6 | DENGUE VIRUSES

6.1 | Disease agent

- Dengue viruses (DENV-1, DENV-2, DENV-3, DENV-4)

6.2 | Disease agent characteristics

- Family: *Flaviviridae*; Genus: *Flavivirus*.
- Virion morphology and size: Enveloped, icosahedral nucleocapsid symmetry, spherical particles, 40–60 nm in diameter.
- Nucleic acid: Linear, positive-sense, single-stranded RNA genome, ~11.0 kb in length.
- Four distinct serotypes (DENV 1-4).
- Physicochemical properties: Inactivated by heating for 10 min at >56°C; half-life of 7 h at 37°C; sensitive to treatment with lipid solvents, detergents, ether, trypsin, chloroform, formaldehyde, and β-propiolactone; infectivity reduced after exposure to irradiation and inactivated at pH 1–3.
- Dengue viruses are stable in dried blood and exudates up to several days at room temperature.

6.3 | Disease name

- Dengue, dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS).
  - WHO classifies dengue into dengue, with and without warning signs, and severe dengue, rather than DHF and DSS.
- Sometimes referred to as "break-bone fever" because of the nature of the symptoms.

6.4 | Priority level

- Scientific/Epidemiologic evidence regarding blood safety: Low in the continental US; priority is related to asymptomatic viremia that has been shown to result in transfusion-transmitted disease and potential for emergence in those parts of the continental US where the vector exists but disease is not endemic. This risk is mitigated by the low rate of autochthonous transmission in the continental US, and by existing deferrals that would exclude donors with travel to areas of the world where dengue and malaria coexist. Concern is moderate to high in Puerto Rico and non-US endemic areas.
- Public perception and/or regulatory concern regarding blood safety: Moderate in the continental US where local cases of dengue have been documented; moderate/high in areas of the Americas where the virus is endemic
- Public concern regarding disease agent: Very low in the continental US except areas where transmission has been documented (e.g., South Florida); moderate/high in areas of the Caribbean and other endemic areas of the Americas

6.5 | Background

- More than 130 arboviruses are known to cause human disease; most of public health importance belong to the genera: *Flavivirus*, *Alphavirus* and *Orthobunyavirus*. Many are nationally notifiable via state reporting to the US CDC (ArboNet); for example, DENV, Zika virus, California serogroup viruses, chikungunya virus, eastern equine encephalitis virus, Powassan virus, St Louis encephalitis virus, West Nile virus, western equine encephalitis virus and yellow fever virus.
- Dengue is among the most important mosquito-borne viral diseases in the world. The disease is caused by four genetically distinct serotypes, DENV-1, -2, -3 and -4, that are further divided into genotypes with strain variations and quasispecies that demonstrate phylogenetic complexity.
- In the last 50 years, dengue incidence has increased 30-fold worldwide. It is highly endemic in most tropical and subtropical areas of Asia, Australia and the Americas and exists in Africa or wherever the primary mosquito vector *Aedes aegypti* is present.
- In the United States, dengue is endemic in Puerto Rico, the Virgin Islands and most US-affiliated Pacific Islands; incidence increases during the wet, hot season of each year. The intensity of annual epidemics is cyclical. Throughout the continental US, dengue is the most frequently diagnosed cause of fever in travelers returning from Asia and the Americas.
- Along the US-Mexico border, outbreaks of dengue have been identified in the United States when there are epidemics along the Mexican side of the border. Dengue serological surveys by the CDC have demonstrated an antibody prevalence of 40% along the US side of the Texas-Mexico border and 78% on the Mexico side. This high rate suggests endemic transmission in the United States.
- During 2009–2010, a locally transmitted dengue outbreak was identified in Key West, FL, and isolated autochthonous cases were found farther north in Florida.
- During the summer of 2013, a very localized outbreak of dengue occurred among residents of Martin and

St. Lucie Counties in Florida. Twenty-two cases were eventually reported. The major blood collection organization in the involved counties briefly suspended collections. This response was possible because the relatively few collections required in the epidemic area could be replaced by enhanced collections elsewhere. Whether this approach would be feasible in more extensive outbreaks without jeopardizing the blood supply is unknown and dependent on multiple considerations.

