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Reply to "Inconclusive Reverse Transcription-PCR Assay Comparison for Dengue Virus Detection and Serotyping"

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With great interest, we read the letter entitled "Inconclusive Reverse Transcription-PCR Assay Comparison for Dengue Virus Detection and Serotyping" written by Drs. Muñoz-Jordán and Santiago (1), and we appreciate the opportunity to respond in print. These authors voice their concerns regarding our published comparison of a laboratory-developed, serotype-specific dengue virus (DENV) multiplex assay (referred to as the DENV multiplex) with the CDC DENV-1-4 real-time RT-PCR (2). The DENV multiplex used in that study was a modified version of an earlier assay (3). As expressed in our paper (2), these changes were made to simplify the interpretation of results, namely, to eliminate minor cross-reactions between the DENV-1 probe and other serotypes (2, 3). We did not, however, attribute the higher clinical sensitivity of the DENV multiplex to these changes. In fact, as shown in the manuscript, the clinical sensitivity did not differ from the original version (sensitivity of 97.4%; 151/155 samples detected for each version). As these authors pointed out, we did not present data from the analytical validation of the DENV multiplex, which was equivalent to the original assay. Namely, the linear range extended 6 orders of magnitude for each serotype, and the lower limit of 95% detection ranged from 6 to 21 cDNA equivalents/μl of eluate, depending on the serotype. As the analytical description of the original assay had recently been published, we focused on clinical data for that article.

Two other major points of concern were the use of a crossing threshold (C_T) of 40.00 cycles as the cutoff in the DENV multiplex and the use of samples obtained on day of illness (DOI) ≥5. The authors state that there is no "publication of data demonstrating the reliability of results obtained at this cutoff." However, in order to address this very concern, we used a composite reference in our study (2). In order for a sample to be considered a true positive, DENV RNA had to be detected by at least two different molecular tests out of the four that were included. Samples that tested positive by only one assay were considered false positives. The clinical sensitivity of the DENV multiplex, using this composite reference, was significantly higher than the CDC DENV-1-4 real-time RT-PCR in 82 samples obtained from patients at DOI ≥5. These samples were collected from patients presenting late in the course of illness, principally with DENV-1. We feel that this is a clinically relevant period during which to diagnose and serotype DENV, as there is data that patients with severe dengue may present later, at DOI 6 on average, than patients with dengue fever (4). Drs. Muñoz-Jordán and Santiago further criticize the detection of DENV in samples obtained this late in the course of illness, as "surveillance systems usually detect most suspected dengue cases during the first 5 days of illness." This should not be surprising if the assays being used in such detection systems demonstrate poor clinical sensitivity in samples obtained after that time point.

Finally, we acknowledge that our comparison was performed on a Rotor-Gene Q (RGQ) instrument. The CDC DENV-1-4 RT-PCR was approved for use on the ABI7500. The RGQ is our preferred real-time thermocycler and is available in our laboratory. We feel that this assay will require evaluation on the instruments that are available, as most laboratories will be unable to purchase a new instrument solely for this test. Furthermore, the samples in our study were made up of predominantly DENV serotypes 1 and 3. No DENV-4 and 13 DENV-2 infections were detected in these specimens. We agree that this warrants further evaluation. In particular, an evaluation of DENV-2 isolates should be pursued, as the forward primer and probe used in the CDC DENV-1-4 RT-PCR are reported to match only 80% and 86%, respectively, of the aligned DENV-2 sequences used in their redesign (5).

In closing, while the critiques of our manuscript require careful consideration, our conclusion that the DENV multiplex "demonstrated. . .improved detection of DENV-1, particularly in samples collected from patients presenting ≥5 days after the onset of illness" has not been called into question. Rather, given the global importance of DENV and the need for improved clinical dengue diagnostics, we offer that these assays should be independently evaluated using a set of clinical samples that represent contemporary strains of the four DENV serotypes.

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