages and prevent any drift of sensitivity to virus infection. Quality control protocols for the cell banks that monitor sterility (bacteria, mycoplasma, and fungi) and susceptibility of cells to viral infection should be included.

Vero cell monolayers should be prepared 2–3 d before use. Sub-confluent or just-confluent monolayers should be used to avoid any alteration or loss of cells during the course of the assay. Quality of the cell monolayer is critical for plaque development and therefore to generate accurate results.

Viruses used in the test

Currently there are a variety of DENV strains on which vaccine development or diagnostic testing is based. Table 1 lists the representative viruses from each serotype being used in tetravalent formulations by five different vaccine developers or diagnostic laboratories. Because the well-established dengue laboratory strains used for PRNT implementation were isolated many years ago and have been amplified by several passages in mosquito or various other cell lines, there is no clear rationale to choose one set of viruses over another.

Plaqueing efficiency is an important parameter in determining PRNT results. Using virus stocks containing

TABLE 1. VIRUSES CURRENTLY USED IN DENV VACCINE DEVELOPMENT OR REFERENCE LABS

Virus serotype	Vaccine developer or reference lab				
	1	2	3	4	5
DEN1	Hawaii	16007	16007/PUO-359	West Pacific or PR94	West Pacific
DEN2	New Guinea C	16681	16681/PUO-218	New Guinea C or Tonga 74	S16803
DEN3	H87	16562	16562/PaH881/88	Sleman 78	CH53489
DEN4	H241	1036	1036/1228	814669 (Dominica/81)	TVP360 or 341750

large amounts of inactive virions could result in falsely low PRNT titers. There are a variety of ways to analyze the contents of a viral preparation. These approaches include, but are not limited to: the quantization of viral genomic copies by RT-PCR; the quantization of the total envelope protein mass (by ELISA or other methods); or direct particle counting in an electron microscope. Each of these techniques has limitations. The best practical approach to developing a virus stock with high ratio of infectivity to particles is to carefully monitor the viral growth conditions and harvest at the appropriate time post-infection.

In the context of assessing vaccine immunogenicity (i.e., serological surrogates of protection), it is advisable to determine PRNT activity against a variety of virus strains such as other wild-type or virulent viruses (low passage virus or viruses isolated from human cases of dengue fever or DHF), or recent viral isolates from different DEN-endemic areas (12, 31). These results should be compared to PRNT results using the prototype laboratory strain(s). This being said, there is little evidence of antigenic drift within a given DENV serotype that would result in a strain resistant to a post-vaccinal response to that serotype. Ongoing comparisons of the DEN E protein structure and cross-reaction analyses between laboratory strains and recent field isolates should yield helpful data to develop future recommendations. Regulatory authorities may also choose to specify other strains that might be used in assays measuring vaccine-induced immunity, in addition to the strains used currently by vaccine developers.

Media

Fetal calf or bovine serum should be used for virus and cell growth, and for virus and sample dilutions should be heat-inactivated at 56°C for 30 min, and used at a low final concentration (2–5%) in the PRNT. The medium used for cell growth should be compatible with the cell type used. For Vero cells, minimal essential medium (MEM), Dulbecco's modified MEM (DMEM), M199, or equivalent media are generally sufficient.

PFU target and vessel size

The challenge virus dose and the number of repeats tested for each dilution (serum or virus) are key factors for achieving accurate measurements. The challenge dose should be modified based on the surface area of the cell monolayer (e.g., 6-well versus 24-well plates), to get readily discernable plaques and minimize plaque overlap. The target number of plaques per well can vary by virus strain, however 40–60 PFU per 35-mm dish should permit accurate titrations while minimizing plaque overlap. Plaque overlap results from crowding of plaques in an individual well. Comparing plaque counts in the test system versus input virus that has been “back-titrated” is the most acceptable way to rule out plaque overlap. In order to reach an acceptable precision for the plaque counts, it is recommended that at least three repeat wells for a challenge dose of 50 PFU/reaction or less be used.

Specimen handling

The technique of heat inactivation at 56°C for 30 min of serum specimens targeted for serological evaluation was introduced to limit the effects that complement or adventitious virus may have on the final results. This practice is routine in most laboratories and should be employed. Filtration of serum specimens to remove particulates is not necessary. Neutralization of DENVs does not require complement, so addition of exogenous sources of complement to the PRNT is not necessary.

In functional assays intended to assess vaccinal immunogenicity, the serum sample dilution series for antibody titration should ideally start below the “seroprotective” threshold titer. Regarding DENV-neutralizing antibodies, the seroprotection threshold remains unknown, but numerous laboratories in the context of vaccine immunogenicity assessment consider a seropositivity threshold to be 1:10. A 1:5 starting dilution would be preferred over 1:10 in this context; however, that should be balanced with the increased sample volume required for the test.

The number of dilutions can vary according to the objective of the testing. For screening purposes, sera can be

processed using a dilution corresponding to the seropositive threshold titer; however, the use of at least three dilutions is recommended due to possible cell toxicity or presence of non-specific inhibitors of virus replication in the first serum dilution. For end-point titration, two- to fourfold serum dilutions should be used. These dilution series lead to a more precise estimate of the end-point titer than higher dilution factors. The number of dilutions used depends on the dilution series and what is sufficient to establish the end-point titer in post-vaccinal samples. The appropriate dilution range can be previously determined by preliminary titration from 10-fold serum dilutions.

Virus-antibody incubation periods

Antigen-antibody reactions are quite rapid, with a period of 1–2 h at 37°C sufficient for the PRNT. Extending this period could result in partial virus inactivation. An overnight incubation at 4°C is also acceptable; however, switching between a variety of incubation periods within a given laboratory should be avoided.

Virus adsorption to cells and incubation of plates

Virus adsorption to Vero cells occurs rapidly at 37°C, with 90% of the infectious virus attaching within the first 30 mins (11). Longer adsorption periods, while not necessarily detrimental to infection, are not needed for maximal viral adsorption. It is important to ensure coverage of the cell monolayer with media during viral adsorption. This is accomplished by tilting the plates at recurring intervals to ensure consistent media coverage of each well. The days of incubation for the plaquing plates will depend on the growth characteristics of the virus strains used. Typically DENV requires 4–7 d for plaques to be visible. Temperature of incubation for the plaquing plates should be compatible with the cells used. For Vero and LLC-MK2 cells, an incubation temperature of 37°C is appropriate, although lower temperatures (e.g., 35°C) are acceptable.

Overlay

In the case of conventional PRNT an overlay is added onto the cell monolayer to limit the virus diffusion within the plate and promotes plaque formation. The overlay can be added to the cell monolayer either after aspiration of the serum/virus mixtures, or without elimination of the mixtures (e.g., yellow fever PRNT). The approach used should be consistent and documented within each laboratory. Semi-solid media such as carboxymethyl cellulose (CMC), methylcellulose (MC), and agarose are acceptable. Lower-grade agar solutions may contain charged inhibitors that may inhibit plaque formation. If

these types of chemicals are used, they should first be tested to determine the presence of such inhibitors. Since the quality of the overlay medium is critical to ensure data reliability, a new batch of commercial reagent should be qualified by comparison with a previous batch for plaque-forming efficacy. Selection of the overlay medium is based on the techniques used for plaque visualization, and whether or not the overlay must be removed for staining. In the latter case, CMC is used at 2–3% and MC is used at 0.8–1.5% final concentration. Agarose solutions are typically used at 1–2% final concentrations, and are primarily used when plaques are visualized with a vital dye such as neutral red. For flaviviruses, a two-overlay approach is preferred. The first overlay is added after the virus adsorption period, and does not contain neutral red. After an appropriate growth period, a second overlay containing 0.5% neutral red is added to visualize plaques.

Plaque visualization

Direct staining of cells. There are a variety of ways to reveal plaques for enumeration in the conventional PRNT. Cell coloration with vital dyes added in the first or second overlay (such as neutral red) permits monitoring of the development of viral plaques as uncolored holes in the cell monolayer. However, this method has some limitations. Neutral red is cytotoxic at high concentrations and light sensitive, therefore the dye concentration in the overlay is necessarily limited, and plates stained with neutral red should be kept in light-tight containers or incubators. This is especially important for the single-overlay technique. Because of this the contrast between the colored cell monolayer and uncolored plaques may be weak, and thus affects the accuracy of plaque enumeration. On the other hand, keeping cells alive with a neutral red overlay makes it possible to introduce flexibility into incubation time that may sharpen plaques or even bring out new plaques. Other dyes (such as amido-black or crystal violet) can be used to improve the cell monolayer staining and to allow possible automation for counting plaques. These stains cannot be added directly to the overlay and require overlay removal for staining. Even though dying the cell monolayers requires an additional coloration step after plaque development and before plaque counting, this approach has some other advantages: (1) the area of the cell monolayer required to reach the minimal recommended plaque count can be reduced because of the increased capacity to visualize smaller plaques; (2) the strong contrast between the cell sheet and the clear plaque permits photographic capture of plate images, and storage of images as raw data records for compliance purposes; (3) chemical fixation of cells inactivates DENV so the staining and counting steps can be implemented under the biological safety level (BSL)

1 containment (instead of BSL2 or BSL3 for dengue vaccine or wild-type viruses); and (4) plaques do not have to be counted immediately.

Direct staining of viral plaques. Viral plaques can also be immunostained using DENV-reactive polyclonal antisera or monoclonal antibodies. Staining plaques with DENV serotype-specific monoclonal antibodies allows an additional identity test of the viral serotype used in the assay. Immunostaining also permits the detection of viruses that plaque poorly, or the use of cell lines in which the virus does not demonstrate cytopathic effects (e.g., C6/36 cells). As with staining of the cell sheet with amido-black or crystal violet, immunostaining requires chemical fixation of cells that inactivates DENV. Subsequent counting can be implemented under BSL1 containment. Plaques do not have to be counted immediately, and the strong contrast between the cell sheet and the stained plaque permits photographic capture of plate images and storage of images as raw data records for compliance purposes.

Counting plaques

Regardless of the method of visualization, plaques are usually counted manually. Depending on the visualization method, plaques can be counted immediately, or later, as in the case of immunostaining or cell monolayer coloration.

Data analysis

The criteria needed to validate an individual test are: (1) integrity of uninfected cell monolayer control; (2) appropriate plaque counts per well as determined by back-titration of input virus; (3) little or no reduction in plaque counts with negative serum control; (4) appropriate PRNT titer of positive control sera; and (5) no serum toxicity observed with low serum dilutions.

PRNT end-point titers are expressed as the reciprocal of the last serum dilution showing the desired percentage reduction in plaque counts. A serum standard has been evaluated by the WHO and is available; however, it has never been characterized as a precise international standard, and at best serves as an internal positive control specimen. Currently no international reference sera are available for routine testing. The PRNT titer should be calculated based on a 50% or greater reduction in plaque counts (PRNT₅₀). A PRNT₅₀ titer is preferred over titers using higher cut-offs (e.g., PRNT₉₀) for vaccinee sera, providing more accurate results from the linear portion of the titration curve. However, PRNT₅₀ titers are more variable. The more stringent PRNT₉₀ titers are more useful in DENV-endemic areas for epidemiological studies

or diagnostic purposes, by decreasing the background serum cross-reactivities among flaviviruses.

There are several ways to calculate PRNT titers. The simplest and most widely used way to calculate titers is to count plaques and report the titer as the reciprocal of the last serum dilution to show $\geq 50\%$ reduction of the input plaque count as based on the back-titration of input plaques (see above). One area for titer variability between labs is whether or not the dilution made by mixing virus and antibody dilution (usually in a 1:1 volume ratio, resulting in a further 1:2 dilution of antibody) is included in the final titer calculations. Because there is no consistency in whether the 1:2 antigen:antibody dilution is included in the final titer, reporting of the data should explicitly state how this dilution was handled. Use of curve-fitting methods from several serum dilutions may permit calculation of a more precise result. There are a variety of computer analysis programs available for this (e.g., SPSS or GraphPad Prism). That being said, consistency in the interpretation method is as important as the method chosen for analysis.

NEW TESTS TO MEASURE VIRUS NEUTRALIZATION

Modifications in the standard PRNT assays are being investigated to shorten the incubation period required to see plaques, stain infected cell cultures to enhance plaque visibility, eliminate the plaque enumeration step, or reduce or eliminate the reliance on cell culture. Any new approach to assessing virus-neutralizing antibodies will have to be validated against the standard PRNT, as outlined in this document, so that the relationship and equivalence of the new and old test is fully understood. The first modification in the PRNT is the reduction of the size of vessel used for plaquing; 24-well plates seem to be the smallest reasonably sized vessel for plaquing when conventional cell staining techniques are used.

Immunostaining of virus-infected cells, instead of directly staining cells with histochemical stains or vital dyes, offers some advantages over these standard procedures. Immunostaining relies on adding enzyme-conjugated antiviral antibody (direct test) or non-conjugated antiviral antibody (later detected with an enzyme-conjugated anti-species antibody indirect test) to virus-infected cell cultures. The binding of the enzyme conjugate to the cell sheet is detected with an immunohistochemical stain specific for the enzyme used (usually horseradish peroxidase or alkaline phosphatase). The availability of DENV virus serotype-specific monoclonal antibodies permits enumeration of plaques within complex mixtures of serotypes using this approach. While using mixtures of DEN viruses in this way may reduce the total number of

plates needed to quantitate virus plaques, issues of plaque overlap still need to be considered. Use of immunochemical staining, which results in signal amplification because of the presence of the enzyme-conjugate, may also facilitate identification of plaques at an earlier stage post-infection, resulting in reduced plaquing incubation times.

Perhaps the most promising technique is to quantitate infectious virus using small numbers of virions and a 96-well plate format (micro-neutralization assay) (52). In this assay, individual plaques are not enumerated, but rather the viral growth measured in any given well is related to the optical density observed in that well using a soluble enzyme substrate. The most difficult parameters to control using this assay design are determining the appropriate amount of input virus and the incubation times between virus infection and ELISA detection. Since viral spread is not limited by semisolid overlays, waiting too long before staining can result in overgrowth of virus. Since not all viruses grow at the same rate, the incubation periods will be virus-specific. Standardizing this assay for four virus serotypes is possible; however, if cross-reactivities need to be measured against a variety of viral isolates, the task becomes more difficult. It is also possible to convert this assay from an immunochemical assay to a nucleic acid detection assay. In this way, viral RNA produced by non-neutralized virus can be detected in a quantitative assay (from culture supernatants and/or cell monolayers). The issues of input virus and time of incubations are still relevant for this process.

