Author's response to reviews

Title: Clinical features and pitfalls in the laboratory diagnosis of dengue in travellers

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Author's response to reviews: see over
Submission of revised manuscript

Dear Editors,

we have revised our manuscript “Clinical features and pitfalls in the laboratory diagnosis of dengue in travelers” in light of the reviewers' comments and want to submit herewith the revised version for consideration for publication as a research article in BMC Infectious Diseases.

We are aware of your policy to allow a maximum of two revisions on manuscripts under consideration only. Therefore, we made every effort to fully address the criticisms during this revision.

We believe that all research results required by the referees are available and that especially the major point of referee 2 is based on a misunderstanding due to ambiguous wording in our manuscript. We hope to have solved this problem by re-writing the specific paragraph in the result section more clearly and to address this misunderstanding in the point-to-point response.

The detailed point-by-point response to the referees is included at the end of this letter.

Yours sincerely

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Referee 1

Comment 1: “The revised manuscript by Wichmann et al. is better understood now, but there are still some statements that are hard to agree with. Even though a specific positive predictive value is a function of the prevalence of the respective disease, stating that the reason for their false-positives is the low cut-off value of this ELISA kit, since that would fit better for suspected cases from endemic areas than from travellers of non-endemic areas, does not seem to be correct. In endemic areas, flavivirus infections are common and a low cut-off would result in a higher percentage of false-positives due to the high cross-reactivity among these viruses. Also, Vaughn et al. found a high specificity for this ELISA in patients without Flavivirus infections, a population feature compared to the one studied here.”

→ We agree to this point and deleted the statement regarding the low cut-off value from the discussion section. As mentioned in comment 3, the major reason might be that only single serum samples have been used.

Comment 2: “The reason this kit has yielded a low PPV in the authors’ hands is not clear, but as mentioned on the previous review, it seems that their major problem is the fact that they searched for IgM in about 20% of their samples collected before the third day of the disease. These samples are expected to be negative for IgM antibodies. According to figure 1, a rough analysis of the samples collected from the 4th to the 15th days after disease onset results in a higher PPV than that found when they analysed all samples. The fact that they needed to provide an answer to the acutely ill patients is experienced by most physicians working on travel clinics. They should consider performing a RT-PCR for cases seeking medical care up to the fifth day following the beginning of the symptoms.”

→ This point has been included in the discussion section on page 14, line 3-11, and has also been included in the conclusion section as a major finding of our study.

Comment 3: “The authors should also discuss the fact their study lacks a second sample that could confirm the diagnosis, and that they have used "more specific tests" not completely validated in most areas of the world. These facts could be the explanations for the low PPV found in their study.”

→ As recommended by the referee, we pointed out that especially the fact that only one serum sample has been used, might be the major reason for the low PPV (bottom of page 14/ first sentence page 15, and line 19-23 on page 15). However, this has been subject to our study to investigate the PPV in single serum samples. We also agree that 3 of these 4 tests used for confirmation are not widely used. However, these tests have showed excellent correlation with the neutralization test as discussed on page 14 and cited as reference 20. Furthermore, we performed a 4th test (IFA) which was concordant with the other 3 in 91%. Samples with discordant results have been excluded as “non-interpretable”.

Referee 2:

Comment 1: “Still the manuscript needs some revisions and clarifications. The study is not easy to follow up. In results, authors mention that in 115/127 samples they obtained concordant results when compared PanBIO IgM/IgG results with the four tests used for confirmation (paragraph 3). However, later analysis shows some discordant results. According to this only in
64 samples (instead of 115) dengue was confirmed according criteria established by authors. Because this incongruence, it’s not easy to understand the discussion”

→ 127 samples were tested positive in the PanBio-ELISA. Four confirmatory tests (E/M-specific and NS1 serotype-specific capture IgM ELISAs, NS1 serotype-specific IgG ELISA, and IFA) were further performed on each of these 127 samples:

In 115 samples, results of these four confirmatory tests were concordant in all four tests: 61 concordant positive and 54 concordant negative. That means that these 54 concordant negatives were discordant with the positive PanBio-ELISA result. This classifies the PanBio-result as “false positive”, since all four confirmatory tests were concordant negative.

In 12 samples the four confirmatory tests were discrepant among each other. These were then classified as “not interpretable”, except for three that were shown to be positive in RT-PCR (=true positive).

We tried to present this result more clearly in regard to the concordant negative and concordant positive results of the 4 confirmatory tests: Result section, bottom of page 9 and top of page 10

Comment 2: “A negative PCR not necessarily indicates a non dengue infection”
→ We agree with this statement and included this point in the discussion section, page 14, line 10-11.

Comment 3: “Rash as thrombocytopenia is observed only in a number of dengue cases. In many dengue patients both are not observed”
→ Both points are included in the discussion section on page 16, line 3 to 8.