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Enhancing the concordance of two commercial dengue IgG ELISAs by exchange of the calibrator sample

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ABSTRACT

Background: Dengue IgG testing is being recommended before dengue vaccination. Presently, the diagnostic method of choice is the dengue IgG ELISA.
Objective: Determine the test performance and concordance of two commercial dengue IgG ELISA kits.
Study design: A diagnostic study to examine the sensitivity, specificity, accuracy and concordance of the Panbio Dengue Indirect IgG ELISA kit and the NovaLisa Dengue IgG ELISA kit. Sera (483) were from dengue-endemic regions in Sudan. Test performance characteristics were determined when tests were performed as indicated in the test kits and when the Panbio calibrator sample was used for both tests.
Results: The sensitivity of the Panbio and the NovaLisa ELISA was 91.1% and 99.0% and the specificity was 79.4% and 50.9%. The Panbio test was slightly more accurate (87.5% compared with 84.0%). Quantitative measurement readings of the tests correlated. The calibrator samples gave different cutoff values. Replacing the NovaLisa cutoff sample with the Panbio calibrator sample raised the accuracy of the NovaLisa assay to 88% and increased the concordance of the tests from 82.8 to 93%.
Conclusions: The study shows that the two dengue IgG ELISAs differed clearly in sensitivity and specificity and gave discordant results for 17.2% of the sera. For the most part the discrepancy depended on the calibrator sample. The findings indicate that an optimized dengue IgG calibrator standard can enhance accuracy and concordance of commercial dengue ELISAs. An optimized standard calibrator would make dengue IgG seroprevalence testing more reliable.

1. Background and objective

Dengue fever is a viral infection transmitted from human to human by mosquitoes of the genus *Aedes*. The infection is endemic in tropical and subtropical regions of the world causing an estimated 390 million infections per year [1]. A live attenuated dengue vaccine, CYD-TDV, has recently been shown "to be efficacious and safe in persons who have had a previous dengue virus (DENV) infection but it carries an increased risk of severe dengue in those who experience their first natural dengue infection after vaccination" [2]. The World Health Organization (WHO) recommends vaccination only if the risk among seronegative individuals can be minimized either by pre-vaccination screening or, if screening is not feasible, in areas with seroprevalence rates of at least 80% by age 9 years [3]. This strategy requires accurate dengue IgG tests for screening and assessment of seroprevalence. The most sensitive and specific antibody test for DENV is the neutralization test (NT). The NT is impractical for surveys of seroprevalence because work with the virus requires biosafety level 2 or 3 containment and the test is time-consuming and labor-intensive.

A more convenient way to examine the DENV IgG response are dengue IgG enzyme-linked immunosorbent assays (ELISAs) and rapid diagnostic tests (RDT). Dengue point-of-care RDTs are the preferred tests for pre-vaccination dengue screening but to date no suitable dengue IgG RDTs are available. Currently existing dengue RDTs are designed for diagnosing acute dengue fever but are not suitable to correctly assess the DENV serostatus [3,4]. However, several DENV IgG ELISA kits are commercially available. The dengue ELISA kits vary in sensitivity and specificity. For instance, a comparison of the Panbio dengue virus IgG capture ELISA and the Standard Diagnostics dengue IgG ELISA showed sensitivities of 56.4 and 88.9%, and specificities of

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95.3 and 63.5%. With an in-house reference ELISA the tests agreed by only 81.7 and 59.3% [5]. In a comparison of six immunoassays for dengue IgG detection, the Panbio IgG Indirect ELISA had a sensitivity of 100% and a specificity of 98% based on overall agreement between the six assays [6]. In a dengue seroprevalence study in travellers the Focus Diagnostics anti-DENV IgG ELISA showed a sensitivity of 100% and a specificity of 24% compared with a dengue virus neutralization test [7] A comparison of the performance of dengue IgG tests in 42 laboratories showed an average sensitivity of dengue IgG ELISAs of 84.4% and a specificity of 83% [8].

The variable accuracy of commercial dengue virus IgG tests prompted us to evaluate and compare two dengue IgG ELISA kits, the Panbio Dengue IgG Indirect ELISA and the NovaLisa Dengue Virus IgG ELISA and to examine the role of the calibrator samples for the performance of the tests.