Another new approach to measuring virus-neutralizing antibodies is to dispense with virus completely and use an “infectious” vector that expresses the E protein or E/prM proteins with or without genes that encode “reporter” molecules (38). These assays are based on the observation that essentially all virus-neutralizing antibodies are elicited by the E protein. A variety of reporter molecules (e.g., green fluorescent protein) can be used to detect residual “virus” activity. However, such approaches suffer the same drawbacks as the micro-neutralization assays. In addition, this system requires the construction of a new vector for each strain to be tested.

SUMMARY

The levels of flavivirus-neutralizing antibody titers in the serum of vaccinated or infection-immune individuals correlates best with protection from subsequent viral infection. However, the lack of a standardized PRNT poses a hurdle for comparing data between vaccine trials and testing laboratories, and defining a threshold value to use as a true serological correlate of protection. The purpose of these guidelines is to: (1) provide scientific insight into the biol-

ogy of the flavivirus neutralization test; (2) provide guidelines for test coordination; and (3) provide minimal recommendations for a test protocol for laboratories that might be interested in establishing the test. It must be remembered that the PRNT is above all a biological assay, and as such will always have a certain degree of inter-laboratory variation. Due to this and other lab-to-lab variability, such as specific vaccine design approaches, strains used for vaccine development, and other unique manufacturing requirements, these guidelines will not mandate the use of a single standardized protocol. Ultimately, the definition of a protective level of vaccine-induced neutralizing antibody by whatever suitable assay will need to be validated in a vaccine efficacy trial. Standardization of the procedure using appropriate reference material, followed by each laboratory's own qualification and validation, may result in better inter-laboratory comparisons of results. These guidelines outline the variables that are important to consider in performing this biological assay, and suggest a procedure that can be used by those laboratories interested in using the PRNT. It is important to note that virus-neutralizing antibody titer, as determined by the PRNT, is considered to be the best immune correlate of protection for flaviviral infections. The fact remains, however, that a true definition of protective levels of vaccine-induced DENV-neutralizing antibody will require vaccine-efficacy trials using fully validated assays.

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REFERENCES

1. An J, Kimura-Kuroda J, Hirabayashi Y, and Yasui K: Development of a novel mouse model for dengue virus infection. *Virology* 1999;263:70–77.
2. Blaney JE Jr., Matro JM, Murphy BR, and Whitehead SS: Recombinant, live-attenuated tetravalent dengue virus vaccine formulations induce a balanced, broad, and protective neutralizing antibody response against each of the four serotypes in rhesus monkeys. *J Virol* 2005;79: 5516–5528.
3. Bressanelli S, Stiasny K, Allison SL, et al.: Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO J* 2004;23:728–738.

4. Brown MG, King CA, Sherren C, Marshall JS, and Anderson R: A dominant role for FcγRII in antibody-enhanced dengue virus infection of human mast cells and associated CCL5 release. *J Leukoc Biol* 2006;80:1242–1250.
5. Burke DS, and Kliks S: Antibody-dependent enhancement in dengue virus infections. *J Infect Dis* 2006;193:601–603; author reply 3–4.
6. Calvert AE, Huang CY, Kinney RM, and Roehrig JT: Non-structural proteins of dengue 2 virus offer limited protection to interferon-deficient mice after dengue 2 virus challenge. *J Gen Virol* 2006;87:339–346.
7. Chu JJ, Rajamanonmani R, Li J, Bhuvanankantham R, Lescar J, and Ng ML: Inhibition of West Nile virus entry by using a recombinant domain III from the envelope glycoprotein. *J Gen Virol* 2005;86:405–412.
8. Costa SM, Freire MS, and Alves AM: DNA vaccine against the non-structural 1 protein (NS1) of dengue 2 virus. *Vaccine* 2005;24:195–205.
9. Costa SM, Paes MV, Barreto DF, et al.: Protection against dengue type 2 virus induced in mice immunized with a DNA plasmid encoding the non-structural 1 (NS1) gene fused to the tissue plasminogen activator signal sequence. *Vaccine* 2006;24:195–205.
10. Crill WD, and Chang GJ: Localization and characterization of flavivirus envelope glycoprotein cross-reactive epitopes. *J Virol* 2004;78:13975–13986.
11. Crill WD, and Roehrig JT: Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *J Virol* 2001;75:7769–7773.
12. Endy TP, Nisalak A, Chunsuttiwat S, et al.: Relationship of preexisting dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a prospective cohort study of DV infection in Thailand. *J Infect Dis* 2004;189:990–1000.
13. Gollins SW, and Porterfield JS: A new mechanism for the neutralization of enveloped viruses by antiviral antibody. *Nature* 1986;321:244–246.
14. Groen J, Velzing J, Copra C, et al.: Diagnostic value of dengue virus-specific IgA and IgM serum antibody detection. *Microbes Infect* 1999;1:1085–1090.
15. Gromowski GD, and Barrett AD: Characterization of an antigenic site that contains a dominant, type-specific neutralization determinant on the envelope protein domain III (ED3) of dengue 2 virus. *Virology* 2007;366:349–360.
16. Gubler DJ, and Kuno G: *Dengue and Dengue Hemorrhagic Fever*. CAB International, New York, 1997.
17. Guirakhoo F, Pugachev K, Zhang Z, et al.: Safety and efficacy of chimeric yellow fever-dengue virus tetravalent vaccine formulations in nonhuman primates. *J Virol* 2004;78:4761–4775.
18. Halstead S, Chow J, and Marchette N: Immunologic enhancement of dengue virus replication. *Nature New Biology* 1973;243:24–26.
19. Halstead SB. Etiologies of the experimental dengues of Siler and Simmons. *Am J Trop Med Hyg* 1974;23:974–982.
20. Halstead SB, and O'Rourke EJ: Antibody-enhanced dengue virus infection in primate leukocytes. *Nature* 1977;265:739–741.
21. Halstead SB, and O'Rourke EJ: Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 1977;146:201–217.
22. Halstead SB, O'Rourke EJ, and Allison AC: Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting *in vitro* infection. *J Exp Med* 1977;146:218–229.
23. Halstead SB, and Papaevangelou G: Transmission of dengue 1 and 2 viruses in Greece in 1928. *Am J Trop Med Hyg* 1980;29:635–637.
24. Huang CY, Butrapet S, Tsuchiya KR, Bhamarapravati N, Gubler DJ, and Kinney RM: Dengue 2 PDK-53 virus as a chimeric carrier for tetravalent dengue vaccine development. *J Virol* 2003;77:11436–11447.
25. Johnson AJ, and Roehrig JT: New mouse model for dengue virus vaccine testing. *J Virol* 1999;73:783–786.
26. Kanesa-athan N, Sun W, Kim-Ahn G, et al.: Safety and immunogenicity of attenuated dengue virus vaccines (Aventis Pasteur) in human volunteers. *Vaccine* 2001;19:3179–3188.
27. Kinney RM, Butrapet S, Chang GJ, et al.: Construction of infectious cDNA clones for dengue 2 virus: strain 16681 and its attenuated vaccine derivative, strain PDK-53. *Virology* 1997;230:300–308.
28. Kliks S: Antibody-enhanced infection of monocytes as the pathogenetic mechanism for severe dengue illness. *AIDS Res Hum Retroviruses* 1990;6:993–998.
29. Kliks SC, Nimmanitya S, Nisalak A, and Burke DS: Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am J Trop Med Hyg* 1988;38:411–419.
30. Kliks SC, Nisalak A, Brandt WE, Wahl L, and Burke DS: Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am J Trop Med Hyg* 1989;40:444–451.
31. Kochel TJ, Watts DM, Halstead SB, et al.: Effect of dengue-1 antibodies on American dengue-2 viral infection and dengue haemorrhagic fever. *Lancet* 2002;360:310–312.
32. Kouri GP, Guzman MG, Bravo JR, and Triana C: Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. *Bull World Health Organ* 1989;67:375–380.
33. Kuhn RJ, Zhang W, Rossmann MG, et al.: Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell* 2002;108:717–725.

34. Littau R, Kurane I, and Ennis FA: Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. *J Immunol* 1990;144:3183–3186.
35. Modis Y, Ogata S, Clements D, and Harrison SC: A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc Natl Acad Sci USA* 2003;100:6986–6991.
36. Modis Y, Ogata S, Clements D, and Harrison SC: Structure of the dengue virus envelope protein after membrane fusion. *Nature* 2004;427:313–319.
37. Modis Y, Ogata S, Clements D, and Harrison SC: Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. *J Virol* 2005;79:1223–1231.
38. Pierson TC, Sanchez MD, Puffer BA, et al.: A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection. *Virology* 2006;346:53–65.
39. Pierson TC, Xu Q, Nelson S, et al.: The stoichiometry of antibody-mediated neutralization and enhancement of West Nile virus infection. *Cell Host Microbe* 2007;1:135–145.
40. Rey FA, Heinz FX, Mandl C, Kunz C, and Harrison SC: The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. *Nature* 1995;375:291–298.
41. Robert Putnak J, Collier BA, Voss G, et al.: An evaluation of dengue type-2 inactivated, recombinant subunit, and live-attenuated vaccine candidates in the rhesus macaque model. *Vaccine* 2005;23:4442–4452.
42. Rodrigo WW, Jin X, Blackley SD, Rose RC, and Schlesinger JJ: Differential enhancement of dengue virus immune complex infectivity mediated by signaling-competent and signaling-incompetent human FcγRI (CD64) or FcγRII (CD32). *J Virol* 2006;80:10128–10138.
43. Russell PK, Nisalak A, Sukhachana P, and Vivona S: A plaque reduction test for dengue virus neutralizing antibodies. *J Immunol* 1967;99:285–290.
44. Sa-Ngasang A, Anantapreecha S, Chanama S, et al.: Specific IgM and IgG responses in primary and secondary dengue virus infections determined by enzyme-linked immunosorbent assay. *Epidemiol Infect* 2006;134:820–825.
45. Sabin AB: *Viral and Rickettsial Infection of Man*. JB Lippincott, Philadelphia, 1959, pp. 361.
46. Schlesinger JJ, Brandriss MW, and Walsh EE: Protection of mice against dengue 2 virus encephalitis by immunization with the dengue 2 virus non-structural glycoprotein NS1. *J Gen Virol* 1987;68(Pt 3):853–857.
47. Schlesinger JJ, and Chapman SE: Influence of the human high-affinity IgG receptor FcγRI (CD64) on residual infectivity of neutralized dengue virus. *Virology* 1999;260:84–88.
48. Stiasny K, Bressanelli S, Lepault J, Rey FA, and Heinz FX: Characterization of a membrane-associated trimeric low-pH-induced form of the class II viral fusion protein E from tick-borne encephalitis virus and its crystallization. *J Virol* 2004;78:3178–3183.
49. Stiasny K, and Heinz FX: Flavivirus membrane fusion. *J Gen Virol* 2006;87:2755–2766.
50. Sun W, Edelman R, Kanesa-Thanan N, et al.: Vaccination of human volunteers with monovalent and tetravalent live-attenuated dengue vaccine candidates. *Am J Trop Med Hyg* 2003;69:24–31.
51. Throsby M, Geuijen C, Goudsmit J, et al.: Isolation and characterization of human monoclonal antibodies from individuals infected with West Nile virus. *J Virol* 2006;80:6982–6992.
52. Vorndam V, and Beltran M: Enzyme-linked immunosorbent assay-format microneutralization test for dengue viruses. *Am J Trop Med Hyg* 2002;66:208–212.
53. Whitehead SS, Hanley KA, Blaney JE Jr., Gilmore LE, Elkins WR, and Murphy BR: Substitution of the structural genes of dengue virus type 4 with those of type 2 results in chimeric vaccine candidates which are attenuated for mosquitoes, mice, and rhesus monkeys. *Vaccine* 2003;21:4307–4316.
54. Zhang W, Chipman PR, Corver J, et al.: Visualization of membrane protein domains by cryo-electron microscopy of dengue virus. *Nat Struct Biol* 2003;10:907–912.
55. Zhang Y, Corver J, Chipman PR, et al.: Structures of immature flavivirus particles. *EMBO J* 2003;22:2604–2613.
56. Zhang Y, Zhang W, Ogata S, et al.: Conformational changes of the flavivirus E glycoprotein. *Structure* 2004;12:1607–1618.
57. Zhang YM, Hayes EP, McCarty TC, et al.: Immunization of mice with dengue structural proteins and nonstructural protein NS1 expressed by baculovirus recombinant induces resistance to dengue virus encephalitis. *J Virol* 1988;62:3027–3031.

