Reply to “Universal PCR Primers Are Critical for Direct Sequencing-Based Enterovirus Genotyping”

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We really appreciate being able to respond to the comments made in this letter, as it can only clarify some issues to other readers.

Clearly, we have used the 8th version of the ICTV virus taxonomy report, but undeniably, we did address both enteroviruses as well as rhinoviruses.

The study was initiated some time ago, with primers that so far have shown excellent performance in detection of enteroviruses. The fact that some of the detected enteroviruses were not typeable was a result of insufficient viral load. Since we carry out routine sequencing, we also can attest to the specificity of the primers. Our initial enterovirus assay was based on consensus primers, based on the—at that time—known enterovirus sequences from the NCBI database as well as our own database with VP4-VP2 sequences from both enteroviruses and rhinoviruses. With the correct melting temperature ($T_m$) optimization, this primer set would in theory be able to detect all enteroviruses.

Obviously, the situation may be different in the future, but this level of sensitivity and specificity is usually unobtainable with commercial tests.

In addition to the above-mentioned enterovirus detection PCR, we also performed a specific rhinovirus assay (1). Once we started to sequence a number of viruses for the determination of the specific genotype, we noticed that a number of rhinoviruses were actually detected and typed as enteroviruses.

This caught our interest, and while focusing on this, more assays from the literature were used, and we were able to detect more specifically 5’ untranslated region (5’-UTR), VP4-VP2, and VP1-VP3 from both enteroviruses as well as rhinoviruses.

This resulted in the detection of these rarely identified enteroviruses of species C. These types, C104, C105 to C109, and C117, were not detected abundantly in previous studies, and we were more than surprised that we could detect them in our population. As can be seen in Fig. 5 in our article, also the rhinoviruses of types A to C were included, and this figure was more specifically addressed at the request of the reviewers.

In summary, we were initially puzzled by the significant percentage of these rarely identified enteroviruses of species C in our population and are aware that more and better diagnostic assays are needed to address this. Our main concern is for diagnostic laboratories and diagnostic companies to increase their effort in developing good diagnostic and well-characterized assays to enable good diagnoses necessary for patient care.

REFERENCES