2. Study design

2.1. Enzyme immunoassays

The Panbio® Dengue IgG Indirect ELISA (Abbott Inc., manufactured by Standard Diagnostics Inc., 65, Borahagal-ro, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea) and the NovaLisa® Dengue Virus IgG ELISA kit (Novatec Immundiagnostica GmbH, Dietzenbach, Germany) were examined. Tests were performed according to the instruction manuals. The NovaLisa IgG ELISA uses purified DENV-2 (strain 16681) as antigen. To obtain the cutoff OD value the NovaLisa ELISA contains a cutoff sample. The OD value obtained with this sample represents the cutoff OD. The Panbio ELISA uses a mixture of all four dengue virus serotypes. The test kit contains a calibrator sample to calculate the cutoff. To obtain the cutoff, the OD value obtained with the calibrator sample is being multiplied by a correction factor that is being specified in the user manual. For both tests, OD results were normalized by dividing the sample OD through the cutoff OD. Test units were defined as the ratio of the OD of the sera and the OD of the cutoff times 10. Values above 11 units were positive, values between 9 and 11 were considered equivocal. Values below 9 were negative.

2.2. Dengue virus neutralization assay

Neutralization tests were performed as previously described [9]. Briefly, Vero VFM cells (25000/well) were seeded in 96-well culture plates and incubated overnight. Sera were diluted 1:20 with medium (DMEM, 1% fetal calf serum, penicillin, streptomycin) and incubated with dengue virus (approximately 16 $TCID_{50}$) for 1 h. We used the following DENV strains isolated from travellers: DENV-1 (isolate 2522/10), acquired presumably in Eritrea, DENV- 2 (isolate 3229/11), Thailand, DENV-3 (isolate 3140/09), Senegal, and DENV-4 (isolate 3274/09), India. Most sera were tested with the four DENV serotypes. When it was observed that 133 of 137 DENV-neutralizing sera (97.1%) neutralized either DENV-2 or DENV-3 or both serotypes, a residual of 40 sera from group 3 was only tested with DENV-2 and DENV-3. Serum-virus dilutions were added to the Vero cells in triplicate cultures and cell culture plates were incubated for 7 or 8 (dengue virus 2, 3, 4) to 13 or 14 (dengue virus 1) days to permit sufficient infection time that differs by virus type. Cytopathic effects were monitored microscopically. The incubation was stopped by removing the cell culture medium from the cells, washing the cells twice with phosphate-buffered saline (PBS) and fixing the cells with methanol for 10 min at −20 °C. Subsequently, the plates were stained by an ELISA protocol using the anti-flavivirus glycoprotein monoclonal antibody 4G2 and HRP-conjugated rabbit-anti-mouse IgG antibody (diluted 1:1000, DAKO, product no. P0260). TMB substrate (TMB Soluble Reagent, TM4500, ScyTek Laboratories, Inc) was added and the enzymatic reaction was stopped by adding 1 N H_2SO_4. The 50% neutralization titer (NT_{50}) was calculated using the Spearman-Kaerber equation. As controls, each test contained uninfected cells, a negative control serum and a back-titration of the virus working dilution. The mean OD of uninfected wells plus 3 standard deviations was used as the cutoff for infected cultures and lack of neutralization. Sera were considered neutralizing when 2 or 3 of the replicate wells were not infected. Results were indeterminate if a single well was negative or had OD values < 50% of the non-neutralizing serum control. The serum was judged not neutralizing when viral infection was not inhibited in any of the wells.

2.3. Serum samples

The study included serum samples from 483 donors with an acute febrile illness from Sudan as previously described. We have previously determined the dengue seroprevalence in Sudan using the same set of sera minus sera that were IgG and IgM positive [9]. In this study, we used all sera. The study was approved by the Department of Microbiology, Faculty of Science, International University of Africa. Sera were obtained in December 2012 and January 2013 in five towns in the states Red Sea, Kassala, and North Kordofan in central and eastern Sudan. The participants were between approximately 1 and 91 years and the median age was 40.7 years There was no age limit for participation [9].

2.4. Statistical analyses

Specificity, sensitivity, predictive values and accuracy were calculated using Microsoft Excel software. Median values and the correlation coefficient of the measurement values were determined using statistical functions of the Excel program. The difference between two groups was compared with the Mann-Whitney U test [10].