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1. Harapan Harapan, Kritu Panta, Alice Michie, Timo Ernst, Suzi McCarthy, Muhsin Muhsin, Safarianti Safarianti, Tjut Mariam Zanaria, Mudatsir Mudatsir, R. Tedjo Sasmono, Allison Imrie. 2022. Hyperendemic Dengue and Possible Zika Circulation in the Westernmost Region of the Indonesian Archipelago. *Viruses* 14:2, 219. [\[Crossref\]](#)
2. Iury Amancio Paiva, Débora Familiar-Macedo, Jéssica Badolato-Corrêa, Fabiana Rabe Carvalho, Helver Gonçalves Dias, Alex Pauvolid-Corrêa, Caroline Fernandes dos Santos, Andréa Alice Silva, Elzinandes Leal de Azeredo, Renata Artimos de Oliveira Vianna, Claudete Aparecida Araújo Cardoso, Alba Grifoni, Alessandro Sette, Daniela Weiskopf, Luzia Maria de-Oliveira-Pinto. 2022. Involvement of Th1Th17 Cell Subpopulations in the Immune Responses of Mothers Who Gave Birth to Children with Congenital Zika Syndrome (CZS). *Viruses* 14:2, 250. [\[Crossref\]](#)
3. Monique R. Q. Lima, Priscila C. G. Nunes, Flávia B. dos Santos. of 173-196. [\[Crossref\]](#)
4. Arun Sankaradoss, Suraj Jagtap, Junaid Nazir, Shefta E. Moula, Ayan Modak, Joshuah Fialho, Meenakshi Iyer, Jayanthi S. Shastri, Mary Dias, Ravisekhar Gadepalli, Alisha Aggarwal, Manoj Vedpathak, Sachee Agrawal, Awadhesh Pandit, Amul Nisheetha, Anuj Kumar, Mahasweta Bordoloi, Mohamed Shafi, Bhagyashree Shelar, Swathi S. Balachandra, Tina Damodar, Moses Muia Masika, Patrick Mwaura, Omu Anzala, Kar Muthumani, Ramanathan Sowdhamini, Guruprasad R. Medigeshi, Rahul Roy, Chitra Pattabiraman, Sudhir Krishna, Easwaran Sreekumar. 2022. Immune profile and responses of a novel dengue DNA vaccine encoding an EDIII-NS1 consensus design based on Indo-African sequences. *Molecular Therapy* 383. . [\[Crossref\]](#)
5. Dallas Vue, Qiyi Tang. 2021. Zika Virus Overview: Transmission, Origin, Pathogenesis, Animal Model and Diagnosis. *Zoonoses* 1:1. . [\[Crossref\]](#)
6. Koemchhoy Khim, Yong Jun Bang, Sao Puth, Yoonjoo Choi, Youn Suhk Lee, Kwangjoon Jeong, Shee Eun Lee, Joon Haeng Rhee. 2021. Deimmunization of flagellin for repeated administration as a vaccine adjuvant. *npj Vaccines* 6:1. . [\[Crossref\]](#)
7. José Javier Morales-Núñez, José Francisco Muñoz-Valle, Paola Carolina Torres-Hernández, Jorge Hernández-Bello. 2021. Overview of Neutralizing Antibodies and Their Potential in COVID-19. *Vaccines* 9:12, 1376. [\[Crossref\]](#)
8. Wei-Liang Liu, Chia-Wei Hsu, Shih-Peng Chan, Pei-Shi Yen, Matthew P. Su, Jian-Chiuan Li, Hsing-Han Li, Lie Cheng, Cheng-Kang Tang, Shih-Hsun Ko, Huai-Kuang Tsai, Zing Tsung-Yeh Tsai, Omar S. Akbari, Anna-Bella Failloux, Chun-Hong Chen. 2021. Transgenic refractory Aedes aegypti lines are resistant to multiple serotypes of dengue virus. *Scientific Reports* 11:1. . [\[Crossref\]](#)
9. Pragati Sharma, Kaustuv Nayak, Elluri Seetharami Reddy, Humaira Farooqi, Kaja Murali-Krishna, Anmol Chande. 2021. Optimization of Flow-Cytometry Based Assay for Measuring Neutralizing Antibody Responses against Each of the Four Dengue Virus Serotypes. *Vaccines* 9:11, 1339. [\[Crossref\]](#)
10. So Lee Park, Yan-Jang S. Huang, Amy C. Lyons, Victoria B. Ayers, Susan M. Hettenbach, D. Scott McVey, Leela E. Noronha, Kenneth R. Burton, Wei-Wen Hsu, Stephen Higgs, Dana L. Vanlandingham. 2021. Mosquito Saliva Modulates Japanese Encephalitis Virus Infection in Domestic Pigs. *Frontiers in Virology* 1. . [\[Crossref\]](#)
11. Umaporn Limothai, Sasipha Tachaboon, Janejira Dinhuzen, Taweewun Hunsawong, Prapapun Ong-ajchaowlerd, Budsaya Thaisomboonsuk, Stefan Fernandez, Supachoke Trongkamolchai, Mananya Wanpaisitkul, Chatchai Chulapornisiri, Anongrat Tiawilai, Thawat Tiawilai, Terapong Tantawichien, Usa Thisyakorn, Nattachai Srisawat. 2021. Dengue pre-vaccination screening test evaluation for the use of dengue vaccine in an endemic area. *PLOS ONE* 16:9, e0257182. [\[Crossref\]](#)
12. Sheliza Halani, Panashe E. Tombindo, Ryan O'Reilly, Rafael N. Miranda, Laura K. Erdman, Clare Whitehead, Joanna M. Bielecki, Lauren Ramsay, Raphael Ximenes, Justin Boyle, Carsten Krueger, Shannon Willmott, Shaun K. Morris, Kellie E. Murphy, Beate Sander. 2021. Clinical manifestations and health outcomes associated with Zika virus infections in adults: A systematic review. *PLOS Neglected Tropical Diseases* 15:7, e0009516. [\[Crossref\]](#)
13. Md Alamgir Kabir, Hussein Zilouchian, Muhammad Awais Younas, Waseem Asghar. 2021. Dengue Detection: Advances in Diagnostic Tools from Conventional Technology to Point of Care. *Biosensors* 11:7, 206. [\[Crossref\]](#)
14. Seokho Hong, Sang Hwan Seo, Sun-Je Woo, Yonghoon Kwon, Manki Song, Nam-Chul Ha. 2021. Epigallocatechin Gallate Inhibits the Uridylate-Specific Endoribonuclease Nsp15 and Efficiently Neutralizes the SARS-CoV-2 Strain. *Journal of Agricultural and Food Chemistry* 69:21, 5948-5954. [\[Crossref\]](#)
15. Matheus N. Weber, Ana C. S. Mosena, Letícia F. Baumbach, Mariana S. da Silva, Raissa Canova, Débora R. L. dos Santos, Renata da F. Budaszewski, Livia V. de Oliveira, Michel M. Soane, Natália B. Saraiva, Fernanda T. Bellucco, Bruno Amaral Mazurek, Gustavo N. Diehl, Laura H. V. G. Gil, Mauro R. Borba, Luis G. Corbellini, Cláudio W. Canal. 2021. Serologic evidence of West Nile virus and Saint Louis encephalitis virus in horses from Southern Brazil. *Brazilian Journal of Microbiology* 52:2, 1021-1027. [\[Crossref\]](#)

16. Carlos A DiazGranados, Edith Langevin, Matthew Bonaparte, Saranya Sridhar, Tifany Machabert, Gustavo Dayan, Rémi Forrat, Stephen Savarino. 2021. CYD Tetravalent Dengue Vaccine Performance by Baseline Immune Profile (Monotypic/Multitypic) in Dengue-Seropositive Individuals. *Clinical Infectious Diseases* 72:10, 1730-1737. [[Crossref](#)]
17. Luana Damasceno, Ana Carolina B. Terzian, Trevon Fuller, Cassia F. Estofotele, Adriana Andrade, Erna G. Kroon, Andrea A. Zin, Zilton Vasconcelos, Jose P. Pereira, Márcia C. Castilho, Isa Cristina R. Piaulino, Nikos Vasilakis, Maria E. Moreira, Karin Nielsen-Saines, Flor E. Martinez Espinosa, Maurício L. Nogueira, Patricia Brasil. 2021. Why Did ZIKV Perinatal Outcomes Differ in Distinct Regions of Brazil? An Exploratory Study of Two Cohorts. *Viruses* 13:5, 736. [[Crossref](#)]
18. Anthony Uchenna Emeribe, Idris Nasir Abdullahi, Odunayo O. R. Ajagbe, Charles Egede Ugwu, Solomon Oloche Onoja, Sharafudeen Dahiru Abubakar, Chikodi Modesta Umeozuru, Olawale Sunday Animasaun, Pius Omoruyi Omosigbo, Umar Mukhtar Danmusa, Mala Alhaji Baba Mallam, Maijiddah Saidu Aminu, Hadiza Yahaya, Silifat Oyewusi. 2021. Incidence, drivers and global health implications of the 2019/2020 yellow fever sporadic outbreaks in Sub-Saharan Africa. *Pathogens and Disease* 79:4. . [[Crossref](#)]
19. Yi-Hua Pan, Mei-Ying Liao, Yu-Wen Chien, Tzong-Shiann Ho, Hui-Ying Ko, Chin-Rur Yang, Shu-Fen Chang, Chia-Yi Yu, Shu-Yu Lin, Pin-Wei Shih, Pei-Yun Shu, Day-Yu Chao, Chao-Ying Pan, Hong-Ming Chen, Guey-Chuen Perng, Chia-Chi Ku, Chwan-Chuen King. 2021. Use of seroprevalence to guide dengue vaccination plans for older adults in a dengue non-endemic country. *PLOS Neglected Tropical Diseases* 15:4, e0009312. [[Crossref](#)]
20. Guoqiang Liu, James F. Rusling. 2021. COVID-19 Antibody Tests and Their Limitations. *ACS Sensors* 6:3, 593-612. [[Crossref](#)]
21. Jessica Badolato-Corrêa, Fabiana Rabe Carvalho, Iury Amancio Paiva, Débora Familiar-Macedo, Helver Gonçalves Dias, Alex Pauvalid-Corrêa, Caroline Fernandes-Santos, Monique da Rocha Queiroz Lima, Mariana Gandini, Andréa Alice Silva, Silvia Maria Baeta Cavalcanti, Solange Artimos de Oliveira, Renata Artimos de Oliveira Vianna, Elzinandes Leal de Azeredo, Claudete Aparecida Araújo Cardoso, Alba Grifoni, Alessandro Sette, Daniela Weiskopf, Luzia Maria de-Oliveira-Pinto. 2021. Differential Longevity of Memory CD4 and CD8 T Cells in a Cohort of the Mothers With a History of ZIKV Infection and Their Children. *Frontiers in Immunology* 12. . [[Crossref](#)]
22. Alberto A. Amarilla, Naphak Modhiran, Yin Xiang Setoh, Nias Y. G. Peng, Julian D. J. Sng, Benjamin Liang, Christopher L. D. McMillan, Morgan E. Freney, Stacey T. M. Cheung, Keith J. Chappell, Alexander A. Khromykh, Paul R. Young, Daniel Watterson. 2021. An Optimized High-Throughput Immuno-Plaque Assay for SARS-CoV-2. *Frontiers in Microbiology* 12. . [[Crossref](#)]
23. Tabitha E. Hoornweg, Willem Schaftenaar, Gilles Maurer, Petra B. van den Doel, Fieke M. Molenaar, Alexandre Chamouard-Galante, Francis Vercammen, Victor P. M. G. Rutten, Cornelis A. M. de Haan. 2021. Elephant Endotheliotropic Herpesvirus Is Omnipresent in Elephants in European Zoos and an Asian Elephant Range Country. *Viruses* 13:2, 283. [[Crossref](#)]
24. Débora Familiar-Macedo, Iury Amancio Paiva, Jessica Badolato-Corrêa da Silva, Fabiana Rabe de Carvalho, Helver Gonçalves Dias, Alex Pauvalid-Corrêa, Caroline Fernandes dos Santos, Mariana Gandini, Andréa Alice Silva, Silvia Maria Baeta Cavalcanti, Solange Artimos de Oliveira, Renata Artimos de Oliveira Vianna, Elzinandes Leal de Azeredo, Alba Grifoni, Alessandro Sette, Daniela Weiskopf, Claudete Aparecida Araújo Cardoso, Luzia Maria de-Oliveira-Pinto. 2021. Evaluation of the Expression of CCR5 and CX3CR1 Receptors and Correlation with the Functionality of T Cells in Women infected with ZIKV during Pregnancy. *Viruses* 13:2, 191. [[Crossref](#)]
25. Yusha Araf, Md. Asad Ullah, Nairita Ahsan Faruqui, Sadrina Afrin Mowna, Durdana Hossain Prium, Bishajit Sarkar. 2021. Dengue Outbreak is a Global Recurrent Crisis: Review of the Literature. *Electronic Journal of General Medicine* 18:1, em267. [[Crossref](#)]
26. Eng Eong Ooi. 2021. Challenges in prevaccination screening for previous dengue infection. *The Lancet Global Health* 9:1, e4-e5. [[Crossref](#)]
27. Aitana Juan-Giner, Derick Kimathi, Kyra H Grantz, Mainga Hamaluba, Patrick Kazooba, Patricia Njuguna, Gamou Fall, Moussa Dia, Ndeye S Bob, Thomas P Monath, Alan D Barrett, Joachim Hombach, Edgar M Mulogo, Immaculate Ampeire, Henry K Karanja, Dan Nyehangane, Juliet Mwanga-Amumpaire, Derek A T Cummings, Philip Bejon, George M Warimwe, Rebecca F Grais. 2021. Immunogenicity and safety of fractional doses of yellow fever vaccines: a randomised, double-blind, non-inferiority trial. *The Lancet* 397:10269, 119-127. [[Crossref](#)]
28. Anne J. Jääskeläinen. Validation of Zika virus infections: Nonmolecular aspects, immunoassays, and beyond 95-105. [[Crossref](#)]
29. Theerut Densathaporn, Rassamee Sangthong, Monvaris Sakolnapa, Smonrapat Surasombatpattana, Marisa Kemapunmanus, Promsin Masrinoul, Sutee Yoksan, Edward B. McNeil, Virasakdi Chongsuvivatwong. 2020. Survey on neutralizing antibodies against Zika virus eighteen months post-outbreak in two southern Thailand communities. *BMC Infectious Diseases* 20:1. . [[Crossref](#)]
30. Sorawat Sangkaew, Li Kiang Tan, Lee Ching Ng, Neil M. Ferguson, Ilaria Dorigatti. 2020. Using cluster analysis to reconstruct dengue exposure patterns from cross-sectional serological studies in Singapore. *Parasites & Vectors* 13:1. . [[Crossref](#)]