- Hawaii has experienced recurring dengue outbreaks in 2001, 2011 and 2015-6. 264 cases were reported in the 2015-6 outbreak, similar in scope to the 2001 cycle. It was the largest outbreak of dengue in a non-endemic area of the US since 1946. There was additional concern since the vector, the *Aedes* mosquito, transmits both dengue and Zika viruses, and this was contemporaneous with the Zika outbreaks but there were no autochthonous Zika cases. No confirmed cases of transfusion transmission were documented.
- In 2022 (through Nov 2), 888 dengue infections were reported in US states and 316 (309 autochthonous) in territories. Among 41 cases of locally acquired dengue in Florida, 37 occurred in Miami-Dade and 2 in Broward counties. This was in addition to 679 total cases in individuals with travel history to a dengue endemic area in the 2 weeks prior to onset. Over 90% were typed as DENV-3 with most travelers having acquired infection in Cuba.
- Evidence exists for additional circulation of genetic variants in nonhuman primates.

6.6 | Common human exposure routes

- Vector-borne; transmission occurs through a mosquito-human cycle
- The importance of transmission from the sylvatic mosquito-nonhuman-primate cycle is minimal

6.7 | Likelihood of secondary transmission

- Isolated cases document parenteral transmission; aerosol transmission does not occur.

6.8 | At-risk populations

- Tropical areas of Asia, Oceania, Africa, and the Americas usually in the monsoon or rainy season, especially among persons residing in substandard living conditions.
- Small outbreaks occur in Queensland Australia.
- Travelers to endemic regions (e.g., 3.4 cases/1000 Israeli travelers to Thailand) with highest proportionate morbidity for travelers to Southeast Asia and the Caribbean
- Secondary infections with a different serotype are associated with an increased risk of severe dengue.

6.9 | Vector and reservoir involved

- *Aedes* species mosquitoes.
- Both urban (mosquito-human) and sylvatic (mosquito-primate) cycles are observed.

6.10 | Blood phase

- Asymptomatic viremia is recognized. Viremia typically begins 2–3 days before the onset of symptoms, and it continues for 4–5 days during acute illness.
- Viremia for DENV-1, 2, and 3 infections ranges from barely detectable to 10^8 virions/mL for 2–12 days (median of 4–5 days); titers for DENV-4 are about 100-fold lower.
- NAT prevalence studies among blood donors in endemic areas (Brazil, Puerto Rico, and Honduras) have shown rates of 0.06%, 0.07%–0.2%, and 0.40%, respectively. Virus was cultured from some of these donors.
- In a large Brazilian study during the 2012 epidemic, 0.51%–0.80% of healthy donors were RNA reactive.

6.11 | Survival/persistence in blood products

- Frozen plasma, red cells and platelets have been associated with transfusion transmission; one case involved a 38-day old RBC unit.

6.12 | Transmission by blood transfusion

- The first documented transfusion-associated case of dengue occurred in 2002 during a local outbreak in Ma Wan, Hong Kong, an area that is not endemic for dengue. The index recipient was a 76-year-old seronegative woman who developed fever without rash 2 days after receiving a unit of packed RBCs collected from a 17-year-old donor who was diagnosed with dengue (generalized rash) 7 days postdonation. The blood had been stored at 4–8°C for 38 days prior to transfusion.

