

# 1 Supplemental Material

## 2 Establishment of the improved sandwich ELISA method

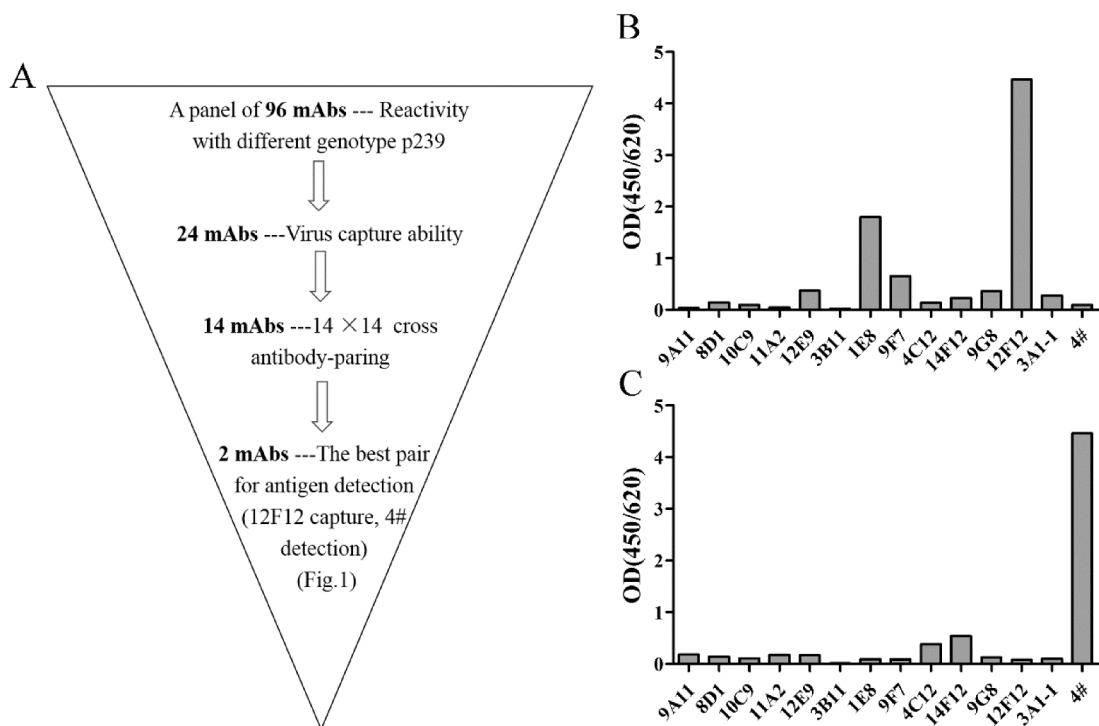
3 The reactivity of mAbs with different genotypes against p239 was performed using an  
4 indirect ELISA. The p239 proteins from different genotypes (100 ng per well) were  
5 coated in a 96-well microplate and incubated with serial 10-fold dilutions of  
6 antibodies (starting at 100 µg) at 37 °C for 30 min. Subsequently, the plate was  
7 washed five times with PBST and the wells were incubated with 100 µL  
8 HRP-conjugated GAM for 30 min at 37 °C. After five washes, 100 µL TMB substrate  
9 was added and incubated for 10 min at 37 °C. The reaction was stopped by adding 50  
10 µL 2 M H<sub>2</sub>SO<sub>4</sub>, and the OD was measured at 450 nm with a reference wavelength of  
11 620 nm.

12 The capacity of mAbs to capture HEV virus in solution was analyzed using a  
13 quantitative real-time RT-PCR assay. Antibodies (100 µg per well) were coated in  
14 96-well microplates. Stool suspensions were added to the antibody-coated wells and  
15 incubated at 37 °C for 2 h to allow the immune complex formation. Subsequently, the  
16 plates were washed ten times with PBST and the HEV viral load in each well captured  
17 by mAbs was determined using a real-time RT-PCR assay as described in the  
18 “Detection of HEV RNA by quantitative reverse transcript PCR” section.

19 Cross antibody-pairing was also performed by sandwich ELISA. Briefly, microtiter  
20 plates were coated with 500 ng per well of each mAb, then washed once with PBST and  
21 blocked with 200 µL PBS containing 2% (w/v) BSA. A total of 50 µL ( $6 \times 10^6$   
22 copies/mL) of genotype 1 stool samples were added and incubated for 60 min at 37 °C.  
23 The plates were incubated with 100 µL per well mAb-HRP solution at 37 °C for 30  
24 min. The color was developed as described in the “Antigen detection protocol for the  
25 sandwich ELISA with 12F12 as the capture Ab” section.

