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FLAVIDOT: AN AUTOMATED VIRUS PLAQUE COUNTER FOR MEASUREMENT OF THE SEROLOGICAL NEUTRALIZATION RESPONSE AGAINST ZIKA AND DENGUE VIRUSES

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Immunoassays, such as plaque reduction neutralization test (PRNT), allow us to quantify the neutralization capability of antisera. This assay is considered the gold standard for estimating the amount of infective viral particles in a sample but requires researchers to count individual plaques in each of the wells either by eye or utilizing expensive equipment and proprietary software. This process is time-consuming (even more so on 96-well plates), difficult to standardize among analysts, and not easy to customize when working with a wide variety of viruses. We report the development of a free, open-source, automated alternative, the Flavidot plaque counter. The Flavidot is capable of counting viral plaques from a variety of phenotypes, is easy to customize for different staining methods, and has a user-friendly interface. The counter was created using R statistical software, and Shiny to provide a user interface where different parameters may be adjusted. The counter was optimized by analyzing plaque images of 150+ DENV strains of all four serotypes. PRNT assays were performed on 96-well plates coated with C6/36 cells. The program's performance was tested on C6/36 and Vero cells infected with DENV and ZIKV. We compared the plaque counts obtained by unaided eye and the counts produced by the Flavidot. Counts obtained by Flavidot automatic settings were similar to those obtained by eye (Analyst 1, average plaque difference between methods: -1.89 [95%CI -4.00 - 0.22]; Analyst 2: -2.64 [95% CI -5.02 - 0.25]), which is consistent with the average plaque difference in counts by eye between analysts (-0.75 [95%CI -2.29- 0.79]). The Flavidot plaque counter was developed as a tool to assist researchers using PRNTs on DENV and ZIKV to quantify protective antibodies. It is easy to install and requires no prior coding experience. With further improvements, this counter may be used in clinical settings, providing analysts with the speed and consistency needed to respond to emerging global health threats.

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DENGUE SEROTYPE AND DISEASE SEVERITY TRENDS AMONG INFANTS AND YOUNG CHILDREN IN INDIA, 2012-2015: IMPLICATIONS FOR DENGUE VACCINE STUDIES

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India is a major epicenter for dengue. We aimed to provide baseline information for dengue vaccines by studying clinical and serotype patterns among children with dengue infection hospitalized at a tertiary-care center in southern India. Dengue was confirmed by positive NS1 and/or anti-dengue-IgM direct ELISA. Dengue severity was graded using the 2009 WHO classification. Dengue serotypes were determined by RT-PCR and sequencing. Between 2012-2015, among 1,064 children with suspected dengue, laboratory-confirmed dengue was seen in 726; 157 in the Nov 2012-Dec 2013 period, and 569 in Sept 2014-Dec 2015. The male:female ratio remained constant at 1.2:1. Mean age decreased over time: 9.1yrs (2012-13) and 7.1yrs (2014-15). The proportion of infants (age<1yr) increased over time; 4.2% to 8.6% (p<0.01). Severe dengue occurred among 25.1% and 28.8% of children during the two time periods. Dengue severity increased with age (p<0.001); infants were more likely to have severe dengue than older children (62.2% vs 24.9%, p<0.001). Moderate-to-severe thrombocytopenia, present in 62.8%, was significantly associated with severe dengue (p<0.001) and DENV-1 serotype (p<0.05). In 2012-13 (PCR and sequencing-confirmed, n=113), serotype 3 (38.9%) and 2 (38.1%) predominated; DENV-1 was less common (19.5%). In 2014-15 (n=401), DENV-1 (64.6%) predominated, followed by DENV-2 (21.2%), DENV-3 (6.7%) and DENV-4 (2.0%). Co-infections with multiple serotypes were seen in 5.5% (n=22). There was no correlation between disease severity and multiple-serotype co-infection. Our results indicate a high burden of dengue among Indian children, particularly infants, and increasing disease severity with dynamic serotype replacement, highlighting the need for a safe vaccine for young children. Ongoing population-based epidemiological surveillance is critical for dengue vaccine studies.

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BURDEN OF DENGUE IN OUAGADOUGOU, BURKINA FASO

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To address a gap in knowledge about dengue burden in Africa, the Dengue Vaccine Initiative conducted passive health care facility-based fever surveillance and seroprevalence study in Ouagadougou, Burkina Faso from Dec 2014 to Apr 2017. In the passive fever surveillance component, 3184 patients between 1-55 years of age, with current fever or history of fever (≤7 days) with negative malaria rapid test, were enrolled at 5 health care centers; 505 were found to be positive either by dengue IgM ELISA or dengue rapid test (NS1 or IgM). Most of these cases occurred in the 15-29 year old age group. During the 26-month study period, dengue cases peaked in Sep-Dec 2015 and 2016. Selected acute serum samples, collected between Dec 2014 and June 2016, underwent further testing using Bioneer's DENV/ZIKV/CHIKV triplex PCR commercial kit. There were 157 acute samples selected for testing; 20 of these samples also had seroconversion by IgM ELISA between acute and convalescent visits; 17 were NS1 Ag positive by rapid test. Twelve (60%) of the 20 IgM seroconverters were dengue PCR positive; 14 (82%) of the 17 NS1 Ag positive patients were dengue PCR positive. In the seroprevalence component, blood samples were collected at 6 month intervals in a cohort aged 1-55 years, with 3 sets of blood samples collected in 3,066 subjects who were initially enrolled in June 2015. At enrollment, 66.4% of subjects were IgG ELISA positive. In the 2nd (n=2,473) and 3rd bleeds (n=2, 248), 70.4% and 67.4% of subjects were IgG positive, respectively. The seroconversion rate was highest at 7.2% in 10-14 year olds, followed by 5-9 year olds (7.1%) and 30-34 year olds (6.9%). During the study period, a dengue outbreak was declared by WHO in Ouagadougou from Sep 2016 to Jan 2017. An additional seroprevalence blood collection was being performed in Mar/Apr 2017 on about 2,000 subjects who had participated in all three previous seroprevalence bleeds. Our study indicates that substantial endemic transmission of dengue is occurring in Burkina Faso.