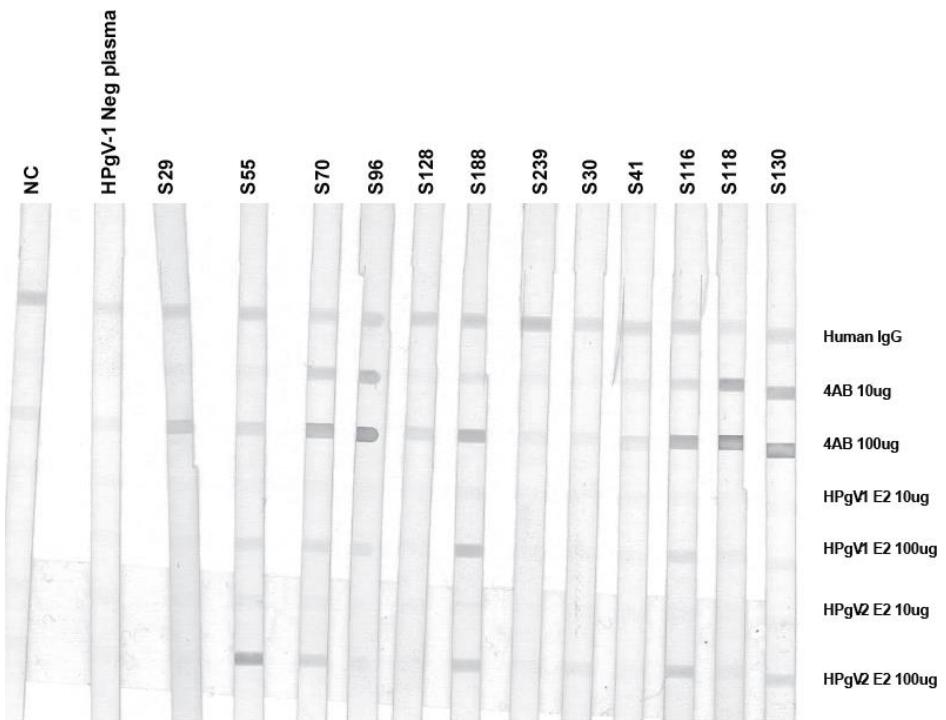


Supplemental Figures:

Supplemental Figure 1: Slot blot of HPgV-2 RNA positive samples. HPgV-2 RNA positive samples were tested for reactivity to NS4AB, HPgV-2 E2, or HPgV-1 E2 by slot blot.

Supplemental Figure 2: A. Alignment of HPgV-2 peptide 16 sequence with corresponding region in HPgV-1 and HCV antigen 5-1-1. B. Alignment of HPgV-2, HPgV-1, and HCV E2.