26 As shown in Fig. S1, the reactivity EC<sub>50</sub> values for all of the mAbs against p239 from  
27 different genotypes were analyzed. A total of 72 mAbs were excluded from the panel  
28 due to a lack of reaction with different genotypic p239 proteins or low reactivity (EC<sub>50</sub>  
29  $\geq 100$  ng/mL) (Fig. S1 (A)). Based on further analysis of the virus capture ability of the

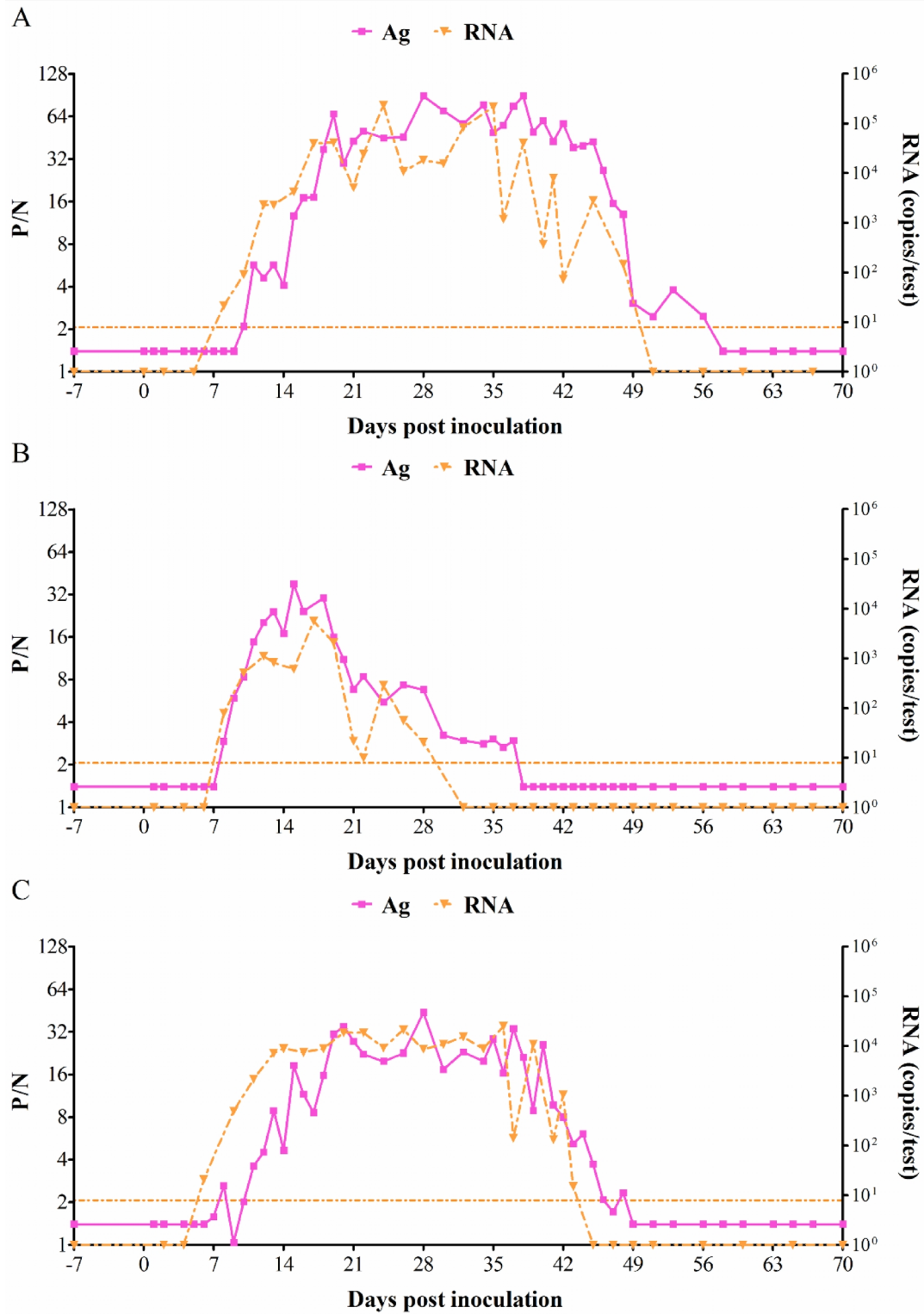
30 antibodies, 10 additional mAbs were excluded. Next, a 14×14 cross antibody-pairing  
 31 was performed; a portion of the results are shown in Fig. S1 (B) and Fig. S1 (C). As  
 32 shown in Fig. S1 (B) and Fig. S1 (C), 12F12 was identified as the antibody with the  
 33 highest ability to capture the virus and the 4# was identified as the best detection mAb.



