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

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A recent comparative study by Waggoner et al. (1) claimed their reverse transcription-PCR (RT-PCR) assay was more sensitive than the CDC DENV-1-4 RT-PCR assay. Although a complete evaluation of the CDC assay was published several weeks before this letter to the editor (2), most of the analytic data were available to the authors in the package insert (3). The CDC assay was optimized and extensively evaluated as a diagnostic test for persons with suspected dengue in order to give unequivocal results in its positive range. Its limit of detection was set at a cutoff value (crossing threshold [C_T]) of 37.00 to ensure very high confidence in a positive result, with low likelihood of a false-positive result (2). The Waggoner et al. assay employs a C_T of 40.00 (4) without publication of data demonstrating the reliability of results obtained at this cutoff and do not represent false-positive results. Most CDC diagnostic PCR assays (FDA approved or not) find C_Ts above 37.00, but these values are often in the equivocal range of the assay (5–9). Waggoner's comparison reported several positive samples in their assay, mostly at >5 days of illness, which were considered negative by the CDC assay because they exceeded the 37.00-C_T threshold. Surveillance systems usually detect most suspected dengue cases during the first 5 days of illness; however, the authors' study sample included 61 specimens obtained <5 days after the onset of illness and 82 specimens obtained ≥5 days after the onset of illness. Although the authors indicate this was a random sample, this distribution of specimen collection times is skewed toward late dengue case presentations and is not reflective of the situation in most settings or geographic areas where dengue is endemic. In addition, the distribution of DENV serotypes allowed for a comparison of only dengue virus serotype 1 (DENV-1) and DENV-3. Compared to their evaluation of the assay published just 3 months earlier, both the DENV-1 probe (18/37 nucleotide differences) and their thermocycling protocol were significantly altered (1, 4). The authors offer no explanation for these modifications and claim to have unpublished data demonstrating no changes in performance (1). However, in their discussion, they attribute the improved performance of their assay to the changes in the probe for DENV-1, the predominant serotype in their evaluation specimens. It is interesting that in their discussion, the authors criticize FDA-approved assays for requiring later modifications, when they have done this for their own assay prior to comparing it to the CDC assay. It is notable that the authors often indicate "results not shown" for critical elements of their test validations (1, 4, 10), while an FDA-approved test provides these analytic results in the package insert. Finally, it is important to recognize that Waggoner et al. performed the CDC assay essentially "off-label". They used the Qiagen Rotor-Gene Q, an instrument that was not approved by the FDA for use with the CDC assay; the package insert states that the ABI 7500 FAST Dx is the only equipment approved for the assay (3). The use of less than robust comparison methods calls into question the conclusions of this study.

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