3. Results

3.1. Performance characteristics of the Panbio and NovaLisa dengue ELISA and concordance of the tests

The Panbio Dengue IgG Indirect and the NovaLisa dengue IgG ELISAs were tested with sera from 483 febrile patients from different regions in Sudan. 334 sera were positive in the Panbio ELISA (69.1%) and 403 (83.4%) were positive in the NovaLisa ELISA (Table 1). The sera were grouped into four groups according to the outcome in the two tests (Table 2). Two thirds of the sera (327 (67.7%)) were concordant positive in both tests (group 1 sera), 7 sera (1.45%) were positive only in the Panbio ELISA (group 2), 76 (15.7%) were positive in the NovaLisa test alone (group 3) and 73 sera (15.1%) were concordant negative or equivocal (group 4). A subgroup of the sera was tested by dengue virus neutralization assay as a reference test to determine the sensitivity and specificity of the ELISAs. 109 of the 118 sera from group 1 that were tested by neutralization test (92.3%), 1 of the 7 sera of group 2 (14.3%), 26 of 73 sera of group 3 (35.6%) and 1 of 30 sera from group 4 (3.3%) neutralized one or more of the dengue virus strains. The number of positives and negatives/equivocals in groups 1, 3 and 4 were grossed up to all sera in the groups and the test performance characteristics were calculated. The sensitivity and specificity of the Panbio test were 91.1% and 79.4%. The sensitivity and specificity of the NovaLisa ELISA

Table 1
Proportion of positive, negative and equivocal sera in the Panbio and NovaLisa test.

	Panbio IgG ELISA (% of total number)	NovaLisa IgG ELISA (% of total number)
positive	334 (69.1)	403 (83.4)
negative	130 (26.9)	72 (14.9)
equivocal	19 (3.9)	8 (1.6)
total	483	483

Table 2
Number of sera that were positive in one, both or none of the ELISAs, fractions of the sera in each group that were tested in the neutralization assay, measured and grossed up numbers of neutralizing and non-neutralizing sera in each group.

Group	Description	Number of sera		Neutralization assay			
		Total (% of all)	Tested in NT (% of group)	Measured positive (% of tested sera)	Grossed up positive*	Measured negative (% of tested sera)	Grossed up negative*
G1	Concordant positive	327 (67.7)	118 (36.0)	109 (92.4)	302	9 (7.6)	25
G2	Panbio pos./NovaLisa neg. or equivocal	7 (1.45)	7 (100)	1 (14.3)	1	6 (85.7)	6
G3	NovaLisa pos./Panbio neg. or equivocal	76 (15.7)	73 (96.0)	26 (35.6)	27	47 (64.3)	49
G4	Concordant negative or equivocal	73 (15.1)	30 (41.0)	1 (3.3)	2	29 (96.7)	71
G 1-4	All sera	483	228	121 (68.4)	339	56 (31.6)	144

* Grossed up numbers = % of positively or negatively measured sera x total number of sera in the group. E. g. 92.4% of the 118 sera tested from G1 were positive and we assume that 92.4% or 302 of the 327 sera in G1 were positive if they were all tested.

were 99.0% and 50.9%. The accuracy of the Panbio and the NovaLisa ELISA were 87.5% and 84.0% (Table 3).

3.2. Correlation of quantitative measurement readings of the Panbio and NovaLisa ELISA

To examine the cause of the discrepancies in the performance, we compared the quantitative results of the two ELISAs. Comparison of the Panbio and the NovaLisa test results showed a positive correlation with a coefficient of correlation R^2 of 0.70 (Fig. 1). We then compared the NovaLisa test readings of the concordant positive sera (group 1) with those of sera that were positive only in the NovaLisa test (group 3). The NovaLisa values in group 1 were significantly higher than in group 3 (Fig. 2).

3.3. Comparison of the NovaLisa and Panbio calibrator sample

Technically, the calibrator samples are dengue antibody positive sera diluted in a way to give OD values at the threshold (NovaLisa cutoff sample) or slightly above the threshold for positive results (Panbio calibrator sample). To evaluate if the ELISAs used different cutoffs for positivity we tested the calibrator sample of the Panbio ELISA and the cutoff sample of the NovaLisa test in both assays and calculated the cutoffs with each calibrator sample. With both assays, the Panbio calibrator sample gave higher cutoff values than the NovaLisa cutoff sample. The theoretical cutoff of the NovaLisa ELISA obtained with the Panbio calibrator sample was 2.05 times higher (Fig. 3).

