Reply to Naccache et al: Viral sequences of NIH-CQV virus, a contamination of DNA extraction method

Naccache et al. confirm our finding that NIH-CQV is a previously unidentified hybrid virus, with a replication-associated protein gene (*rep*) homologous to circoviruses and capsid protein gene (*cp*) homologous to parvoviruses (1–3), and overall has a structure with characteristics of *Parvoviridae* (1).

Naccache et al. suggest that the NIH-CQV virus is a contaminant of the nucleic acid extraction kit (3). We evaluated several Qiagen kits: NIH-CQV is indeed a contaminant of the columns, as determined by water elution and mock extractions followed by quantitative PCR (qPCR) (1) and by endpoint PCR (3). By qPCR, the quantity of NIH-CQV varied among lots and within lots of Qiagen columns. Evaluating sequential elutions from the same columns, NIH-CQV could be detected in at least one eluate, sometimes in all three eluates, which are results that may partially explain our original observation of viral titers varying among individual patient samples (1). The Virus minElute kit appeared to contain the lowest level (<1 copy/μL) of NIH-CQV, and levels were undetectable using Qiagen ultraclean production (UCP) columns. Using the Virus minElute kit and UCP columns, we were unable to replicate detection of NIH-CQV from patient samples. Contamination of silica-based columns with NIH-CQV was confirmed by others (4) and was suspected if not documented in an early deep sequencing effort (5). In conclusion, NIH-CQV's apparent presence in human plasma is likely spurious, secondary to the method of DNA extraction.

Search of the National Heart, Lung, and Blood Institute nonredundant nucleotide/protein collection is the current standard for virus discovery by deep sequencing; other public databases have been less frequently exploited. Naccache et al. speculated that NIH-CQV might have its source in diatoms in silica, as they observed small fragments of NIH-CQV in two marine metagenomic databases (3). However, viruses not of marine origin are present in marine ecosystems, introduced, for instance, by sewage: adenoviruses and enteroviruses are examples. Although diatoms may be expected within silica, silica is subject to extreme heating in the manufacture of Qiagen columns, during which time, DNA should be destroyed.

We further queried for the presence of protein sequences encoded by *rep* and *cp* of NIH-CQV in the NCBI genomic Blast website (tblastn). We observed hits sporadically distributed across the phylogenies of *Phytophthora* and *Phyium* [identity >30% and expect (E) value < $1E^{-5}$]. For example, in a supercontig of *Phytophthora parasitica*, two tandem ORFs showed significant homologies to *rep* and *cp* of NIH-CQV (65% and 54% at the nucleotide level and 66% and 38% at the protein level, respectively). Integration of NIH-CQV relatives in these species' genomes suggests an infectious relationship. Both *Phytophthora* and *Phyium* are oomycetes, organisms that are ubiquitous in soil and aquatic environments and that infect a range of plants and animals (6). In manufacture, silica in DNA extraction kits is subject to extensive water washing, during which DNA contamination from oomycetes might be introduced. Hits for *rep* and *cp* gene of NIH-CQV in *Phytophthora* and *Phyium* were not observed in previous analyses (1, 3), likely due to their recent publication and annotation, and were not available when the draft genome of NIH-CQV was assembled.

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The authors declare no conflict of interest.

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