Supplemental Figure 3: S/CO values for testing HCV and HBV seroconversion panels for anti-E2. The following HCV and HBV seroconversion panels were tested: [panel number (bleed number)]: HCV: 100071 (1, 7), 10043 (1, 10), 913 (1, 4), 914 (1, 9), 919 (1, 7), 921 (1, 11), 922 (1, 6), 6224 (1, 6) 6229 (1, 8), 9044 (1, 6), 9047 (1, 10), 9054 (1, 10), 9058 (1, 5), 10025 (1, 11), 10041 (1, 3), 10165 (1, 9), 9055 (1, 11), 10017 (1, 16), 10002 (1, 7), 10004 (1, 6), 10008 (1, 10), 10011 (1, 13), 10020 (1, 13), 10023 (1, 21), 10029 (1, 16), 1051 (1, 14), 9054 (1, 8), 10016 (1, 12), last bleed only: 6222, 6227, 9041, 6216, 6213, 6225, 6228, 10026, 10062, 10021, 10003, 915, 6226, 10000. HBV: 6272 (1, 26), 26982/14399 (1, 25), 11007 (1, 14) 43527/3453 (1, 26), PHM935A (1, 20), 11009 (1, 23), 1808 (1, 21), 11028 (1, 10), 1002 (1, 6), 6282 (1, 14), 11031 (1, 16), 11012 (1, 6), 9279 (1, 7), 11005 (1, 14), 6271 (5), PHM925 (1, 5), 6284 (1, 19), 6274 (1, 7), 6273 (1, 6), 11017 (1, 14), 11000(1, 9), PHM (928 (1, 7), PHM912 (1, 9), PHM924 (1, 5), PHM908 (1, 8), PHM918 (1, 3), PHM904 (1, 3), PHM933(2, 6), PHM906(1, 5), PHM934 (1,6), 11014 (1(12), 11027 (1, 14), 6281 (1, 12), 6278 (1, 9), 6290 (1, 12), 11008 (1, 18), 11059 (1, 9), 11013 (1, 35), 11029 (1, 13), 11024 (1, 14), 11062 (1, 12), 11016 (1, 10), 11052 (1, 13), 11069 (1), 11006 (17), 6277 (1, 11), 6275 (1, 7), 6286 (1, 9), 1672/3471 (1, 9), 13867/3482 (1, 31), 11056 (1, 11), PHM917 (1, 3), 1807/3463 (1, 25), 26022/14518 (1,2), 9074 (1, 20), PHM927 (1, 6), PHM926 (1, 8), PHM929 (1,9), 11001 (1,8), 0994/3457 (1,23), 11026 (1,16), PHM935B (21, 32), last bleed only: 11011, 6278, 11003, 6285, PHM930, 11056. Seroconversion panels tested for HPgV-2 RNA: 6222, 6224, 6225, 6226, 6227, 6228, 9041, 9045, 9047, and 9054.



Supplemental Figure 1: Slot blot of HPgV-2 RNA positive samples. HPgV-2 RNA positive samples were tested for reactivity to NS4AB, HPgV-2 E2, or HPgV-1 E2 by slot blot.

A

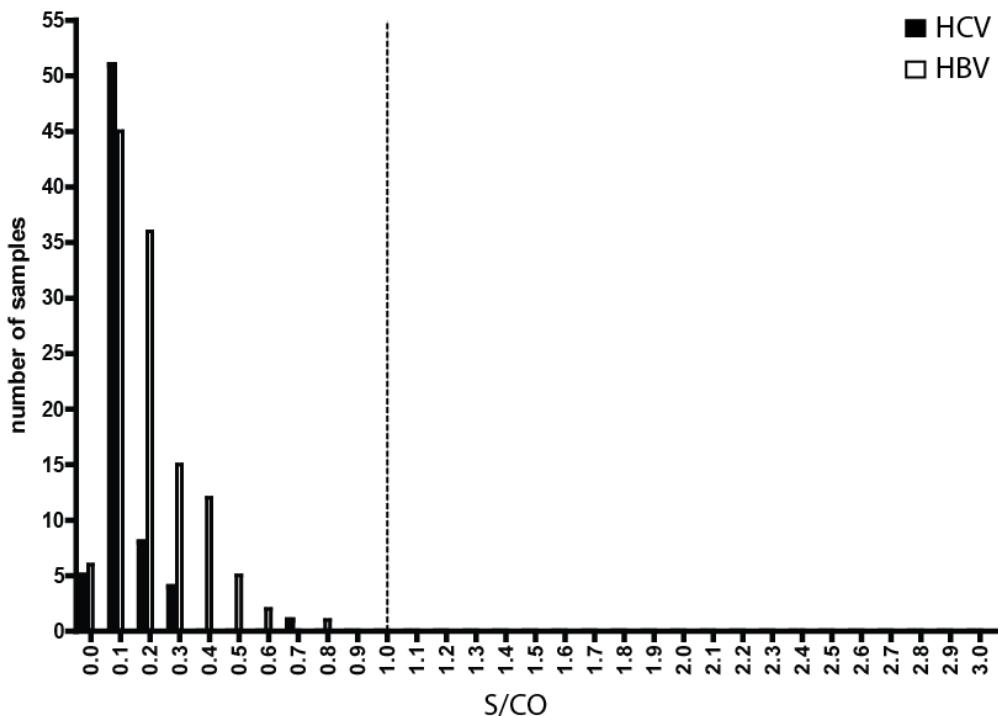
| | 1850 | 1860 | 1870 | 1880 | 1890 | 1900 | 1910 | 1920 | |
|-------------|--|------|------|------|------|------|------|------|------|
| HPgV-2 | ----- SVEVR ----- | | | | | | | | 1889 |
| HPgV-1 | VVVTDWDVKGGSNPLYRNGDQATPQFVVQVPPVDHRPGGESAPSDAKTVTDAAAIQVNCDWSVMTLSIGEVLAQAKTABAYT | | | | | | | | 1630 |
| 5-1-1 (HCV) | -----IIPDREVLYREFDEMEECSQHLPYIEQGMMMLAEQFKQKALGLL | | | | | | | | 43 |