RT-PCR testing of the recovered donor plasma and archived specimens from the donor and recipient were found to be positive for DENV-1. IgM-specific antibody also developed in the recipient posttransfusion.
- The second documented transfusion transmission was a cluster reported from Singapore, an area endemic for dengue in which three cases occurred from one donation from an infected donor. The donor was a 52-year-old male whose components were transfused to three recipients. The donor reported fever the day following donation, and a stored serum sample was positive for DENV-2 by PCR. Both the RBC and FFP recipients reported fever 1–2 days posttransfusion and tested positive by PCR for DENV-2. Direct sequencing of PCR amplified products from the donor and two recipients showed alignment of envelope segments for DENV-2. The platelet recipient was asymptomatic for dengue. All three recipients tested antibody positive for IgM and/or IgG with documented seroconversion in the RBC recipient 11 days posttransfusion.
- Another report of transfusion transmission was in Puerto Rico in 2007 following tracing of a patient who received an RNA-positive packed RBC unit from a donor in which greater than 10^8 copies/mL were detected. Donor screening was part of a research study investigating the frequency of donor viremia in Puerto Rico during the 2007 dengue outbreak. Recipient samples were available from the CDC as part of a repository of all clinical cases reported on the island. Both the donor and recipient samples had identical sequences of DENV-2 covering 1500 nucleotides in the envelope region. The recipient developed DHF 4 days posttransfusion.
- Many other case reports of dengue transfusion transmission worldwide have been published.
- Although severe illness has been reported after transfusion transmission of DENV, in a prospective Brazilian study of 39,134 donors, 16 RNA-positive components were infused into 16 susceptible recipients. In five, transfusion transmission occurred, in one it was possible and in ten there was no transmission. The infected recipients were clinically indistinguishable from controls.
- Transmission also has been observed after needle stick exposure and in bone marrow and kidney transplant recipients.

6.13 | Cases/frequency in population

- The incidence has grown dramatically, with half of the world's population at risk. Worldwide, the WHO estimates between 100 and 400 million cases of infection annually, with 80% of those mild or asymptomatic.
- In endemic areas, over 90% of the population may be antibody positive.
- In the United States, dengue became nationally notifiable in 2010. Local outbreaks have occurred most recently in Hawaii (2015), Florida (2013, 2020, 2022) and Texas (2013). The first case of local transmission in Arizona was reported in Nov 2022 in Maricopa County. Most outbreaks in the United States are small and limited to small areas. However, local spread is possible in any area when *Aedes* mosquitoes are present.

6.14 | Incubation period

- 3–14 days (usually 4–7 days)

6.15 | Likelihood of clinical disease

- Low; the US CDC estimates that one-half or more of all dengue-infected individuals are asymptomatic. Other reports indicate significant variability with documented asymptomatic to symptomatic ratios of 2:1–13:1 depending on circumstances surrounding the acquisition of the data.
- Homologous immunity to a single serotype is complete and probably lifelong, but cross-protection between serotypes lasts less than 12 weeks.

6.16 | Primary disease symptoms

- Dengue fever presents as an abrupt onset of fever sustained for up to 5–7 days, accompanied by a transient maculopapular rash that occurs in up to 50% of the patients around day 4, followed by severe headache, retrobulbar pain, lumbosacral aching pain ("breakbone fever"), conjunctivitis, and facial flushing accompanied by myalgia or bone pain, anorexia, nausea, vomiting, weakness, and prostration.
- The rash begins on the legs and trunk and spreads centripetally but spares the soles and palms. It may desquamate. In some cases, a biphasic course may occur.
- Severe dengue (DHF, DSS) is quite rare with only 0.5% of secondary cases progressing to this outcome. It occurs more often in persons previously infected with another serotype of DENV. The distinctive feature of severe dengue is the rapid onset of capillary leakage (pleural effusion, ascites, or hypoproteinemia) leading

to hypotension and hemoconcentration. This results in shock with a subset presenting with hemorrhagic manifestations (petechiae, epistaxis, gastrointestinal bleeding, and menorrhagia) that occurs 4–7 days after onset of the disease often following a period of defervescence.
- Central neurologic disorders (encephalopathy, peripheral mononeuropathy, polyneuritis, etc.) may occur in severe dengue although this relationship is not fully accepted by all experts.
- Prolonged postinfectious fatigue and depression syndrome can persist for weeks.
- Differentiation from chikungunya is sometimes difficult, although excruciating, symmetrical joint pain, as opposed to myalgia, is more consistent with chikungunya.