3.4. Performance of the NovaLisa dengue IgG ELISA with the Panbio calibrator sample

The 2.05 times elevated cutoff was applied to the values measured with the NovaLisa test. This changed the number of sera in the four groups. Two sera from group 1 moved to group 2 and 51 sera from group 3 shifted to group 4. The number of concordant sera increased from 82.8% (400 sera) to 93% (449 sera, Table 4). Comparison with the neutralization test showed that this led to a loss of detection of 14 true positive and 35 true negative sera. As a result, replacement of the NovaLisa cutoff sample with the Panbio calibrator reduced the sensitivity of the NovaLisa ELISA to 93.7% and increased the specificity to 75%. The accuracy of the NovaLisa ELISA increased to 88.0% (Table 3).

4. Discussion

A first vaccine against dengue, Dengvaxia, has been licensed in several dengue-endemic countries in Asia and Latin America. The vaccine is protective but there is an increased risk of severe disease for children who subsequently contract natural DENV infection. Therefore, the current recommendation of the WHO is to only vaccinate individuals that have had laboratory-confirmed primary infection or, to use the vaccine only in an area with a recently documented dengue seroprevalence of at least 80% in children at the age of 9 years [3]. To correctly identify previous dengue virus infections, accurate dengue virus IgG tests are needed. Numerous commercial and in-house dengue IgG ELISAs are currently in use. The performance of some of them has previously been compared and it was shown that the test sensitivities and specificities vary considerably [5,6,11]. In this study, we compared the performance of the Panbio and the NovaLisa dengue IgG ELISA kits. These tests are frequently used in Europe. In a recent external quality control assessment among 48 members of the European Network for Imported Viral Diseases (ENIVD) 17 of 39 laboratories (43.6%) that used commercial tests for dengue IgG measurements used one of these assays [8].

Based on previous studies reported in the technical summary sheet of the kit, the Panbio dengue IgG ELISA has a specificity of 100% and a

Table 3
Performance characteristics of the dengue IgG ELISAs.

	Panbio Dengue IgG ELISA/Panbio calibrator	NovaLisa Dengue IgG ELISA/NovaLisa cutoff	NovaLisa Dengue IgG ELISA/Panbio calibrator*
Sensitivity (%)	91.1	99.0	93.7
Specificity (%)	79.4	50.9	75.0
Positive predictive value (%)	90.7	81.7	89.4
Negative predictive value (%)	80.2	95.7	84.1
Accuracy (%)	87.5	84.0	88.0

* Values were determined by shifting the cutoff by a factor of 2.05. No neutralization data were available for one serum that shifted from G1 to G2 and for 3 sera that shifted from G3 to G4. For the calculations, the sera were assumed to be positive.

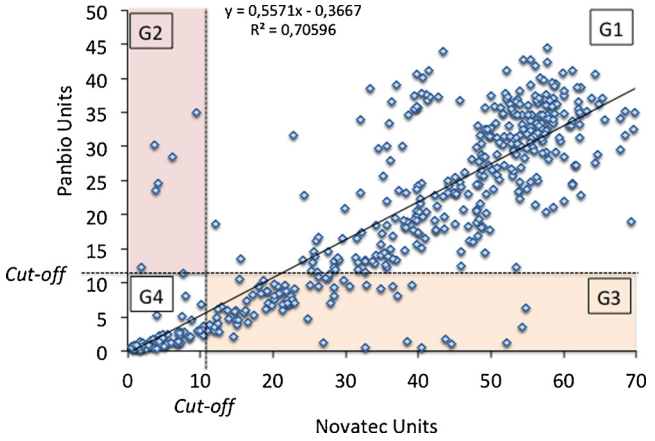

Fig. 1. Correlation of the Panbio and NovaLisa ELISA test results. Serum samples (N = 483) were tested in the Panbio and NovaLisa dengue IgG ELISAs. ELISA unit values were calculated and compared. Linear regression was used to calculate the correlation coefficient. R2: Correlation coefficient. Dotted lines indicate the cutoff values for the Panbio and NovaLisa ELISA. G1 to G4 indicate the graph regions of the double positive, single ELISA positive and double negative sera.