31. Lian Yih Pong, Peng Nian Yew, Wai Leng Lee, Yau Yan Lim, Syed Hassan Sharifah. 2020. Anti-dengue virus serotype 2 activity of tannins from porcupine dates. *Chinese Medicine* 15:1. . [[Crossref](#)]
32. J. Erin Staples, Alan D. T. Barrett, Annelies Wilder-Smith, Joachim Hombach. 2020. Review of data and knowledge gaps regarding yellow fever vaccine-induced immunity and duration of protection. *npj Vaccines* 5:1. . [[Crossref](#)]
33. Jue Hou, Shubham Shrivastava, Hooi Linn Loo, Lan Hiong Wong, Eng Eong Ooi, Jianzhu Chen. 2020. Sequential immunization induces strong and broad immunity against all four dengue virus serotypes. *npj Vaccines* 5:1. . [[Crossref](#)]
34. Pablo Manrique-Saide, Natalie E. Dean, M. Elizabeth Halloran, Ira M. Longini, Matthew H. Collins, Lance A. Waller, Hector Gomez-Dantes, Audrey Lenhart, Thomas J. Hladish, Azael Che-Mendoza, Oscar D. Kirstein, Yamila Romer, Fabian Correa-Morales, Jorge Palacio-Vargas, Rosa Mendez-Vales, Pilar Granja Pérez, Norma Pavia-Ruz, Guadalupe Ayora-Talavera, Gonzalo M. Vazquez-Prokopec. 2020. The TIRS trial: protocol for a cluster randomized controlled trial assessing the efficacy of preventive targeted indoor residual spraying to reduce Aedes-borne viral illnesses in Merida, Mexico. *Trials* 21:1. . [[Crossref](#)]
35. Shumpei Watanabe, Shuetsu Fukushi, Toshihiko Harada, Masayuki Shimojima, Tomoki Yoshikawa, Takeshi Kurosu, Yoshihiro Kaku, Shigeru Morikawa, Masayuki Saijo. 2020. Effective inactivation of Nipah virus in serum samples for safe processing in low-containment laboratories. *Virology Journal* 17:1. . [[Crossref](#)]
36. Maja Haut, Philipp Grl, Beate Oswald, Thomas Romig, Anna Obiegala, Gerhard Dobler, Martin Pfeffer. 2020. The Red Fox (*Vulpes vulpes*) as Sentinel for Tick-Borne Encephalitis Virus in Endemic and Non-Endemic Areas. *Microorganisms* 8:11, 1817. [[Crossref](#)]
37. Junfeng Lv, Xiaoxiao Liu, Shulin Cui, Lixin Yang, Shenghua Qu, Runze Meng, Baolin Yang, Chonglun Feng, Xiaoyan Wang, Dabing Zhang. 2020. The Neutralizing Antibody Response Elicited by Tembusu Virus Is Affected Dramatically by a Single Mutation in the Stem Region of the Envelope Protein. *Frontiers in Microbiology* 11. . [[Crossref](#)]
38. Mark Turner, Athanasia Papadimitriou, Peter Winkle, Nathan Segall, Michael Levin, Matthew Doust, Casey Johnson, Gregg Lucksinger, Carlos Fierro, Paul Pickrell, Marsha Raanan, Vianney Tricou, Astrid Borkowski, Derek Wallace. 2020. Immunogenicity and safety of lyophilized and liquid dengue tetravalent vaccine candidate formulations in healthy adults: a randomized, phase 2 clinical trial. *Human Vaccines & Immunotherapeutics* 16:10, 2456-2464. [[Crossref](#)]
39. Claudio Siqueira, Valéria Féres, Livia Coutinho, Isabela Junqueira, Luziane Bento, Larissa Montes, João Bosco Siqueira. 2020. Six Cases of Zika/Dengue Coinfection in a Brazilian Cohort, 2015–2019. *Viruses* 12:10, 1201. [[Crossref](#)]
40. Young-Il Kim, Mark Anthony B. Casel, Se-Mi Kim, Seong-Gyu Kim, Su-Jin Park, Eun-Ha Kim, Hye Won Jeong, Haryoung Poo, Young Ki Choi. 2020. Development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) thermal inactivation method with preservation of diagnostic sensitivity. *Journal of Microbiology* 58:10, 886-891. [[Crossref](#)]
41. Daraka Tongthainan, Nanthanida Mongkol, Kultida Jiamsomboon, Sarocha Suthisawat, Pornchai Sanyathitiserree, Manakorn Sukmak, Worawidh Wajjwalku, Yong Poovorawan, Gittiyaporn Ieamsaard, Bencharong Sangkharak, Kanokwan Taruyanon, Wirasak Fungfuang, Phitsanu Tulayakul, Kobporn Boonnak. 2020. Seroprevalence of Dengue, Zika, and Chikungunya Viruses in Wild Monkeys in Thailand. *The American Journal of Tropical Medicine and Hygiene* 103:3, 1228-1233. [[Crossref](#)]
42. Laura Lazo. 2020. Dengue virus 4: the ‘black sheep’ of the family?. *Expert Review of Vaccines* 19:9, 807-815. [[Crossref](#)]
43. Chee-Sieng Khor, Nurul-Farhana Mohd-Rahim, Habibi Hassan, Kim-Kee Tan, Nurhafiza Zainal, Boon-Teong Teoh, Sing-Sin Sam, Jing-Jing Khoo, Hai-Yen Lee, Yvonne Ai-Lian Lim, Szaly Abubakar. 2020. Serological evidence of DENV, JEV, and ZIKV among the indigenous people (Orang Asli) of Peninsular Malaysia. *Journal of Medical Virology* 92:8, 956-962. [[Crossref](#)]
44. Tey Putita Ou, Chanvannak Yun, Heidi Auerswald, Saraden In, Rithea Leang, Rekol Huy, Rithy Choeung, Philippe Dussart, Veasna Duong. 2020. Improved detection of dengue and Zika viruses using multiplex RT-qPCR assays. *Journal of Virological Methods* 282, 113862. [[Crossref](#)]
45. Arianna Mahely Hurtado-Monzón, Carlos Daniel Cordero-Rivera, Carlos Noe Farfan-Morales, Juan Fidel Osuna-Ramos, Luis Adrián De Jesús-González, José Manuel Reyes-Ruiz, Rosa María Ángel. 2020. The role of anti-flavivirus humoral immune response in protection and pathogenesis. *Reviews in Medical Virology* 30:4. . [[Crossref](#)]
46. Yunlong Cao, Bin Su, Xianghua Guo, Wenjie Sun, Yongqiang Deng, Linlin Bao, Qinyu Zhu, Xu Zhang, Yinghui Zheng, Chenyang Geng, Xiaoran Chai, Runsheng He, Xiaofeng Li, Qi Lv, Hua Zhu, Wei Deng, Yanfeng Xu, Yanjun Wang, Luxin Qiao, Yafang Tan, Liyang Song, Guopeng Wang, Xiaoxia Du, Ning Gao, Jiangning Liu, Junyu Xiao, Xiao-dong Su, Zongmin Du, Yingmei Feng, Chuan Qin, Chengfeng Qin, Ronghua Jin, X. Sunney Xie. 2020. Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients’ B Cells. *Cell* 182:1, 73-84.e16. [[Crossref](#)]
47. Ilaria Vicenti, Filippo Dragoni, Alessia Giannini, Federica Giammarino, Michele Spinicci, Francesco Saladini, Adele Boccuto, Maurizio Zazzi. 2020. Development of a Cell-Based Immunodetection Assay for Simultaneous Screening of Antiviral Compounds Inhibiting Zika and Dengue Virus Replication. *SLAS DISCOVERY: Advancing the Science of Drug Discovery* 25:5, 506-514. [[Crossref](#)]

48. Jean Claude Balingit, Minh Huong Phu Ly, Mami Matsuda, Ryosuke Suzuki, Futoshi Hasebe, Kouichi Morita, Meng Ling Moi. 2020. A Simple and High-Throughput ELISA-Based Neutralization Assay for the Determination of Anti-Flavivirus Neutralizing Antibodies. *Vaccines* **8**:2, 297. [[Crossref](#)]
49. Vianney Tricou, Xavier Sáez-Llorens, Delia Yu, Luis Rivera, José Jimeno, Ana Cecilia Villarreal, Epiphany Dato, Onix Saldaña de Suman, Nathali Montenegro, Rodrigo DeAntonio, Sonia Mazara, Maria Vargas, Debbie Mendoza, Martina Rauscher, Manja Brose, Inge Lefevre, Suely Tuboi, Astrid Borkowski, Derek Wallace. 2020. Safety and immunogenicity of a tetravalent dengue vaccine in children aged 2–17 years: a randomised, placebo-controlled, phase 2 trial. *The Lancet* **395**:10234, 1434–1443. [[Crossref](#)]
50. Didier Musso, Philippe Despres. 2020. Serological Diagnosis of Flavivirus-Associated Human Infections. *Diagnostics* **10**:5, 302. [[Crossref](#)]
51. Zilton Vasconcelos, Renata Campos Azevedo, Andrea Zin, Luiza Neves, Daniela Prado Cunha. ZIKV Diagnostics: Current Scenario and Future Directions . [[Crossref](#)]
52. Kyoko Saito, Masayoshi Fukasawa, Yoshitaka Shirasago, Ryosuke Suzuki, Naoki Osada, Toshiyuki Yamaji, Takaji Wakita, Eiji Konishi, Kentaro Hanada. 2020. Comparative characterization of flavivirus production in two cell lines: Human hepatoma-derived Huh7.5.1-8 and African green monkey kidney-derived Vero. *PLOS ONE* **15**:4, e0232274. [[Crossref](#)]
53. Matthew H. Collins, Omar Zepeda, Bryan Blette, Ramesh Jadi, Marlen Morales, Rigoberto Pérez, Guei-Jiun Alice Liou, Magelda Montoya-Cruz, Eva Harris, Sylvia Becker-Dreps, Aravinda M. de Silva, Jeffrey Stringer, Filemon Bucardo, Elizabeth Stringer. 2020. Serologic surveillance of maternal Zika infection in a prospective cohort in Leon, Nicaragua during the peak of the Zika epidemic. *PLOS ONE* **15**:4, e0230692. [[Crossref](#)]
54. Gustavo H. Dayan, Edith Langevin, Peter B. Gilbert, Yukun Wu, Zoe Moodie, Rémi Forrat, Brenda Price, Carina Frago, Alain Bouckennooghe, Margarita Cortes, Fernando Noriega, Carlos A. DiazGranados. 2020. Assessment of the long-term efficacy of a dengue vaccine against symptomatic, virologically-confirmed dengue disease by baseline dengue serostatus. *Vaccine* **38**:19, 3531–3536. [[Crossref](#)]
55. Wen-Hung Wang, Aspiro Nayim Urbina, Chia-Ching Wu, Chih-Yen Lin, Arunee Thitithanyanont, Wanchai Assavalapsakul, Po-Liang Lu, Yen-Hsu Chen, Sheng-Fan Wang. 2020. An epidemiological survey of the current status of Zika and the immune interaction between dengue and Zika infection in Southern Taiwan. *International Journal of Infectious Diseases* **93**, 151–159. [[Crossref](#)]
56. Toshihiko Harada, Shuetsu Fukushi, Takeshi Kurosu, Tomoki Yoshikawa, Masayuki Shimojima, Kiyoshi Tanabayashi, Masayuki Saijo. 2020. Inactivation of severe fever with thrombocytopenia syndrome virus for improved laboratory safety. *Journal of Biosafety and Biosecurity* **2**:1, 31–35. [[Crossref](#)]
57. Vianney Tricou, Jenny G. Low, Helen M. Oh, Yee-Sin Leo, Shirin Kalimuddin, Limin Wijaya, Junxiong Pang, Li Min Ling, Tau Hong Lee, Manja Brose, Yane Hutagalung, Martina Rauscher, Astrid Borkowski, Derek Wallace. 2020. Safety and immunogenicity of a single dose of a tetravalent dengue vaccine with two different serotype-2 potencies in adults in Singapore: A phase 2, double-blind, randomised, controlled trial. *Vaccine* **38**:6, 1513–1519. [[Crossref](#)]
58. Jessica L. Smith, Alec J. Hirsch. Analysis of Serum Anti-Zika Virus Antibodies by Focus Reduction Neutralization Test 73–80. [[Crossref](#)]
59. Isaura Beatriz Borges Silva, Aldacilene Souza da Silva, Mariana Sequetin Cunha, Aline Diniz Cabral, Kelly Cristina Alves de Oliveira, Elizabeth De Gaspari, Carlos Roberto Prudencio. 2020. Zika virus serological diagnosis: commercial tests and monoclonal antibodies as tools. *Journal of Venomous Animals and Toxins including Tropical Diseases* **3**. . [[Crossref](#)]
60. Junfeng Lv, Lixin Yang, Shenghua Qu, Runze Meng, Qingxiangzi Li, Huicong Liu, Xiaoyan Wang, Dabing Zhang. 2019. Detection of Neutralizing Antibodies to Tembusu Virus: Implications for Infection and Immunity. *Frontiers in Veterinary Science* **6**. . [[Crossref](#)]
61. E. Chepkorir, D. P. Tchouassi, S. L. Konongoi, J. Lutemiah, C. Tigoi, Z. Irura, F. Eyase, M. Venter, R. Sang. 2019. Serological evidence of Flavivirus circulation in human populations in Northern Kenya: an assessment of disease risk 2016–2017. *Virology Journal* **16**:1. . [[Crossref](#)]
62. Chukiat Sirivichayakul, Kriengsak Limkittikul, Pornthep Chanthavanich, Sutee Yoksan, Anuttarasakdi Ratchatatat, Jacqueline Kyungah Lim, Watcharee Arunsodsai, Arunee Sabchareon. 2019. Monoclonal antibody-based capture ELISA in the diagnosis of previous dengue infection. *Virology Journal* **16**:1. . [[Crossref](#)]
63. Nader Raafat, Stuart D Blacksell, Richard J Maude. 2019. A review of dengue diagnostics and implications for surveillance and control. *Transactions of The Royal Society of Tropical Medicine and Hygiene* **113**:11, 653–660. [[Crossref](#)]
64. Alice F. Versiani, Raissa Prado Rocha, Tiago A. O. Mendes, Glauco C. Pereira, Jordana Graziella A. Coelho dos Reis, Daniella C. Bartholomeu, Flávio G. da Fonseca. 2019. Identification of B-Cell Epitopes with Potential to Serologically Discriminate Dengue from Zika Infections. *Viruses* **11**:11, 1079. [[Crossref](#)]