B

| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | |
|-----------|--|-----|-----|-----|-----|-----|-----|-----|-----|
| HPgV-2 E2 | YKHQSSESYLKCYCTITNTSTSMMNCDFCGFTTRNTESRFSIPRFCPVKINNSSTFICSGWSWWFAENITRPFYT-----DV | | | | | | | | 74 |
| HPgV-1 E2 | --GAPASVLGSRPFEAGLTWQSCSCRNSGRVFTGERVWERGNVTLLCDCPNGPWWVWPALCQAIGWGDPITHW---SH | | | | | | | | 74 |
| HCV E2 | --HVTGGNAG---RTTAGLVLGLITPGAKQNIQLINTNGSWHINSTALNCNESLN-TGWLALFYQHKFNSSGCPERLASCR | | | | | | | | 75 |
| | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | |
| HPgV-2 E2 | GMPGPAPISALCYIYSNN-----DPPPWYHNT-----TIIPQNCRNSTVDPPTAPCRD-----KWGN--ATACI | | | | | | | | 130 |
| HPgV-1 E2 | GQNQWPLSCPQFVYGAWSVTCVGWSVWFASTGGRDSKVWDVWSLVPVGSASCTIAALGSSDRDTVVELSEWGIP-CATCI | | | | | | | | 153 |
| HCV E2 | RLTDFAQGWGPISYANGSGLDE-RPYCWHPPPRPCGIVPAKSVCVCPVYCFPSPVVVGTTDRSG-APTYSWGANDTDVFV | | | | | | | | 153 |
| | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 | |
| HPgV-2 E2 | LDRRSRFCGDCYGGCFYTNGSHDRSWRD--CGIG--YRDGLIEFVQLGQIRPNISNTTIEL---LAGASLVIASGLRPGF | | | | | | | | 203 |
| HPgV-1 E2 | LDRRPASCCTCVRDCWPETGSVRFPHR--CGAGPRLTRDLEAVEFVNRRTTFPTIRGPLGN--QGRGNPVRSPFLFGPSY | | | | | | | | 228 |
| HCV E2 | LNNTRPPLGNWFGCTWMNSTGFTKVCVGAPPCCVIGGVNNNTLLCPTDCFRKHPEATYSRCGSGPWITPRCMVDYFYRLWHY | | | | | | | | 233 |
| | 250 | 260 | 270 | 280 | 290 | 300 | 310 | 320 | |
| HPgV-2 E2 | GCRAHGVVHCYRCPSYRDLHQFGPGLGKWPPLPGEPVPELCLINFQWARRGFRMSNNPLSLLQTFVEDIFLAPFCNPPTG | | | | | | | | 283 |
| HPgV-1 E2 | TMTKIRDSDLHLVKCPTP---AIEPPGTGTFGFPGVPLNNNCMILLGEVSEVLGGAG---LTGGFYEPILVRR--CSELMG | | | | | | | | 299 |
| HCV E2 | PCTINYTIFKVRMYVGG---VEHRLAACNWTRGERCDLEDRLRSELSPLLSTTQWQVLPSCFTTLPALS-TGLIHL | | | | | | | | 307 |
| | 330 | 340 | 350 | | | | | | |
| HPgV-2 E2 | RVRVCNNNTAFYPRGGGFVQLIGDVQVLTPTN | | | | | | | | 314 |
| HPgV-1 E2 | R-R-----NPVCPGFAWLS | | | | | | | | 312 |
| HCV E2 | HQNIVDVQYLGVGSSIASWAIKWEYVVLFFLLLADA | | | | | | | | 344 |