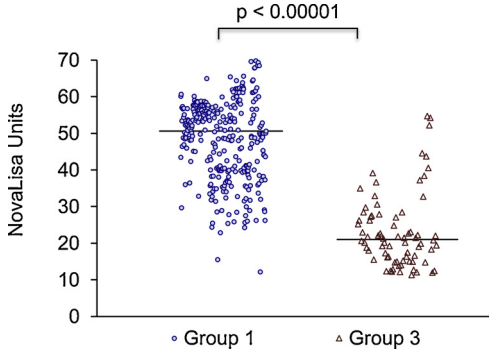

Fig. 2. Comparison of the NovaLisa measurement readings of group 1 (N = 327) and group 3 (N = 76). The lines indicate median values. Median values were: G1 (double ELISA positive): 48.4 NovaLisa units, G3 (NovaLisa positive/Panbio negative or equivocal): 23.2 units. The significance of the differences (p-value) was determined with the Mann-Whitney U test.

sensitivity of 33.3% after primary and of 97.9% after secondary dengue infection (Panbio Dengue IgG Indirect ELISA,Panbio Diagnostics, Cat. No. 01PE30 [12]. In a comparison of the ELISA with five other commercial test kits the sensitivity was 100% and the specificity 98% [6]. Similarly, the NovaLisa dengue IgG ELISA has a diagnostic sensitivity of 100% and a specificity of 98% according to the information provided by the manufacturer [13]

In this study, the diagnostic sensitivity of the NovaLisa test was 99% and the sensitivity of the Panbio ELISA was 91.1%. This agrees with the specifications from the test kits if one assumes that some of the sera were from primary infections. The specificity of the tests was lower

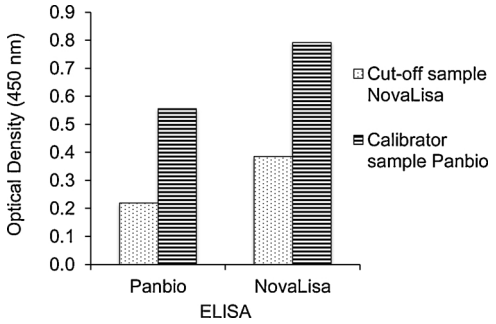

Fig. 3. Comparison of the cutoff OD values in the Panbio and NovaLisa dengue IgG ELISAs using the Panbio calibrator and the NovaLisa cutoff sample. Samples were tested in both assays and the cutoffs were determined as described in the user manuals. With the NovaLisa cutoff sample, the cutoff OD values were the measured OD values. With the Panbio calibrator sample, the cutoff OD values were obtained by multiplying the measured OD values by 0.72, the calibration factor specified in the kit.

Table 4
Sera in groups 1–4, concordance and discordance using the original NovaLisa cutoff sample and the Panbio calibrator for the NovaLisa ELISA.

Group	Description	Number of sera (% of all sera)	
		NovaLisa cutoff	Panbio calibrator
G1	Concordant positive	327 (67.7)	325 (67.2)
G2	Panbio pos./NovaLisa neg. or equivocal	7 (1.45)	9 (1.9)
G3	NovaLisa pos./Panbio neg. or equivocal	76 (15.7)	25 (5.2)
G4	Concordant negative or equivocal	73 (15.1)	124 (25.7)
G1 + G4	Total concordant	400 (82.8)	449 (93.0)
G2 + G3	Total discordant	83 (17.2)	34 (7.0)

than indicated. One in 11 positive results in the Panbio ELISA was false (1-positive predictive value, 9.2%) and approximately one in 5 positive test results was false in the NovaLisa test (18.3%).

On the average, serum samples that were positive only in the NovaLisa ELISA had lower test unit values than sera that were positive in both tests. This suggested that raising the cutoff would reduce the fraction of discrepant results. Subsequent comparison of the calibrator samples from both test kits showed that they led to different cutoffs. When applying the cutoff obtained with the Panbio calibrator sample to the NovaLisa ELISA the sensitivity and specificity of the NovaLisa test shifted to values similar to those obtained with the Panbio ELISA and the concordance of the two tests increased significantly. This shows that for the most part the differences in sensitivity, specificity and the degree of concordance depended on the cutoff and not, for example, on the antigen preparations used in the assay kits.

The two dengue IgG tests led to divergent seropositivity rates. With the Panbio test the dengue seropositivity rate in the sample was 69.1%, whereas it was 83.4% with the NovaLisa ELISA (Table 1). If the ELISA

kits had been used in nine year old children in a pre-vaccination serosurvey, testing with the NovaLisa ELISA, but not with the Panbio test, would have led to recommendation for dengue vaccination [3]. For optimal guidance for dengue vaccination the performance characteristics of dengue IgG ELISAs should be standardized. Comparing and adjusting the calibrator samples of the ELISAs will be important to achieve this.