65. Stephen J. Thomas, In-Kyu Yoon. 2019. A review of Dengvaxia®: development to deployment. *Human Vaccines & Immunotherapeutics* 15:10, 2295-2314. [[Crossref](#)]
66. Susan B. Manoff, Michele Sausser, Amy Falk Russell, Jason Martin, David Radley, Donna Hyatt, Christine C. Roberts, Jason Lickliter, Janakan Krishnarajah, Andrew Bett, Sheri Dubey, Tyler Finn, Beth-Ann Collier. 2019. Immunogenicity and safety of an investigational tetravalent recombinant subunit vaccine for dengue: results of a Phase I randomized clinical trial in flavivirus-naïve adults. *Human Vaccines & Immunotherapeutics* 15:9, 2195-2204. [[Crossref](#)]
67. Frumence, Viranaicken, Gadea, Desprès. 2019. A GFP Reporter MR766-Based Flow Cytometry Neutralization Test for Rapid Detection of Zika Virus-Neutralizing Antibodies in Serum Specimens. *Vaccines* 7:3, 66. [[Crossref](#)]
68. Rebecca M. Casey, Jennifer B. Harris, Steve Ahuka-Mundeke, Meredith G. Dixon, Gabriel M. Kizito, Pierre M. Nsele, Grace Umutesi, Janeen Laven, Olga Kosoy, Gilson Paluku, Abdou S. Gueye, Terri B. Hyde, Raimi Ewetola, Guylain K.M. Sheria, Jean-Jacques Muyembe-Tamfum, J. Erin Staples. 2019. Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak — Final Report. *New England Journal of Medicine* 381:5, 444-454. [[Crossref](#)]
69. Matthew H. Collins, Jesse J. Waggoner. 2019. Detecting Vertical Zika Transmission: Emerging Diagnostic Approaches for an Emerged Flavivirus. *ACS Infectious Diseases* 5:7, 1055-1069. [[Crossref](#)]
70. Jue Hou, Shubham Shrivastava, Christopher C. Fraser, Hooi Linn Loo, Lan Hiong Wong, Victor Ho, Katja Fink, Eng Eong Ooi, Jianzhu Chen. 2019. Dengue Mosaic Vaccines Enhance Cellular Immunity and Expand the Breadth of Neutralizing Antibody Against All Four Serotypes of Dengue Viruses in Mice. *Frontiers in Immunology* 10. . [[Crossref](#)]
71. Tyler M. Sharp, Marc Fischer, Jorge L. Muñoz-Jordán, Gabriela Paz-Bailey, J. Erin Staples, Christopher J. Gregory, Stephen H. Waterman. 2019. Dengue and Zika Virus Diagnostic Testing for Patients with a Clinically Compatible Illness and Risk for Infection with Both Viruses. *MMWR. Recommendations and Reports* 68:1, 1-10. [[Crossref](#)]
72. Matthew H. Collins. 2019. Serologic Tools and Strategies to Support Intervention Trials to Combat Zika Virus Infection and Disease. *Tropical Medicine and Infectious Disease* 4:2, 68. [[Crossref](#)]
73. Rajendra Raut, Aravinda M de Silva. 2019. Structural differences between dengue viruses circulating in humans and viruses used for vaccine research. *Future Virology* 14:6, 379-381. [[Crossref](#)]
74. Adrienne Guignard, François Haguinet, Stéphanie Wéry, Phirangkul Kerdpanich. 2019. Prevalence and Persistence of Maternal Dengue Neutralizing Antibodies in Infants From Central and Southern Thailand: A Retrospective Cohort Study. *Asia Pacific Journal of Public Health* 31:4, 288-295. [[Crossref](#)]
75. Andrea K.M. Furuya, Danielle Hunt, Kirsten St. George, Alan P. Dupuis, Laura D. Kramer, Pei-Yong Shi, Susan Wong. 2019. Use of the immunoglobulin G avidity assay to differentiate between recent Zika and past dengue virus infections. *Clinical Science* 133:7, 859-867. [[Crossref](#)]
76. Priscila M. S. Castanha, Wayner V. Souza, Cynthia Braga, Thalia Velho Barreto de Araújo, Ricardo A. A. Ximenes, Maria de Fátima P. M. Albuquerque, Ulisses R. Montarroyos, Demócrito B. Miranda-Filho, Marli T. Cordeiro, Rafael Dhalia, Ernesto T. A. Marques, Laura C. Rodrigues, Celina M. T. Martelli. 2019. Perinatal analyses of Zika- and dengue virus-specific neutralizing antibodies: A microcephaly case-control study in an area of high dengue endemicity in Brazil. *PLOS Neglected Tropical Diseases* 13:3, e0007246. [[Crossref](#)]
77. Juan Samuel Sulca Herencia. Laboratory Tests Used in the Diagnostic and Research of Dengue Virus: Present and Future . [[Crossref](#)]
78. Renato AS Oliveira, Edmilson F de Oliveira-Filho, Ana IV Fernandes, Carlos AA Brito, Ernesto TA Marques, Marli C Tenório, Laura HGV Gil. 2019. Previous dengue or Zika virus exposure can drive to infection enhancement or neutralisation of other flaviviruses. *Memórias do Instituto Oswaldo Cruz* 114. . [[Crossref](#)]
79. Melissa C. Whiteman, Leah Bogardus, Danila G. Giaccone, Leonard J. Rubinstein, Joseph M. Antonello, Dengyun Sun, Sarah Daijogo, Kevin B. Gurney. 2018. Virus Reduction Neutralization Test: A Single-Cell Imaging High-Throughput Virus Neutralization Assay for Dengue. *The American Journal of Tropical Medicine and Hygiene* 99:6, 1430-1439. [[Crossref](#)]
80. So Lee Park, Yan-Jang S. Huang, Amy C. Lyons, Victoria B. Ayers, Susan M. Hettenbach, D. Scott McVey, Kenneth R. Burton, Stephen Higgs, Dana L. Vanlandingham. 2018. North American domestic pigs are susceptible to experimental infection with Japanese encephalitis virus. *Scientific Reports* 8:1. . [[Crossref](#)]
81. Minh Huong Phu Ly, Meng Ling Moi, Thi Bich Hau Vu, Mya Myat Ngwe Tun, Todd Saunders, Cam Nhat Nguyen, Anh Kieu Thi Nguyen, Hung Manh Nguyen, Than Huu Dao, Do Quyen Pham, Thi Thu Thuy Nguyen, Thi Quynh Mai Le, Futoshi Hasebe, Kouichi Morita. 2018. Dengue virus infection-enhancement activity in neutralizing antibodies of healthy adults before dengue season as determined by using FcγR-expressing cells. *BMC Infectious Diseases* 18:1. . [[Crossref](#)]
82. Young Chan Kim, Cesar Lopez-Camacho, Joanne E. Nettleship, Nahid Rahman, Michelle L. Hill, Laura Silva-Reyes, Georgina Ortiz-Martinez, Gloria Figueroa-Aguilar, María Antonieta Mar, Héctor Vivanco-Cid, Christine S. Rollier, Nicole Zitzmann,

- Martha Eva Viveros-Sandoval, Raymond J. Owens, Arturo Reyes-Sandoval. 2018. Optimization of Zika virus envelope protein production for ELISA and correlation of antibody titers with virus neutralization in Mexican patients from an arbovirus endemic region. *Virology Journal* 15:1. . [\[Crossref\]](#)
83. Hélène Boigard, Velasco Cimica, Jose M. Galarza. 2018. Dengue-2 virus-like particle (VLP) based vaccine elicits the highest titers of neutralizing antibodies when produced at reduced temperature. *Vaccine* 36:50, 7728-7736. [\[Crossref\]](#)
 84. L.C. Hygino da Cruz, O.J.M. Nascimento, F.P.P.L. Lopes, I.R.F. da Silva. 2018. Neuroimaging Findings of Zika Virus–Associated Neurologic Complications in Adults. *American Journal of Neuroradiology* 39:11, 1967-1974. [\[Crossref\]](#)
 85. Emily N. Gallichotte, Thomas J. Baric, Usha Nivarthi, Matthew J. Delacruz, Rachel Graham, Douglas G. Widman, Boyd L. Yount, Anna P. Durbin, Stephen S. Whitehead, Aravinda M. de Silva, Ralph S. Baric. 2018. Genetic Variation between Dengue Virus Type 4 Strains Impacts Human Antibody Binding and Neutralization. *Cell Reports* 25:5, 1214-1224. [\[Crossref\]](#)
 86. Dieudonné Mumba, Ramesh Jadi, Aravinda M. de Silva, Matthew H. Collins, Steven R. Meshnick, Antoinette Tshefu, Jonathan B. Parr, Corinna Keeler, Melchior Kashamuka, Alexandra C. Willcox. 2018. Seroepidemiology of Dengue, Zika, and Yellow Fever Viruses among Children in the Democratic Republic of the Congo. *The American Journal of Tropical Medicine and Hygiene* 99:3, 756-763. [\[Crossref\]](#)
 87. Joshua Nealon, Anne-Frieda Taurel, Sutee Yoksan, Annick Moureau, Matt Bonaparte, Luong Chan Quang, Maria R Capeding, Ari Prayitno, Sri Rezeki Hadinegoro, Danaya Chansinghakul, Alain Bouckennooghe. 2018. Serological Evidence of Japanese Encephalitis Virus Circulation in Asian Children From Dengue-Endemic Countries. *The Journal of Infectious Diseases* 10. . [\[Crossref\]](#)
 88. E. F. de Oliveira-Filho, R. A. S. Oliveira, D. R. A. Ferreira, P. O. Laroque, L. J. Pena, M. M. Valença-Montenegro, R. A. Mota, L. H. V. G. Gil. 2018. Seroprevalence of selected flaviviruses in free-living and captive capuchin monkeys in the state of Pernambuco, Brazil. *Transboundary and Emerging Diseases* 65:4, 1094-1097. [\[Crossref\]](#)
 89. R. Tedjo Sasmono, Anne-Frieda Taurel, Ari Prayitno, Hermin Sitompul, Benediktus Yohan, Rahma F. Hayati, Alain Bouckennooghe, Sri Rezeki Hadinegoro, Joshua Nealon. 2018. Dengue virus serotype distribution based on serological evidence in pediatric urban population in Indonesia. *PLOS Neglected Tropical Diseases* 12:6, e0006616. [\[Crossref\]](#)
 90. Alberto Anastacio Amarilla, Marcilio Jorge Fumagalli, Mario Luis Figueiredo, Djalma S. Lima-Junior, Nilton Nascimento Santos-Junior, Helda Liz Alfonso, Veronica Lippi, Amanda Cristina Trabuco, Flavio Lauretti, Vanessa Danielle Muller, David F. Colón, João P. M. Luiz, Andreas Suhrbier, Yin Xiang Setoh, Alexander A. Khromykh, Luiz Tadeu Moraes Figueiredo, Victor Hugo Aquino. 2018. Ilheus and Saint Louis encephalitis viruses elicit cross-protection against a lethal Rocio virus challenge in mice. *PLOS ONE* 13:6, e0199071. [\[Crossref\]](#)
 91. A. Cosano-Quero, V. Velasco-Tirado, M.P. Sánchez Seco, L. Manzanedo-Bueno, M. Belhassen-García. 2018. Virus Zika: manifestaciones cutáneas en 3 pacientes. *Actas Dermo-Sifiliográficas* 109:3, e13-e16. [\[Crossref\]](#)
 92. Huiqiang Yang, Huan Yang, Zhushi Li, Lina Liu, Wei Wang, Ting He, Fengming Fan, Yan Sun, Jie Liu, Yuhua Li, Xianwu Zeng. 2018. Japanese encephalitis virus/yellow fever virus chimera is safe and confers full protection against yellow fever virus in intracerebrally challenged mice. *Vaccine* 36:18, 2450-2455. [\[Crossref\]](#)
 93. Maïna L'Azou, Jade Assoukpa, Karen Fanouillere, Eric Plennevaux, Matthew Bonaparte, Alain Bouckennooghe, Carina Frago, Fernando Noriega, Betzana Zambrano, R Leon Ochiai, Bruno Guy, Nicholas Jackson. 2018. Dengue seroprevalence: data from the clinical development of a tetravalent dengue vaccine in 14 countries (2005–2014). *Transactions of The Royal Society of Tropical Medicine and Hygiene* 112:4, 158-168. [\[Crossref\]](#)
 94. A. Cosano-Quero, V. Velasco-Tirado, M.P. Sánchez Seco, L. Manzanedo-Bueno, M. Belhassen-García. 2018. Zika Virus: Cutaneous Manifestations in 3 Patients. *Actas Dermo-Sifiliográficas (English Edition)* 109:3, e13-e16. [\[Crossref\]](#)
 95. Veronique Barban, Nathalie Mantel, Aymeric De Montfort, Anke Pagnon, Fabrine Pradezynski, Jean Lang, Florence Boudet. 2018. Improvement of the Dengue Virus (DENV) Nonhuman Primate Model via a Reverse Translational Approach Based on Dengue Vaccine Clinical Efficacy Data against DENV-2 and -4. *Journal of Virology* 92:12, e00440-18. [\[Crossref\]](#)
 96. Andrea Cristine Koishi, Andréia Akemi Suzukawa, Camila Zanluca, Daria Elena Camacho, Guillermo Comach, Claudia Nunes Duarte dos Santos. 2018. Development and evaluation of a novel high-throughput image-based fluorescent neutralization test for detection of Zika virus infection. *PLOS Neglected Tropical Diseases* 12:3, e0006342. [\[Crossref\]](#)
 97. Andrew Laughhunn, Yan-Jang S. Huang, Dana L. Vanlandingham, Marion C. Lanteri, Adonis Stassinopoulos. 2018. Inactivation of chikungunya virus in blood components treated with amotosalen/ultraviolet A light or amustaline/glutathione. *Transfusion* 58:3, 748-757. [\[Crossref\]](#)
 98. Angela M. Thomm, Anna M. Schotthoefer, Alan P. Dupuis, Laura D. Kramer, Holly M. Frost, Thomas R. Fritsche, Yvette A. Harrington, Konstance K. Knox, Sue C. Kehl. 2018. Development and Validation of a Serologic Test Panel for Detection of Powassan Virus Infection in U.S. Patients Residing in Regions Where Lyme Disease Is Endemic. *mSphere* 3:1. . [\[Crossref\]](#)

