Hepatitis E Virus Genotype 3 in Shellfish, United Kingdom

Technical Appendix

```
LB21          CGACTGTTAAATTATACACCTCCGTTGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
ABN26         CGACTGTTAAATTATACACCTCCGTTGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
FB29          CGACTGTTAAATTATACACCTCCGTTGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
swUK          CGGCTGTTAAATTATATACTTCCGTCGAGAATGCACAGCAGGATAAGGGCATTGCCATAC 60
huFrance      CAACTGTTAAGTTATACACCTCTGTCGAGAATGCACAGCAGGATAAGGGTATTGCTATAC 60
AB40          CGACTGTCAAATTATACACATCTGTCGAGAATGCACAGCAGGACAAGGGCATTGCCATAC 60
LB23          CGACTGTCAAATTATACACATCTGTCGAGAATGCGCAGCAGGACAAGGGCATTGCCATAC 60
AB7           CGACAGTAAAGTTATATACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
YE26          CGACAGTAAAGTTATATACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
YE29          CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
huUK          CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB31          CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB22          CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB23          CGACAGTAAAGTTATACACATCCGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
AB24          CGACAGTAAAGTTATACACATCTGTAGAGAATGCGCAGCAAGACAAGGGCATTACCATTC 60
HEVgenotype3  CAACAGTAAAGTTATACACATCTGTTGAGAATGCGCAGCAAGACAAGGGCATCACCATTC 60
huUS          CAACAGTAAAGTTATATACATCTGTTGAGAATGCGCAGCAAGACAAGGGCATTACCATCC 60
HEVgenotype1  CGACTGTTAAGTTGTATACATCTGTAGAGAATGCTCAGCAGGATAAGGGTATTGCAATCC 60
HEVgenotype2  CAACCGTGAAGCTCTATACATCAGTGGAGAATGCTCAGCAGGATAAGGGTGTTGCTATCC 60
HEVgenotype4  -GACAGTGAAACTTTACACTTCAGTCGAGAACGCTCAGCAGGACAAGGGTGTAGCTATTC 59
              *  ** **   * ** ** ** ** ***** ** ***** ** *****  *  * ** *

LB21          CTCATGATATAGACTTAGGGGACTCTCGCGTGGTTATC 98
ABN26         CTCATGATATAGACTTAGGGGACTCTCGCGTGGTTATC 98
FB29          CTCACGATATAGACTTAGGGGACTCTCGCGTGGTTATC 98
swUK          CTCACGATATAGACCTAGGGGATTCCCGCGTGGTTATC 98
huFrance      CACACGATATAGACCTAGGGGATTCCCGTGTGGTTGTA 98
AB40          CACATGATATAGATCTGGGAGATTCTCGTGTGGTTATT 98
LB23          CACATGATATAGATCTGGGAGATTCTCGTGTGGTTATT 98
AB7           CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
YE26          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
YE29          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
huUK          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB31          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB22          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB23          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
AB24          CACACGATATAGATTTGGGTGATTCCCGTGTGGTTATT 98
HEVgenotype3  CACACGACATAGATTTAGGTGACTCCCGTGTGGTTATC 98
huUS          CACATGATATAGATCTGGGTGACTCCCGTGTGGTTATC 98
HEVgenotype1  CGCATGACATTGACCTCGGAGAATCTCGTGTGGTTATT 98
HEVgenotype2  CCCACGATATCGATCTTGGTGATTCGCGTGTGGTCATT 98
HEVgenotype4  CACATGATATTGACCTTGGTGAGTCCCGTGTGGTTATT 97
              *  **  ** ** **   *  ** ** ** **  *****    *
```

Technical Appendix Figure. ClustalW alignment of sequences used to generate the phylogenetic tree in the Figure (see article text). Sequences were generated from RNA isolated from 50–100 mg of digestive gland or gill. Tissue was homogenized in 300 µL phosphate-buffered saline, and viral RNA was isolated by using a viral RNA kit (QIAGEN, Crawley, UK), and PCR was conducted by amplifying nucleotides

6332–6476 as described (*1*). The nucleotide sequences were aligned and bootstrapped, and phylogenetic neighbor-joining trees were constructed by using the ClustalW software (www.ebi.ac.uk/Tools/msa/clustalw2).

Reference

1. Erker JC, Desai SM, Mushahwar IK. Rapid detection of hepatitis E virus RNA by reverse transcription–polymerase chain reaction using universal oligonucleotide primers. J Virol Methods. 1999;81:109. PubMed http://dx.doi.org/10.1016/S0166-0934(99)00052-X