Supplemental Figure 2

Supplemental Figure 2: A. Alignment of HPgV-2 peptide 16 sequence with corresponding region in HPgV-1 and HCV antigen 5-1-1. Peptide 16 sequence is highlighted. B. Alignment of HPgV-2, HPgV-1, and HCV E2.



Supplemental Figure 3: S/CO values for testing HCV and HBV seroconversion panels for anti-E2. The following HCV and HBV seroconversion panels were tested: [panel number (bleed number)]: HCV: 100071 (1, 7), 10043 (1, 10), 913 (1, 4), 914 (1, 9), 919 (1, 7), 921 (1, 11), 922 (1, 6), 6224 (1, 6) 6229 (1, 8), 9044 (1, 6), 9047 (1, 10), 9054 (1, 10), 9058 (1, 5), 10025 (1, 11), 10041 (1, 3), 10165 (1, 9), 9055 (1, 11), 10017 (1, 16), 10002 (1, 7), 10004 (1, 6), 10008 (1, 10), 10011 (1, 13), 10020 (1, 13), 10023 (1, 21), 10029 (1, 16), 1051 (1, 14), 9054 (1, 8), 10016 (1, 12), last bleed only: 6222, 6227, 9041, 6216, 6213, 6225, 6228, 10026, 10062, 10021, 10003, 915, 6226, 10000. HBV: 6272 (1, 26), 26982/14399 (1, 25), 11007 (1, 14) 43527/3453 (1, 26), PHM935A (1, 20), 11009 (1, 23), 1808 (1, 21), 11028 (1, 10), 1002 (1, 6), 6282 (1, 14), 11031 (1, 16), 11012 (1, 6), 9279 (1, 7), 11005 (1, 14), 6271 (5), PHM925 (1, 5), 6284 (1, 19), 6274 (1, 7), 6273 (1, 6), 11017 (1, 14), 11000(1, 9), PHM (928 (1, 7), PHM912 (1, 9), PHM924 (1, 5), PHM908 (1, 8), PHM918 (1, 3), PHM904 (1, 3), PHM933(2, 6), PHM906(1, 5), PHM934 (1,6), 11014 (1(12), 11027 (1, 14), 6281 (1, 12), 6278 (1, 9), 6290 (1, 12), 11008 (1, 18), 11059 (1, 9), 11013 (1, 35), 11029 (1, 13), 11024 (1, 14), 11062 (1, 12), 11016 (1, 10), 11052 (1, 13), 11069 (1), 11006 (17), 6277 (1, 11), 6275 (1, 7), 6286 (1, 9), 1672/3471 (1, 9), 13867/3482 (1, 31), 11056 (1, 11), PHM917 (1, 3), 1807/3463 (1, 25), 26022/14518 (1,2), 9074 (1, 20), PHM927 (1, 6), PHM926 (1, 8), PHM929 (1,9), 11001 (1,8), 0994/3457 (1,23), 11026 (1,16), PHM935B (21, 32), last bleed only: 11011, 6278, 11003, 6285, PHM930, 11056. Seroconversion panels tested for HPgV-2 RNA: 6222, 6224, 6225, 6226, 6227, 6228, 9041, 9045, 9047, and 9054.