99. Femke W. Overbosch, Janke Schinkel, Ineke G. Stolte, Maria Prins, Gerard J. B. Sonder. 2018. Dengue virus infection among long-term travelers from the Netherlands: A prospective study, 2008-2011. *PLOS ONE* 13:2, e0192193. [[Crossref](#)]
100. Elitza S. Theel, D. Jane Hata. 2018. Diagnostic Testing for Zika Virus: a Postoutbreak Update. *Journal of Clinical Microbiology* 56:4. . [[Crossref](#)]
101. Jeffrey W. Perry, Yanhua Chen, Elizabeth Speliotes, Andrew W. Tai. 2018. Functional Analysis of the Dengue Virus Genome Using an Insertional Mutagenesis Screen. *Journal of Virology* 92:7. . [[Crossref](#)]
102. Dvorak Cheryl M.T., Payne Brian J., Seate Jessica L., Murtaugh Michael P. 2018. Effect of Maternal Antibody Transfer on Antibody Dynamics and Control of Porcine Circovirus Type 2 Infection in Offspring. *Viral Immunology* 31:1, 40-46. [[Abstract](#)] [[Full Text](#)] [[PDF](#)] [[PDF Plus](#)]
103. . Clinical Manifestations and Laboratory Diagnosis of Zika Virus Disease 103-115. [[Crossref](#)]
104. Jian Xu, Xixi Zhang, Shuanghai Zhou, Junjun Shen, Dawei Yang, Jing Wu, Xiaoyang Li, Meiling Li, Xiufen Huang, Joshua E. Sealy, Munir Iqbal, Yongqing Li. 2017. A DNA aptamer efficiently inhibits the infectivity of Bovine herpesvirus 1 by blocking viral entry. *Scientific Reports* 7:1. . [[Crossref](#)]
105. Joseph Torresi, Peter C Richmond, Leon G Heron, Ming Qiao, Joanne Marjason, Linda Starr-Spires, Diane van der Vliet, Jing Jin, T Anh Wartel, Alain Bouckennooghe. 2017. Replication and Excretion of the Live Attenuated Tetravalent Dengue Vaccine CYD-TDV in a Flavivirus-Naïve Adult Population: Assessment of Vaccine Viremia and Virus Shedding. *The Journal of Infectious Diseases* 216:7, 834-841. [[Crossref](#)]
106. Nicole P. Lindsey, J. Erin Staples, Krista Powell, Ingrid B. Rabe, Marc Fischer, Ann M. Powers, Olga I. Kosoy, Eric C. Mossel, Jorge L. Munoz-Jordan, Manuela Beltran, W. Thane Hancock, Karrie-Ann E. Toews, Esther M. Ellis, Brett R. Ellis, Amanda J. Panella, Alison J. Basile, Amanda E. Calvert, Janeen Laven, Christin H. Goodman, Carolyn V. Gould, Stacey W. Martin, Jennifer D. Thomas, Julie Villanueva, Mary L. Mataia, Rebecca Sciulli, Remedios Gose, A. Christian Whelen, Susan L. Hills. 2017. Ability To Serologically Confirm Recent Zika Virus Infection in Areas with Varying Past Incidence of Dengue Virus Infection in the United States and U.S. Territories in 2016. *Journal of Clinical Microbiology* 56:1. . [[Crossref](#)]
107. P. M. S. CASTANHA, U. R. MONTARROYOS, S. M. M. SILVEIRA, G. D. M. ALBUQUERQUE, M. J. G. MELLO, K. G. S. LOPES, M. T. CORDEIRO, E. T. A. MARQUES, C. M. T. MARTELLI, C. BRAGA. 2017. Incidence and risk factors for Dengue virus (DENV) infection in the first 2 years of life in a Brazilian prospective birth cohort. *Epidemiology and Infection* 145:14, 2971-2979. [[Crossref](#)]
108. Heather L. Wilson, Thomas Tran, Julian Druce, Myrielle Dupont-Rouzeyrol, Michael Catton. 2017. Neutralization Assay for Zika and Dengue Viruses by Use of Real-Time-PCR-Based Endpoint Assessment. *Journal of Clinical Microbiology* 55:10, 3104-3112. [[Crossref](#)]
109. Osvaldo J.M. Nascimento, Ivan R.F. da Silva. 2017. Guillain-Barré syndrome and Zika virus outbreaks. *Current Opinion in Neurology* 30:5, 500-507. [[Crossref](#)]
110. Shashank S. Pawitwar, Supurna Dhar, Sneham Tiwari, Chet Raj Ojha, Jessica Lapierre, Kyle Martins, Alexandra Rodzinski, Tiyaash Parira, Iru Paudel, Jiaojiao Li, Rajib Kumar Dutta, Monica R. Silva, Ajeet Kaushik, Nazira El-Hage. 2017. Overview on the Current Status of Zika Virus Pathogenesis and Animal Related Research. *Journal of Neuroimmune Pharmacology* 12:3, 371-388. [[Crossref](#)]
111. Irfan A. Rather, Jameel B. Lone, Vivek K. Bajpai, Woon K. Paek, Jeongheui Lim. 2017. Zika Virus: An Emerging Worldwide Threat. *Frontiers in Microbiology* 8. . [[Crossref](#)]
112. Marylene Maistriau, Tea Carletti, Mohammad Khalid Zakaria, Luca Braga, Valentina Faoro, Vasileios Vasileiadis, Alessandro Marcello. 2017. A method for the detection of virus infectivity in single cells and real time: Towards an automated fluorescence neutralization test. *Virus Research* 237, 1-6. [[Crossref](#)]
113. Flor Irene Rodriguez Melo, José Juan Renteria Morales, Abiel Homero Mascareñas De Los Santos, Enrique Rivas, Claire Vigne, Fernando Noriega. 2017. Immunogenicity and Safety of a Booster Injection of DTap-IPV//Hib (Pentaxim) Administered Concomitantly With Tetravalent Dengue Vaccine in Healthy Toddlers 15-18 Months of Age in Mexico. *Pediatric Infectious Disease Journal* 36:6, 602-608. [[Crossref](#)]
114. Janis A. Müller, Mirja Harms, Axel Schubert, Benjamin Mayer, Stephanie Jansen, Jean-Philippe Herbeuval, Detlef Michel, Thomas Mertens, Olli Vapalahti, Jonas Schmidt-Chanasit, Jan Münch. 2017. Development of a high-throughput colorimetric Zika virus infection assay. *Medical Microbiology and Immunology* 206:2, 175-185. [[Crossref](#)]
115. Padmapriya Padmanabhan, Sheriff Khaleefathullah, Krishansamy Kaveri, Gunasekaran Palani, Giriprasath Ramanathan, Sathiah Thennarasu, Uma Tirichurapalli Sivagnanam. 2017. Antiviral activity of Thiosemicarbazones derived from α -amino acids against Dengue virus. *Journal of Medical Virology* 89:3, 546-552. [[Crossref](#)]

116. Esther Shuyi Gan, Donald Heng Rong Ting, Kuan Rong Chan. 2017. The mechanistic role of antibodies to dengue virus in protection and disease pathogenesis. *Expert Review of Anti-infective Therapy* **15**:2, 111-119. [[Crossref](#)]
117. Supawat Chatchen, Arunee Sabchareon, Chukiat Sirivichayakul. 2017. Serodiagnosis of asymptomatic dengue infection. *Asian Pacific Journal of Tropical Medicine* **10**:1, 11-14. [[Crossref](#)]
118. Veasna Duong, Philippe Dussart, Philippe Buchy. 2017. Zika virus in Asia. *International Journal of Infectious Diseases* **54**, 121-128. [[Crossref](#)]
119. Pei Wang, Huiling Wang, Jianhai Yu, Qian Xie, Zhiwei Yao, Zhiran Qin, Weizhi Lu, Jia Li, Minyi Zhang, Guangjin Cao, Zhicheng Zhong, Tianwen He, Danjuan Ma, Bao Zhang, Wei Zhao. 2016. Molecular characterization and phylogenetic analysis of dengue virus type 1 in Guangdong in 2014. *SpringerPlus* **5**:1. . [[Crossref](#)]
120. Angela Ferreira Lopes de Teive e Argolo, Valéria Christina de Rezende Fêres, Marli Tenório Cordeiro, Lucimeire Antonelli da Silveira, Adriana Oliveira Guilarde, Ernesto Torres de Azevedo Marques, Wayner Vieira de Souza, Celina Maria Turchi Martelli. 2016. High frequency of pre-existing neutralizing antibody responses in patients with dengue during an outbreak in Central Brazil. *BMC Infectious Diseases* **16**:1. . [[Crossref](#)]
121. Qinye Song, Suzanne Stone, Donna Drebes, Laura L. Greiner, Cheryl M.T. Dvorak, Michael P. Murtaugh. 2016. Characterization of anti-porcine epidemic diarrhea virus neutralizing activity in mammary secretions. *Virus Research* **226**, 85-92. [[Crossref](#)]
122. Xiaoyu Wang, Dong Yang, Shihua Li, Xurong Xu, Cheng-Feng Qin, Ruikang Tang. 2016. Biomineralized vaccine nanohybrid for needle-free intranasal immunization. *Biomaterials* **106**, 286-294. [[Crossref](#)]
123. Yitades Gebre, Nikkiah Forbes, Teshome Gebre. 2016. Zika virus infection, transmission, associated neurological disorders and birth abnormalities: A review of progress in research, priorities and knowledge gaps. *Asian Pacific Journal of Tropical Biomedicine* **6**:10, 815-824. [[Crossref](#)]
124. Kanti Pabbaraju, Sallene Wong, Kara Gill, Kevin Fonseca, Graham A. Tipples, Raymond Tellier. 2016. Simultaneous detection of Zika, Chikungunya and Dengue viruses by a multiplex real-time RT-PCR assay. *Journal of Clinical Virology* **83**, 66-71. [[Crossref](#)]
125. So Lee Park, Yan-Jang S. Huang, Wei-Wen Hsu, Susan M. Hettenbach, Stephen Higgs, Dana L. Vanlandingham. 2016. Virus-specific thermostability and heat inactivation profiles of alphaviruses. *Journal of Virological Methods* **234**, 152-155. [[Crossref](#)]
126. Huiqiang Yang, Zhushi Li, Hua Lin, Wei Wang, Jian Yang, Lina Liu, Xianwu Zeng, Yonglin Wu, Yongxin Yu, Yuhua Li. 2016. A novel dengue virus serotype 1 vaccine candidate based on Japanese encephalitis virus vaccine strain SA14-14-2 as the backbone. *Archives of Virology* **161**:6, 1517-1526. [[Crossref](#)]
127. Lyle R. Petersen, Denise J. Jamieson, Ann M. Powers, Margaret A. Honein. 2016. Zika Virus. *New England Journal of Medicine* **374**:16, 1552-1563. [[Crossref](#)]
128. Dhanasekaran Govindarajan, Liming Guan, Steven Meschino, Arthur Fridman, Ansu Bagchi, Irene Pak, Jan ter Meulen, Danilo R. Casimiro, Andrew J. Bett. 2016. A Rapid and Improved Method to Generate Recombinant Dengue Virus Vaccine Candidates. *PLOS ONE* **11**:3, e0152209. [[Crossref](#)]
129. Kosoy Olga, Rabe Ingrid, Geissler Aimee, Adjemian Jennifer, Panella Amanda, Laven Janeen, Basile Alison J., Velez Jason, Griffith Kevin, Wong David, Fischer Marc, Lanciotti Robert S.. 2016. Serological Survey for Antibodies to Mosquito-Borne Bunyaviruses Among US National Park Service and US Forest Service Employees. *Vector-Borne and Zoonotic Diseases* **16**:3, 191-198. [[Abstract](#)] [[Full Text](#)] [[PDF](#)] [[PDF Plus](#)]
130. Iris Valdés, Lázaro Gil, Laura Lazo, Ernesto Marcos, Jorge Martín, Edith Suzarte, Jorge Castro, Yaremis Romero, Gerardo Guillén, Lisset Hermida. 2016. Could an experimental dengue virus infection fail to induce solid immunity against homologous viral challenge in non-human primates?. *Archives of Virology* **161**:2, 465-470. [[Crossref](#)]
131. Robert S. Lanciotti, John T. Roehrig. Arboviruses 648-657. [[Crossref](#)]
132. Bruno Guy, Jean Lang, Melanie Saville, Nicholas Jackson. 2016. Vaccination Against Dengue: Challenges and Current Developments. *Annual Review of Medicine* **67**:1, 387-404. [[Crossref](#)]
133. Nor Azila Muhammad Azami, Meng Ling Moi, Tomohiko Takasaki. Neutralization Assay for Chikungunya Virus Infection: Plaque Reduction Neutralization Test 273-282. [[Crossref](#)]
134. Jaime Henrique Amorim, Rúbens Prince dos Santos Alves, Raíza Bizerra, Sara Araújo Pereira, Lennon Ramos Pereira, Denicar Lina Nascimento Fabris, Robert Andreato Santos, Camila Malta Romano, Luís Carlos de Souza Ferreira. 2016. Antibodies are not required to a protective immune response against dengue virus elicited in a mouse encephalitis model. *Virology* **487**, 41-49. [[Crossref](#)]
135. Yingxian Yin, Yi Xu, Ling Su, Xun Zhu, Minxia Chen, Weijin Zhu, Huimin Xia, Xi Huang, Sitang Gong. 2016. Epidemiologic investigation of a family cluster of imported ZIKV cases in Guangdong, China: probable human-to-human transmission. *Emerging Microbes & Infections* **5**:1, 1-7. [[Crossref](#)]