6.17 | Severity of clinical disease

- Moderate to high, especially involving antibody-dependent enhancement (ADE) when preexisting antibodies present from a primary (first) infection bind to a dengue viral particle during a subsequent infection with a different dengue type. The antibodies from the primary infection cannot neutralize the virus. Instead, the Ab-virus complexes attach to Fcγ receptors on circulating monocytes helping the virus to infect the monocytes more efficiently. The outcome is an increase in viral replication and risk of severe dengue.

6.18 | Mortality

- There is no specific treatment for dengue/severe dengue. Early detection of disease progression associated with severe dengue, and access to proper medical care lowers fatality rates of severe dengue to below 1%. Thus, while morbidity may be high, mortality rate is low.
- WHO estimates 21,000 deaths annually worldwide.

6.19 | Chronic carriage

- None

6.20 | Treatment available/efficacious

- Supportive treatment only

6.21 | Agent-specific screening question(s)

- No specific question is in use; however, the current questions related to travel outside United States and Canada for malaria deferral will result in deferral for travel to many dengue endemic areas.
- Travel questions could be broadened to include areas where malaria is not present and dengue outbreaks are occurring.
- Some authorities in the European Union and elsewhere have implemented temporary deferral periods following any travel to the tropics that are not malaria endemic. This would mitigate risk from arboviruses for which no mitigation is in place, by preventing donation until resolution of asymptomatic viremia. However, it could be associated with a significant operational burden and loss of donors.
- A survey in the United States representing a large proportion of blood from 2014 to 2015 looked at 14- versus 28-day deferral periods following travel to endemic areas to estimate donor loss. Results varied by season and geographic area representing regions that would trigger deferrals. Less than a 1% deferral rate was projected for a 14-day deferral limited to the Caribbean and the Americas versus 3% or greater if a 28-day deferral for all travel outside of the United States and Canada was used. The latter donor loss would not be trivial during intervals of severe shortage.
- Another strategy is the use of information sheets to enhance postdonation symptom reporting to facilitate quarantine and withdrawal of potentially infectious components.

6.22 | Laboratory test(s) available

- No FDA-licensed blood donor screening test exists; however, research NAT assays have been used for blood donor prevalence studies and the detection of viral RNA in asymptomatic individuals. One NAT-reactive donor whose index donation was infectious in mosquito cell culture had RNA detected for over 29 days indicating that the duration of DENV RNAemia may be longer than previously demonstrated; however, associated infectivity in the presence of neutralizing antibody would be expected to be considerably shorter.
- NS1 antigen is not as sensitive or specific as virus-specific RNA for blood donor screening, but its detection correlates with high RNA titers.

- DENV-specific IgM antibody can be detected by EIA 4–5 days after onset of symptoms and remains detectable for 3–6 months. The detection rate is lower in secondary DENV infection than in primary infection.
- In blood donors studied retrospectively in Puerto Rico in 2005 and in 2007 and confirmed positive for dengue RNA, 1/12 (8.3%) and 6/29 (20.7%) were IgM anti-DENV positive (using an IgM capture assay), respectively. Variability was likely related to the extent of the dengue outbreaks (2007 > 2005).

6.23 | Currently recommended donor deferral period

- No FDA Guidance or AABB Standard exists.
- The appropriate deferral period for clinical dengue is unknown but would likely be on the order of several weeks after the resolution of symptoms.
- Overall, current available scientific data do not support a temporary deferral for otherwise qualified donors living in nonendemic or non-outbreak areas of the US who have traveled to an outbreak and/or endemic area. However, may be considered in the face of a significant expanding outbreak or reported autochthonous cases as the result of introduction from an outbreak area. Resulting deferrals may reach 1%–3% of presenting donors.