Thirty-four sera (7.0%) were still discordant when the Panbio calibrator sample was used in both ELISAs (Table 4). While part of the discordant results may be due to technical differences between the tests like different antigen preparations, it is possible that this number can be further reduced by additional adjustment of the cutoff. In a previous study, the performance of the Focus Diagnostics anti-DENV IgG ELISA was optimized by ROC analysis. The optimal cutoff was obtained by increasing the cutoff by a factor of 3 [7]. A combination of optimization of the calibrator by ROC analysis and using the optimized calibrator as standard will improve accuracy and concordance of currently used commercial and in-house dengue IgG assays.

In summary, the study shows differences in sensitivity, specificity and accuracy of the Panbio and the NovaLisa DENV IgG ELISAs. For the most part the differences were corrected by exchange of the calibrator sample of the test kits. The findings are relevant for dengue surveillance and to establish the dengue seroprevalence in target populations for dengue vaccination. An optimized standard calibrator sample is desirable to harmonize dengue IgG ELISAs and to improve the accuracy of the tests.

Credit author statement

T. Schüttoff made investigations, curated and analyzed data and reviewed the manuscript. A. Adam selected participants, obtained blood specimens, performed measurements and reviewed the manuscript. S. Reiche developed methodology, gave methodological advise and reviewed the manuscript. C. Jassoy conceptualized the study, developed methodology, analyzed data and wrote the draft manuscript.

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References

[1] S. Bhatt, P.W. Gething, O.J. Brady, J.P. Messina, A.W. Farlow, C.L. Moyes, et al., The global distribution and burden of dengue, Nature 496 (2013) 504–507.
[2] World Health Organization. Dengue and severe dengue; 2018 [cited 2019 Feb 12]. Available from: http://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.
[3] World Health Organization, Dengue vaccine: WHO position paper - September 2018, Weekly Epidemiol. Rep. 93 (2018) 457–476.
[4] K.K. Ariën, A. Wilder-Smith, Dengue vaccine: reliably determining previous exposure, Lancet Glob. Health 6 (2018) e830–e831.
[5] S.D. Blacksell, R.G. Jarman, R.V. Gibbons, A. Tanganuchitcharnchai, M.P. Mammen, A. Nisalak, et al., Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection, Clin. Vaccine Immunol. 19 (2012) 804–810.
[6] J. Groen, P. Koraka, J. Velzing, C. Copra, Osterhaus ADME, Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G antibodies, Clin. Diagn. Lab. Immunol. 7 (2000) 867–871.
[7] K.M. Marrero-Santos, M. Beltrán, J. Carrión-Lebrón, C. Sanchez-Vegas, D.H. Hamer, E.D. Barnett, et al., Optimization of the cutoff value for a commercial anti-dengue virus IgG immunoassay, Clin. Vaccine Immunol. 20 (2013) 358–362.
[8] C. Domingo, M.J. Alves, Ory F. de, A. Teichmann, H. Schmitz, R. Müller, et al., International external quality control assessment for the serological diagnosis of dengue infections, BMC Infect. Dis. 15 (2015) 167.
[9] A. Adam, T. Schüttoff, S. Reiche, C. Jassoy, High seroprevalence of dengue virus indicates that dengue virus infections are frequent in central and eastern Sudan, Trop. Med. Int. Health 23 (2018) 960–967.
[10] Stangroom J. Social Science Statistics. Available at: www.socscistatistics.com.
[11] S. Pal, A.L. Dauner, A. Valks, B.M. Forshey, K.C. Long, B. Thaisomboonsuk, et al., Multicountry prospective clinical evaluation of two enzyme-linked immunosorbent assays and two rapid diagnostic tests for diagnosing dengue fever, J. Clin. Microbiol. 53 (2015) 1092–1102.
[12] Abbott. Panbio Dengue IgG Indirect ELISA: Specifications [cited 2019 Feb 12]. Available from: https://www.alere.com/en/home/product-details/panbio-dengue-igg-indirect-elisa.html.
[13] W.J. McBride, H. Mullner, J.T. LaBrooy, Wronski I. The, Dengue 2 epidemic in North Queensland: a serosurvey and comparison of hemagglutination inhibition with an ELISA, Am. J. Trop. Med. Hyg. 1998 (59) (1993) 457–461.