136. Supawat Chatchen, Kriengsak Limkittikul, Pimolpachr Sriburin, Arunee Sabchareon, Chukiat Sirivichayakul. 2016. Monoclonal Antibody-Based Enzyme Immunoassay for Epidemiological Studies of Asymptomatic Dengue Infection. *Advances in Infectious Diseases* **06:03**, 113-119. [[Crossref](#)]
137. Dhiraj Acharya, Fengwei Bai. An Overview of Current Approaches Toward the Treatment and Prevention of West Nile Virus Infection 249-291. [[Crossref](#)]
138. Huang Yan-Jang S., Hsu Wei-Wen, Higgs Stephen, Vanlandingham Dana L.. 2015. Temperature Tolerance and Inactivation of Chikungunya Virus. *Vector-Borne and Zoonotic Diseases* **15:11**, 674-677. [[Abstract](#)] [[Full Text](#)] [[PDF](#)] [[PDF Plus](#)]
139. James Weger-Lucarelli, Matthew T. Aliota, Attapon Kamlangdee, Jorge E. Osorio. 2015. Identifying the Role of E2 Domains on Alphavirus Neutralization and Protective Immune Responses. *PLOS Neglected Tropical Diseases* **9:10**, e0004163. [[Crossref](#)]
140. Sarah L. George, Mimi A. Wong, Tina J. T. Dube, Karen L. Boroughs, Janae L. Stovall, Betty E. Luy, Aurelia A. Haller, Jorge E. Osorio, Linda M. Eggemeyer, Sharon Irby-Moore, Sharon E. Frey, Claire Y.-H. Huang, Dan T. Stinchcomb. 2015. Safety and Immunogenicity of a Live Attenuated Tetravalent Dengue Vaccine Candidate in Flavivirus-Naïve Adults: A Randomized, Double-Blinded Phase 1 Clinical Trial. *Journal of Infectious Diseases* **212:7**, 1032-1041. [[Crossref](#)]
141. Joseph Torresi, Leon G. Heron, Ming Qiao, Joanne Marjason, Laurent Chambonneau, Alain Bouckennooghe, Mark Boaz, Diane van der Vliet, Derek Wallace, Yane Hutagalung, Michael D. Nissen, Peter C. Richmond. 2015. Lot-to-lot consistency of a tetravalent dengue vaccine in healthy adults in Australia: A randomised study. *Vaccine* **33:39**, 5127-5134. [[Crossref](#)]
142. Scott B. Halstead, Sanford N. Cohen. 2015. Dengue Hemorrhagic Fever at 60 Years: Early Evolution of Concepts of Causation and Treatment. *Microbiology and Molecular Biology Reviews* **79:3**, 281-291. [[Crossref](#)]
143. Dhanasekaran Govindarajan, Steven Meschino, Liming Guan, David E. Clements, Jan H. ter Meulen, Danilo R. Casimiro, Beth-Ann G. Collier, Andrew J. Bett. 2015. Preclinical development of a dengue tetravalent recombinant subunit vaccine: Immunogenicity and protective efficacy in nonhuman primates. *Vaccine* **33:33**, 4105-4116. [[Crossref](#)]
144. Wen-Yang Tsai, Anna Durbin, Jih-Jin Tsai, Szu-Chia Hsieh, Stephen Whitehead, Wei-Kung Wang. 2015. Complexity of Neutralizing Antibodies against Multiple Dengue Virus Serotypes after Heterotypic Immunization and Secondary Infection Revealed by In-Depth Analysis of Cross-Reactive Antibodies. *Journal of Virology* **89:14**, 7348-7362. [[Crossref](#)]
145. Elizabeth Hunsperger. Arboviruses 1644-1659. [[Crossref](#)]
146. Sally R. Robinson, Juan Li, Eric A. Nelson, Michael P. Murtaugh. 2015. Broadly neutralizing antibodies against the rapidly evolving porcine reproductive and respiratory syndrome virus. *Virus Research* **203**, 56-65. [[Crossref](#)]
147. Ge Liu, Langzhou Song, David W. C. Beasley, Robert Putnak, Jason Parent, John Misczak, Hong Li, Lucia Reiserova, Xiangyu Liu, Haijun Tian, Wenzhe Liu, Darlene Labonte, Lihua Duan, Youngsun Kim, Linda Travalent, Devin Wigington, Bruce Weaver, Lynda Tussey. 2015. Immunogenicity and Efficacy of Flagellin-Envelope Fusion Dengue Vaccines in Mice and Monkeys. *Clinical and Vaccine Immunology* **22:5**, 516-525. [[Crossref](#)]
148. Luisa Barzon, Monia Pacenti, Sebastian Ulbert, Giorgio Palù. 2015. Latest developments and challenges in the diagnosis of human West Nile virus infection. *Expert Review of Anti-infective Therapy* **13:3**, 327-342. [[Crossref](#)]
149. B. Katherine Poole-Smith, Ryan R. Hemme, Mark Delorey, Gilberto Felix, Andrea L. Gonzalez, Manuel Amador, Elizabeth A. Hunsperger, Roberto Barrera. 2015. Comparison of Vector Competence of *Aedes mediovittatus* and *Aedes aegypti* for Dengue Virus: Implications for Dengue Control in the Caribbean. *PLOS Neglected Tropical Diseases* **9:2**, e0003462. [[Crossref](#)]
150. Chukiat Sirivichayakul, Arunee Sabchareon, Kriengsak Limkittikul, Sutee Yoksan. 2014. Plaque reduction neutralization antibody test does not accurately predict protection against dengue infection in Ratchaburi cohort, Thailand. *Virology Journal* **11:1**. . [[Crossref](#)]
151. Xi-Xia Ding, Xiao-Feng Li, Yong-Qiang Deng, Yong-Hui Guo, Wei Hao, Xiao-Yan Che, Cheng-Feng Qin, Ning Fu. 2014. Development of a Double Antibody Sandwich ELISA for West Nile Virus Detection Using Monoclonal Antibodies against Non-Structural Protein 1. *PLoS ONE* **9:10**, e108623. [[Crossref](#)]
152. Go Woon Cha, Jung Eun Cho, Young Ran Ju, Young-Jin Hong, Myung Guk Han, Won-Ja Lee, Eui Yul Choi, Young Eui Jeong. 2014. Comparison of Four Serological Tests for Detecting Antibodies to Japanese Encephalitis Virus after Vaccination in Children. *Osong Public Health and Research Perspectives* **5:5**, 286-291. [[Crossref](#)]
153. Carlos A. Sariol, Laura J. White. 2014. Utility, Limitations, and Future of Non-Human Primates for Dengue Research and Vaccine Development. *Frontiers in Immunology* **5**. . [[Crossref](#)]
154. Victor C. Gan. 2014. Dengue: Moving from Current Standard of Care to State-of-the-Art Treatment. *Current Treatment Options in Infectious Diseases* **6:3**, 208-226. [[Crossref](#)]
155. Vivaldo G. da Costa, Ariany C. Marques-Silva, Vitor G. Floriano, Marcos L. Moreli. 2014. Safety, immunogenicity and efficacy of a recombinant tetravalent dengue vaccine: A meta-analysis of randomized trials. *Vaccine* **32:39**, 4885-4892. [[Crossref](#)]

156. Stephen J. Thomas. 2014. Developing a dengue vaccine: progress and future challenges. *Annals of the New York Academy of Sciences* **1323**:1, 140-159. [[Crossref](#)]
157. Henrik Salje, Isabel Rodríguez-Barraquer, Kaitlin Rainwater-Lovett, Ananda Nisalak, Butsaya Thaisomboonsuk, Stephen J. Thomas, Stefan Fernandez, Richard G. Jarman, In-Kyu Yoon, Derek A. T. Cummings. 2014. Variability in Dengue Titer Estimates from Plaque Reduction Neutralization Tests Poses a Challenge to Epidemiological Studies and Vaccine Development. *PLoS Neglected Tropical Diseases* **8**:6, e2952. [[Crossref](#)]
158. Marycelin M Baba, Nina Vidergar, Alessandro Marcello. 2014. Virological point-of-care testing for the developing world. *Future Virology* **9**:6, 595-603. [[Crossref](#)]
159. Mark K. Slifka. 2014. Vaccine-Mediated Immunity Against Dengue and the Potential for Long-Term Protection Against Disease. *Frontiers in Immunology* **5**. . [[Crossref](#)]
160. Hee-Jung Lee, Kyung-Il Min, Ki Hoon Park, Hyo Jung Choi, Min-Kyoung Kim, Chi-Young Ahn, Young-Jin Hong, Young Bong Kim. 2014. Comparison of JEV neutralization assay using pseudotyped JEV with the conventional plaque-reduction neutralization test. *Journal of Microbiology* **52**:5, 435-440. [[Crossref](#)]
161. Abdullah Ahmed Al-alimi, Syed A. Ali, Faisal Muti Al-Hassan, Fauziah Mohd Idris, Sin-Yeang Teow, Narazah Mohd Yusoff. 2014. Dengue Virus Type 2 (DENV2)-Induced Oxidative Responses in Monocytes from Glucose-6-Phosphate Dehydrogenase (G6PD)-Deficient and G6PD Normal Subjects. *PLoS Neglected Tropical Diseases* **8**:3, e2711. [[Crossref](#)]
162. Lisheng Yang, Delei He, Min Tang, Zhiquan Li, Che Liu, Longfa Xu, Yixin Chen, Hailian Du, Qinjian Zhao, Jun Zhang, Tong Cheng, Ningshao Xia. 2014. Development of an Enzyme-Linked Immunosorbent Spot Assay To Measure Serum-Neutralizing Antibodies against Coxsackievirus B3. *Clinical and Vaccine Immunology* **21**:3, 312-320. [[Crossref](#)]
163. Litzba Nadine, Zelená Hana, Kreil Thomas R., Niklasson Bo, Kühlmann-Rabens Ilona, Remoli Maria Elena, Niedrig Matthias. 2014. Evaluation of Different Serological Diagnostic Methods for Tick-Borne Encephalitis Virus: Enzyme-Linked Immunosorbent, Immunofluorescence, and Neutralization Assay. *Vector-Borne and Zoonotic Diseases* **14**:2, 149-159. [[Abstract](#)] [[Full Text](#)] [[PDF](#)] [[PDF Plus](#)]
164. Kah Hin Tan, Kittu Chan Wing Ki, Satoru Watanabe, Subhash G. Vasudevan, Manoj Krishnan. Cell-Based Flavivirus Infection (CFI) Assay for the Evaluation of Dengue Antiviral Candidates Using High-Content Imaging 99-109. [[Crossref](#)]
165. Sadegh Chinikar, Nariman Shah-Hosseini. Dengue Fever in Asia and Africa 193-215. [[Crossref](#)]
166. Gustavo H. Dayan, Pedro Garbes, Fernando Noriega, Ana Daniela Izoton de Sadovsky, Patricia Marques Rodrigues, Camila Giuberti, Reynaldo Dietze. 2013. Immunogenicity and Safety of a Recombinant Tetravalent Dengue Vaccine in Children and Adolescents Ages 9–16 Years in Brazil. *The American Journal of Tropical Medicine and Hygiene* **89**:6, 1058-1065. [[Crossref](#)]
167. Iris Valdés, Lázaro Gil, Jorge Castro, Damián Odoyo, Rikoi Hitler, Elephas Munene, Yaremis Romero, Lucy Ochola, Karelia Cosme, Thomas Kariuki, Gerardo Guillén, Lisset Hermida. 2013. Olive baboons: a non-human primate model for testing dengue virus type 2 replication. *International Journal of Infectious Diseases* **17**:12, e1176-e1181. [[Crossref](#)]
168. Amar-Singh HSS, Mia-Tuang Koh, Kah Kee Tan, Lee Gaik Chan, Lynn Zhou, Alain Bouckennooghe, Denis Crevat, Yane Hutagalung. 2013. Safety and immunogenicity of a tetravalent dengue vaccine in healthy children aged 2–11 years in Malaysia: A randomized, placebo-controlled, Phase III study. *Vaccine* **31**:49, 5814-5821. [[Crossref](#)]
169. Angela FLT Argolo, Valéria CR Féres, Lucimeire A Silveira, Anna Carolina M Oliveira, Luiz A Pereira, João Bosco Siqueira Júnior, Cynthia Braga, Celina MT Martelli. 2013. Prevalence and incidence of dengue virus and antibody placental transfer during late pregnancy in central Brazil. *BMC Infectious Diseases* **13**:1. . [[Crossref](#)]
170. Gustavo H. Dayan, Manoj Thakur, Mark Boaz, Carol Johnson. 2013. Safety and immunogenicity of three tetravalent dengue vaccine formulations in healthy adults in the USA. *Vaccine* **31**:44, 5047-5054. [[Crossref](#)]
171. Luis Á. Villar, Doris M. Rivera-Medina, José Luis Arredondo-García, Mark Boaz, Linda Starr-Spires, Manoj Thakur, Betzana Zambrano, María C. Miranda, Enrique Rivas, Gustavo H. Dayan. 2013. Safety and Immunogenicity of a Recombinant Tetravalent Dengue Vaccine in 9–16 Year Olds. *Pediatric Infectious Disease Journal* **32**:10, 1102-1109. [[Crossref](#)]
172. Sandra Olkowski, Brett M. Forshey, Amy C. Morrison, Claudio Rocha, Stalin Vilcarromero, Eric S. Halsey, Tadeusz J. Kochel, Thomas W. Scott, Steven T. Stoddard. 2013. Reduced Risk of Disease During Postsecondary Dengue Virus Infections. *The Journal of Infectious Diseases* **208**:6, 1026-1033. [[Crossref](#)]
173. Madhuri Namekar, Esther M. Ellis, Maile O'Connell, Joe Elm, Alexandra Gurary, Sarah Y. Park, Allison Imrie, Vivek R. Nerurkar. 2013. Evaluation of a New Commercially Available Immunoglobulin M Capture Enzyme-Linked Immunosorbent Assay for Diagnosis of Dengue Virus Infection. *Journal of Clinical Microbiology* **51**:9, 3102-3106. [[Crossref](#)]
174. Monica A McArthur, Marcelo B Szein, Robert Edelman. 2013. Dengue vaccines: recent developments, ongoing challenges and current candidates. *Expert Review of Vaccines* **12**:8, 933-953. [[Crossref](#)]