6.24 | Impact on blood availability

- Agent-specific screening question(s): Not generally considered applicable due to concerns of donor loss without demonstrated efficacy
- Laboratory test(s) available: Not applicable; data collected using current NAT research tests indicate rates of deferral due to false positivity would be low

6.25 | Impact on blood safety

- Agent-specific screening question(s): Not generally considered applicable due to questions of sensitivity and specificity.
- Laboratory test(s) available: Not applicable; potential impact of NAT may be significant in dengue endemic areas as well as any area of concern in the United States (due to introduction by travel during an expanding outbreak or autochthonous cases).

6.26 | Leukoreduction efficacy

- No data available. Plasma viremia makes a clinically significant impact unlikely.

6.27 | Pathogen reduction efficacy for plasma derivatives

- Multiple pathogen reduction steps used in the fractionation process have been shown to be robust in the removal of enveloped viruses.

6.28 | Other prevention measures

- Mosquito control and avoidance particularly during dawn to dusk hours; repellents and wearing clothing that minimize skin exposure
- The only US FDA approved dengue vaccine is Dengvaxia (Sanofi Pasteur), a live, attenuated tetravalent chimeric vaccine made using recombinant DNA by replacing the PrM (pre-membrane) and E (envelope) structural genes of the yellow fever attenuated 17D strain vaccine with those from the four dengue serotypes. It was FDA approved in May 2019 and recommended for use in June 2021 by the Advisory Committee on Immunization Practices (ACIP) in children and adolescents 9–16 years old with laboratory-confirmed evidence of a dengue virus infection who are living in areas of the US where dengue is common. It is administered in 3 doses. The safety and effectiveness (76%) of the vaccine was determined in three randomized, placebo-controlled studies involving approximately 35,000 individuals in dengue-endemic areas, including Puerto Rico, Latin America and the Asia Pacific region. It is approved in 19 countries and the European Union. The dengue vaccine is the only vaccine available in the United States that requires testing prior to vaccination to confirm prior dengue infection. Evidence indicates that vaccination could lead to a higher risk of severe disease in those who have not been previously infected but who are subsequently infected with a different serotype.
- Several other multivalent vaccines are in various stages of development and clinical evaluation.
- Multiple inactivation procedures involving a chemical treatment with visible light or UVA, or UVC alone have demonstrated the ability to inactivate DENV in plasma and platelet concentrates, respectively.
- Flaviviruses are inactivated by multiple pathogen reduction processes that are available or in development for labile components.