34  
 35 Fig. S1. Establishment of the improved sandwich ELISA method. (A) A schematic of  
 36 the selection process of the paired mAbs for the establishment of the sandwich ELISA.  
 37 (B) The capture ability of 14 mAbs when 4# was used as the detection antibody in the  
 38 sandwich ELISA. (C) The detection ability of 14 mAbs when 12F12 was used as the  
 39 capture antibody in the sandwich ELISA.

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41 **The detection of HEV antigen and RNA in serial fecal samples from rhesus**  
42 **monkeys infected with different genotypes of HEV**



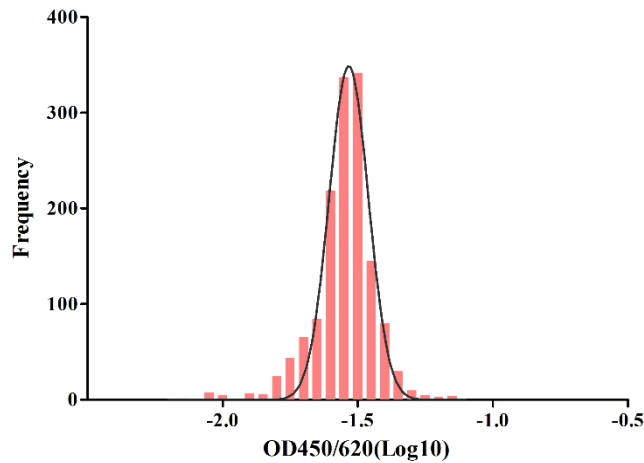
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44 Fig. S2. The relationship between HEV antigen and RNA in serial fecal samples from  
45 rhesus monkeys infected with genotype 1(A), genotype 3(B), and genotype 4(C) HEV.

46 Antigen detection was transformed to positive/negative (P/N). A value of  $P/N \geq 2.1$   
47 was defined as positive.

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#### 49 **Determination of the cut-off value of the sandwich ELISA**



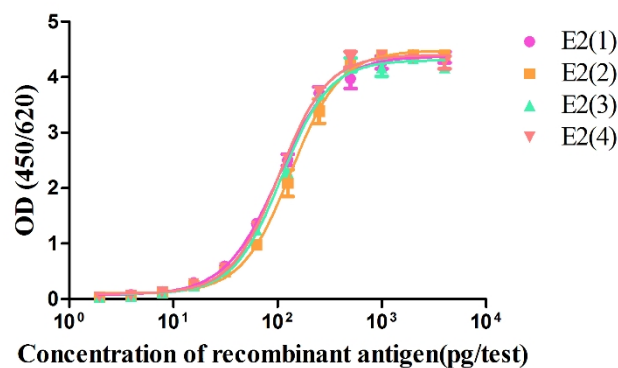
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51 Fig. S3. Frequency distributions of the logarithm of the optical densities of 1424  
52 serum samples. The results were analyzed with GraphPad Prism software and then a  
53 curve was fitted for Gaussian regression. The mean of the logarithm of the optical  
54 densities and standard deviations were -1.533 and 0.07286, respectively. The cut-off  
55 value was set as the mean optical density plus eight SDs, 0.12.

56

#### 57 **The detection ability of the improved ELISA method on genotype 2 proteins**

58 Recombinant capsid E2 proteins from all 4 genotypes were expressed in *E. coli* and  
59 purified as described previously (12). Antigen detection was performed as described  
60 in the “Antigen detection protocol for the sandwich ELISA with 12F12 as the capture  
61 Ab” section.



62

63 Fig. S4. The detection ability of the improved ELISA method on E2 proteins from all 4  
64 genotypes. The results were analyzed with GraphPad Prism software and curves were  
65 fit for non-linear regression. The sandwich ELISA had the same detection ability for  
66 E2 proteins from all genotypes.