175. Tanu Chawla, Kuan Rong Chan, Summer L. Zhang, Hwee Cheng Tan, Angeline P. C. Lim, Brendon J. Hanson, Eng Eong Ooi. 2013. Dengue Virus Neutralization in Cells Expressing Fc Gamma Receptors. *PLoS ONE* **8**:5, e65231. [[Crossref](#)]
176. P. M. S. CASTANHA, M. T. CORDEIRO, C. M. T. MARTELLI, W. V. SOUZA, E. T. A. MARQUES, C. BRAGA. 2013. Force of infection of dengue serotypes in a population-based study in the northeast of Brazil. *Epidemiology and Infection* **141**:5, 1080-1088. [[Crossref](#)]
177. Tatyana M. Timiryasova, Matthew I. Bonaparte, Ping Luo, Rebecca Zedar, Branda T. Hu, Stephen W. Hildreth. 2013. Optimization and Validation of a Plaque Reduction Neutralization Test for the Detection of Neutralizing Antibodies to Four Serotypes of Dengue Virus Used in Support of Dengue Vaccine Development. *The American Journal of Tropical Medicine and Hygiene* **88**:5, 962-970. [[Crossref](#)]
178. W. Sun, K. H. Eckels, J. R. Putnak, A. G. Lyons, S. J. Thomas, D. W. Vaughn, R. V. Gibbons, S. Fernandez, V. J. Gunther, M. P. Mammen, J. D. Statler, B. L. Innis. 2013. Experimental Dengue Virus Challenge of Human Subjects Previously Vaccinated With Live Attenuated Tetravalent Dengue Vaccines. *Journal of Infectious Diseases* **207**:5, 700-708. [[Crossref](#)]
179. Karla M. Marrero-Santos, Manuela Beltrán, Jessica Carrión-Lebrón, Carolina Sanchez-Vegas, Davidson H. Hamer, Elizabeth D. Barnett, Luis M. Santiago, Elizabeth A. Hunsperger. 2013. Optimization of the Cutoff Value for a Commercial Anti-Dengue Virus IgG Immunoassay. *Clinical and Vaccine Immunology* **20**:3, 358-362. [[Crossref](#)]
180. Sathyamangalam Swaminathan, Navin Khanna. Experimental Dengue Vaccines 135-151. [[Crossref](#)]
181. Scott B. Halstead, Stephen J. Thomas. Dengue vaccines 1042-1051. [[Crossref](#)]
182. Akihiko Maeda, Junko Maeda. 2013. Review of diagnostic plaque reduction neutralization tests for flavivirus infection. *The Veterinary Journal* **195**:1, 33-40. [[Crossref](#)]
183. Joshua M. Costin, Elena Zaitseva, Kristen M. Kahle, Cindo O. Nicholson, Dawne K. Rowe, Amanda S. Graham, Lindsey E. Bazzone, Greg Hogancamp, Marielys Figueroa Sierra, Rachel H. Fong, Sung-Tae Yang, Li Lin, James E. Robinson, Benjamin J. Doranz, Leonid V. Chernomordik, Scott F. Michael, John S. Schieffelin, Sharon Isern. 2013. Mechanistic Study of Broadly Neutralizing Human Monoclonal Antibodies against Dengue Virus That Target the Fusion Loop. *Journal of Virology* **87**:1, 52-66. [[Crossref](#)]
184. Ryan S.L. Wu, Kuan Rong Chan, Hwee Cheng Tan, Angelia Chow, John C. Allen, Eng Eong Ooi. 2012. Neutralization of dengue virus in the presence of Fc receptor-mediated phagocytosis distinguishes serotype-specific from cross-neutralizing antibodies. *Antiviral Research* **96**:3, 340-343. [[Crossref](#)]
185. Kaitlin Rainwater-Lovett, Isabel Rodriguez-Barraquer, Derek AT Cummings, Justin Lessler. 2012. Variation in dengue virus plaque reduction neutralization testing: systematic review and pooled analysis. *BMC Infectious Diseases* **12**:1. . [[Crossref](#)]
186. Arunee Sabchareon, Derek Wallace, Chukiat Sirivichayakul, Kriengsak Limkittikul, Pornthep Chanthavanich, Saravudh Suvannadabba, Vithaya Jiwariyavej, Wut Dulyachai, Krisana Pengsaa, T Anh Wartel, Annick Moureau, Melanie Saville, Alain Bouckennooghe, Simonetta Viviani, Nadia G Tornieporth, Jean Lang. 2012. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *The Lancet* **380**:9853, 1559-1567. [[Crossref](#)]
187. Marisol Simões, Luiz Antonio B. Camacho, Anna M.Y. Yamamura, Emily Hime Miranda, Ana Carolina R.A. Cajaraville, Marcos da Silva Freire. 2012. Evaluation of accuracy and reliability of the plaque reduction neutralization test (micro-PRNT) in detection of yellow fever virus antibodies. *Biologicals* **40**:6, 399-404. [[Crossref](#)]
188. Katherine M. Smith, Kavita Nanda, Carla J. Spears, Amanda Piper, Mariana Ribeiro, Michelle Quiles, Caitlin M. Briggs, Gwynneth S. Thomas, Malcolm E. Thomas, Dennis T. Brown, Raquel Hernandez, Victoria McCarl. 2012. Testing of Novel Dengue Virus 2 Vaccines in African Green Monkeys: Safety, Immunogenicity, and Efficacy. *The American Journal of Tropical Medicine and Hygiene* **87**:4, 743-753. [[Crossref](#)]
189. Marli Tenório Cordeiro. 2012. Laboratory diagnosis for dengue. *Revista do Instituto de Medicina Tropical de São Paulo* **54**:suppl 18, 10-12. [[Crossref](#)]
190. Shuetsu Fukushima, Hideki Tani, Tomoki Yoshikawa, Masayuki Saijo, Shigeru Morikawa. 2012. Serological Assays Based on Recombinant Viral Proteins for the Diagnosis of Arenavirus Hemorrhagic Fevers. *Viruses* **4**:10, 2097-2114. [[Crossref](#)]
191. Yee Sin Leo, Annelies Wilder-Smith, Sophia Archuleta, Lynette P. Shek, Chia Yin Chong, Hoe Nam Leong, Chian Yong Low, May-Lin Helen Oh, Alain Bouckennooghe, T. Anh Wartel, Denis Crevat. 2012. Immunogenicity and safety of recombinant tetravalent dengue vaccine (CYD-TDV) in individuals aged 2-45 years. *Human Vaccines & Immunotherapeutics* **8**:9, 1259-1271. [[Crossref](#)]
192. Veronique Barban, Jorge L. Munoz-Jordan, Gilberto A. Santiago, Nathalie Mantel, Yves Girerd, Sandrine Gulia, Jean-Baptiste Claude, Jean Lang. 2012. Broad neutralization of wild-type dengue virus isolates following immunization in monkeys with a tetravalent dengue vaccine based on chimeric Yellow Fever 17D/Dengue viruses. *Virology* **429**:2, 91-98. [[Crossref](#)]

193. Kin Fai Tang, Eng Eong Ooi. 2012. Diagnosis of dengue: an update. *Expert Review of Anti-infective Therapy* **10**:8, 895-907. [[Crossref](#)]
194. Alienys Izquierdo, Iris Valdés, Lázaro Gil, Lisset Hermida, Sheila Gutiérrez, Angélica García, Lidice Bernardo, Alekis Pavón, Gerardo Guillén, María G. Guzmán. 2012. Serotype specificity of recombinant fusion protein containing domain III and capsid protein of dengue virus 2. *Antiviral Research* **95**:1, 1-8. [[Crossref](#)]
195. Brett A. Thibodeaux, Nina C. Garbino, Nathan M. Liss, Joseph Piper, Jacob J. Schlesinger, Carol D. Blair, John T. Roehrig. 2012. A humanized IgG but not IgM antibody is effective in prophylaxis and therapy of yellow fever infection in an AG129/17D-204 peripheral challenge mouse model. *Antiviral Research* **94**:1, 1-8. [[Crossref](#)]
196. William B. Messer, Boyd Yount, Kari E. Hacker, Eric F. Donaldson, Jeremy P. Huynh, Aravinda M. de Silva, Ralph S. Baric. 2012. Development and Characterization of a Reverse Genetic System for Studying Dengue Virus Serotype 3 Strain Variation and Neutralization. *PLoS Neglected Tropical Diseases* **6**:2, e1486. [[Crossref](#)]
197. Anuja Mathew, Kim West, Siripen Kalayanaroj, Robert V. Gibbons, Anon Srikiatkachorn, Sharone Green, Daniel Libraty, Smita Jaiswal, Alan L. Rothman. 2011. B-Cell Responses During Primary and Secondary Dengue Virus Infections in Humans. *The Journal of Infectious Diseases* **204**:10, 1514-1522. [[Crossref](#)]
198. David Shaw, Ming Qiao, Anh Wartel-Tram, Remi Forrat, Jean Lang. 2011. Priming Effect of Dengue and Yellow Fever Vaccination on the Immunogenicity, Infectivity, and Safety of a Tetravalent Dengue Vaccine in Humans. *The American Journal of Tropical Medicine and Hygiene* **85**:4, 724-731. [[Crossref](#)]
199. Bruno Guy, Beatrice Barrere, Claire Malinowski, Melanie Saville, Remy Teyssou, Jean Lang. 2011. From research to phase III: Preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* **29**:42, 7229-7241. [[Crossref](#)]
200. K. R. Chan, S. L.-X. Zhang, H. C. Tan, Y. K. Chan, A. Chow, A. P. C. Lim, S. G. Vasudevan, B. J. Hanson, E. E. Ooi. 2011. Ligation of Fc gamma receptor IIB inhibits antibody-dependent enhancement of dengue virus infection. *Proceedings of the National Academy of Sciences* **108**:30, 12479-12484. [[Crossref](#)]
201. Jie Li, Dong-mei Hu, Xi-xia Ding, Yue Chen, Yu-xian Pan, Li-wen Qiu, Xiao-yan Che. 2011. Enzyme-Linked Immunosorbent Assay-Format Tissue Culture Infectious Dose-50 Test for Titrating Dengue Virus. *PLoS ONE* **6**:7, e22553. [[Crossref](#)]
202. Elizabeth Hunsperger, Manuela Beltran, Luz Nereida Acosta, Jorge Jordan-Munoz, Jomil Torres, Richard Luce, Kay M. Tomashek. 2011. Serological Evaluation of Suspected West Nile Virus Human Cases following Its Introduction during a Dengue Outbreak in Puerto Rico in 2007. *Clinical and Vaccine Immunology* **18**:6, 978-983. [[Crossref](#)]
203. Grace Yap, Bijon Kumar Sil, Lee-Ching Ng. 2011. Use of Saliva for Early Dengue Diagnosis. *PLoS Neglected Tropical Diseases* **5**:5, e1046. [[Crossref](#)]
204. Rosario Z. Capeding, Imelda A. Luna, Emily Bomasang, Socorro Lupisan, Jean Lang, Remi Forrat, Anh Wartel, Denis Crevat. 2011. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: Randomized controlled phase I trial in the Philippines. *Vaccine* **29**:22, 3863-3872. [[Crossref](#)]
205. Gijs G.G. Baaten, Gerard J.B. Sonder, Hans L. Zaaijer, Tom van Gool, Joan A.P.C.M. Kint, Anneke van den Hoek. 2011. Travel-related Dengue Virus Infection, the Netherlands, 2006-2007. *Emerging Infectious Diseases* **17**:5, 821-828. [[Crossref](#)]
206. Xixia Ding, Dongmei Hu, Yue Chen, Biao Di, Jing Jin, Yuxian Pan, Liwen Qiu, Yadi Wang, Kun Wen, Ming Wang, Xiaoyan Che. 2011. Full Serotype- and Group-Specific NS1 Capture Enzyme-Linked Immunosorbent Assay for Rapid Differential Diagnosis of Dengue Virus Infection. *Clinical and Vaccine Immunology* **18**:3, 430-434. [[Crossref](#)]
207. S. J. Thomas. 2011. The Necessity and Quandaries of Dengue Vaccine Development. *Journal of Infectious Diseases* **203**:3, 299-303. [[Crossref](#)]
208. Jorge Poo, Francisco Galan, Remi Forrat, Betzana Zambrano, Jean Lang, Gustavo H. Dayan. 2011. Live-attenuated Tetravalent Dengue Vaccine in Dengue-naïve Children, Adolescents, and Adults in Mexico City. *Pediatric Infectious Disease Journal* **30**:1, e9-e17. [[Crossref](#)]
209. Dennis Trent, Jinho Shin, Joachim Hombach, Ivana Knezevic, Philip Minor. 2010. WHO Working Group on technical specifications for manufacture and evaluation of dengue vaccines, Geneva, Switzerland, 11-12 May 2009. *Vaccine* **28**:52, 8246-8255. [[Crossref](#)]
210. Maria G. Guzman, Scott B. Halstead, Harvey Artsob, Philippe Buchy, Jeremy Farrar, Duane J. Gubler, Elizabeth Hunsperger, Axel Kroeger, Harold S. Margolis, Eric Martínez, Michael B. Nathan, Jose Luis Pelegrino, Cameron Simmons, Sutee Yoksan, Rosanna W. Peeling. 2010. Dengue: a continuing global threat. *Nature Reviews Microbiology* **8**:S12, S7-S16. [[Crossref](#)]
211. Brett M. Forshey, Carolina Guevara, V. Alberto Laguna-Torres, Manuel Céspedes, Jorge Vargas, Alberto Gianella, Efraín Vallejo, César Madrid, Nicolas Aguayo, Eduardo Gotuzzo, Victor Suarez, Ana Maria Morales, Luis Beingolea, Nora Reyes, Juan Perez, Monica Negrete, Claudio Rocha, Amy C. Morrison, Kevin L. Russell, Patrick J. Blair, James G. Olson, Tadeusz J. Kochel. 2010.

Arboviral Etiologies of Acute Febrile Illnesses in Western South America, 2000–2007. *PLoS Neglected Tropical Diseases* 4:8, e787. [[Crossref](#)]

212. Grace Yap, Kwoon-Yong Pok, Yee-Ling Lai, Hapuarachchige-Chanditha Hapuarachchi, Angela Chow, Yee-Sin Leo, Li-Kiang Tan, Lee-Ching Ng. 2010. Evaluation of Chikungunya Diagnostic Assays: Differences in Sensitivity of Serology Assays in Two Independent Outbreaks. *PLoS Neglected Tropical Diseases* 4:7, e753. [[Crossref](#)]
213. Sathyamangalam Swaminathan, Gaurav Batra, Navin Khanna. 2010. Dengue vaccines: state of the art. *Expert Opinion on Therapeutic Patents* 20:6, 819-835. [[Crossref](#)]
214. Ronald B. Reisler, Denise K. Danner, Paul H. Gibbs. 2010. Immunogenicity of an inactivated Japanese encephalitis vaccine (JE-VAX) in humans over 20 years at USAMRIID: Using PRNT50 as an endpoint for immunogenicity. *Vaccine* 28:12, 2436-2441. [[Crossref](#)]
215. Eiji Konishi, Yuko Tabuchi, Atsushi Yamanaka. 2010. A simple assay system for infection-enhancing and -neutralizing antibodies to dengue type 2 virus using layers of semi-adherent K562 cells. *Journal of Virological Methods* 163:2, 360-367. [[Crossref](#)]
216. Philippe Thullier, Dorothea Thea Sesardic. Neutralization Tests 705-722. [[Crossref](#)]
217. Barbara W. Johnson, Olga Kosoy, Elizabeth Hunsperger, Manuela Beltran, Mark Delorey, Farshad Guirakhoo, Thomas Monath. 2009. Evaluation of Chimeric Japanese Encephalitis and Dengue Viruses for Use in Diagnostic Plaque Reduction Neutralization Tests. *Clinical and Vaccine Immunology* 16:7, 1052-1059. [[Crossref](#)]
218. Atsushi Yamanaka, Eiji Konishi. 2009. A simple method for evaluating dengue vaccine effectiveness in mice based on levels of viremia caused by intraperitoneal injection of infected culture cells. *Vaccine* 27:28, 3735-3743. [[Crossref](#)]
219. Grace K Tan, Sylvie Alonso. 2009. Pathogenesis and prevention of dengue virus infection: state-of-the-art. *Current Opinion in Infectious Diseases* 22:3, 302-308. [[Crossref](#)]
220. Stephen J. Thomas, Joachim Hombach, Alan Barrett. 2009. Scientific consultation on cell mediated immunity (CMI) in dengue and dengue vaccine development. *Vaccine* 27:3, 355-368. [[Crossref](#)]
221. Li-wen Qiu, Biao Di, Kun Wen, Xin-shuai Wang, Wei-hua Liang, Ya-di Wang, Yu-xian Pan, Ming Wang, Yan-qing Ding, Xiao-yan Che. 2009. Development of an Antigen Capture Immunoassay Based on Monoclonal Antibodies Specific for Dengue Virus Serotype 2 Nonstructural Protein 1 for Early and Rapid Identification of Dengue Virus Serotype 2 Infections. *Clinical and Vaccine Immunology* 16:1, 88-95. [[Crossref](#)]