SUGGESTED READING

1. Ajariyakhajorn C, Mammen MP Jr, Endy TP, Gettayacamin M, Nisalak A, Nimmannitya S, Libraty DH. Randomized, placebo-controlled trial of nonpegylated and pegylated forms of recombinant human alpha interferon 2a for suppression of dengue virus viremia in rhesus monkeys. Antimicrob Agents Chemother. 2005;49:4508–14.
2. Bausch DG, Ksiazek TG. Viral hemorrhagic fevers including hantavirus pulmonary syndrome in the Americas. Clin Lab Med. 2002;22:981–1020.
3. Bessoff K, Phoutrides E, Delorey M, Acosta LN, Hunsperger EA. Utility of a commercial nonstructural protein 1 antigen capture kit as a dengue virus diagnostic tool. Clin Vacc Immunol. 2010;17:949–53.
4. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496:504–7.
5. Brunkard JM, Lopez JL, Ramirez J, Cifuentes E, Rothenberg SJ, Hunsperger EA, et al. Dengue fever seroprevalence and risk factors, Texas-Mexico border, 2004. Emerg Infect Dis. 2007;10:1477–83.
6. Centers for Disease Control and Prevention. Dengue: 2022 data. https://www.cdc.gov/dengue/statistics-maps/2022.html
7. Chuang WW, Wong TY, Leung YH, et al. Review of dengue fever cases in Hong Kong during 1998-2005. Hong Kong Med J. 2008;14:170–7.
8. Duffy MR, Chen T, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360:2536–43.
9. Freedman DO, Weld LH, Kozarsky PE, Fisk T, Robins R, von Sonnenburg F, et al. Spectrum of disease and relation to place of exposure among ill returned travelers. N Engl J Med. 2006;354:119–30.
10. Gubler DJ, Suharyono W, Tan R, Abidin M, Sie A. Viraemia in patients with naturally acquired dengue infection. Bull WHO. 1981;59:623–30.
11. Guzman MG, Kouri G. Dengue diagnosis, advances and challenges. Int J Infect Dis. 2004;8:69–80.
12. Lew RJ, Tsai W-Y, Wang W-K. Dengue outbreaks in Hawai'i after WWII—A review of public health response and scientific literature. Hawaii J Med Public Health. 2018;77:315–8.
13. Linnen JM, Vinelli E, Sabino EC, Tobler LH, Hyland C, Lee T-H, et al. Dengue viremia in blood donors from Honduras, Brazil, and Australia. Transfusion. 2008;48:1355–62.
14. Matos D, Tomashek KM, Perez-Padilla J, Muñoz-Jordán J, Hunsperger E, Horiuchi K, et al. Probable and possible transfusion-transmitted dengue associated with NS1 antigen negative but RNA cofermed-positive red blood cells. Transfusion. 2015;56:215–22.
15. Mohammed H, Linnen JM, Muñoz-Jordán JL, Tomashek K, Foster G, Broulik AS, et al. Dengue virus in blood donations, Puerto Rico, 2005. Transfusion. 2008;48:1348–54.
16. Mohammed H, Tomashek KM, Stramer SL, Hunsperger E. Prevalence of anti-dengue immunoglobulin G antibodies among American Red Cross blood donors in Puerto Rico, 2006. Transfusion. 2012;52:1652–6.
17. Nemes Z, Kiss G, Madarassi EP, Peterfi Z, Ferenczi E, Bakonyi T, Ternak G. Nosocomial transmission of dengue. Emerg Inf Dis. 2004;10:1880–1.
18. ProMED. Dengue virus, transfusion transmission-China (HK), Archive number 20021011.5526. 2002 [cited May 2009]. Available from: http://www.promedmail.org
19. Sabino EC, Loureino P, Lopes ME, Capuani L, McClure C, Chowdhury D, et al. Transfusion-transmitted dengue and associated clinical symptoms during the 2012 epidemic in Brazil. J Inf Dis. 2016;213:694–702.
20. Simmons CP, Farrar JJ, Nguyen vVC, Wills B. Dengue. New Engl J Med. 2012;366:1423–32.
21. Spencer BR, Stramer S, Dodd R, Kleinman SH, Hollinger B, Krysztof DE, et al. Survey to estimate donor loss to 14- or 28-day travel deferral for mitigation of CHIKV, DENV, and other acute infections. Abstract P1-030A. Transfusion. 2015; 55(S3):3A.
22. Stramer SL, Linnen JM, Carrick JM, Foster GA, Krysztof DE, Zou S, et al. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion. 2012;52: 1657–66.
23. Tambyah PA, Koay ES, Poon ML, Lin RV, Ong BK. Dengue hemorrhagic fever transmitted by blood transfusion. N Engl J Med. 2008;359:1526–7.
24. Wilder-Smith A, Schwartz E. Dengue in travelers. New Engl J Med. 2005;353:924–32.
25. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva, Switzerland: WHO; 2009. https://apps.who.int/iris/handle/10665/44188
26. World Health Organization. Dengue and severe dengue fact sheet. Geneva, Switzerland: WHO; 2013 Available from: http://www.who.int/mediacentre/factsheets/fs117/en/
27. World Health Organization. Global strategy for dengue prevention and control 2012-2020. Geneva, Switzerland: WHO; 2012 Available from: https://www.who.int/publications/i/item/9789241504034