

Supplementary materials

Title: Validation of pretreatment methods for the in-process quantification of foot-and-mouth disease vaccine antigens

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Inventory of Supplemental Information

Supplemental Figures and Legends:

- **Figure S1 (refers to Figure 1)**
- **Figure S2 (refers to Figure 2)**
- **Figure S3 (refers to Table 1)**
- **Figure S4 (refers to Table 2)**
- **Figure S5 (refers to Table 3)**
- **Figure S6 (refers to Table 4)**

Supplemental figures and legends

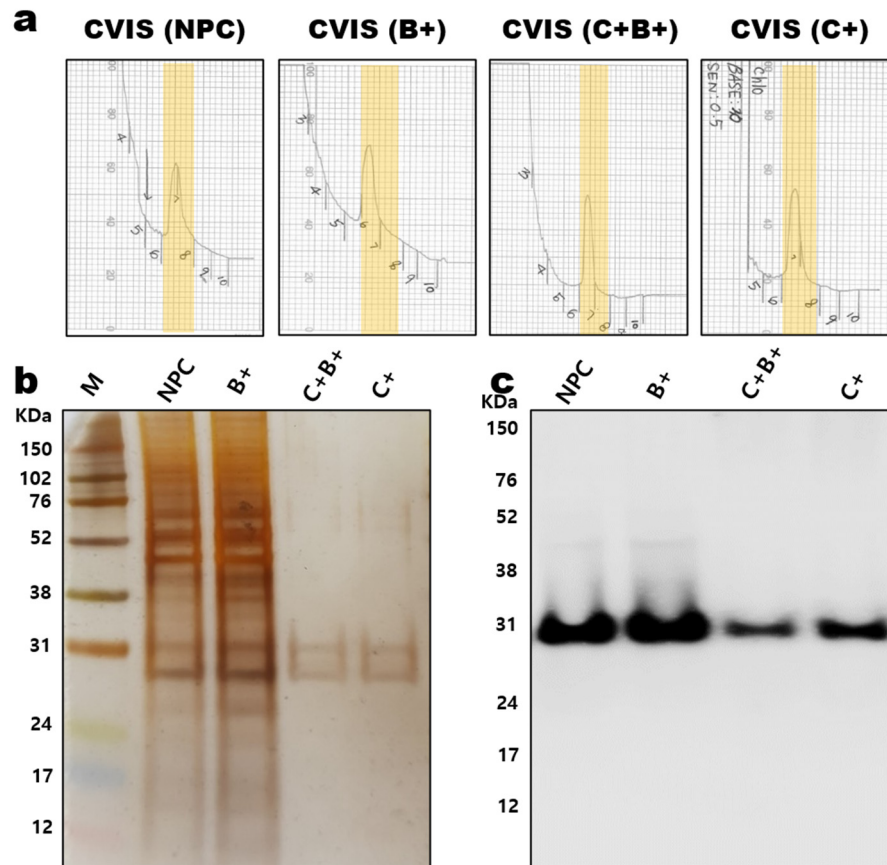


Fig. S1. Purity of 146S antigen peak fractions collected by SDS fractionation from the 10 × concentrates for crude virus infection supernatant (CVIS) of FMDV O SKR/Boeun/2017.

(a) Original chromatograms from SDS fractionation of CVIS (10×) dependent on each pretreatment method. Yellow backgrounds indicate the collected target peak fractions for further purity tests in b and c. (b) A silver-stained gel after SDS-PAGE of SDS ultracentrifugation target peak fraction dependent on each pretreatment method. (c) Western blot result against FMDV type O VP1 on SDS ultracentrifugation target peak fraction dependent on each pretreatment method.

Abbreviations: SDS, sucrose density gradient; NPC, non-pretreated control; B+, benzonase digestion; C+, chloroform extraction; C+B+, combinational pretreatment of chloroform and benzonase; M, marker.

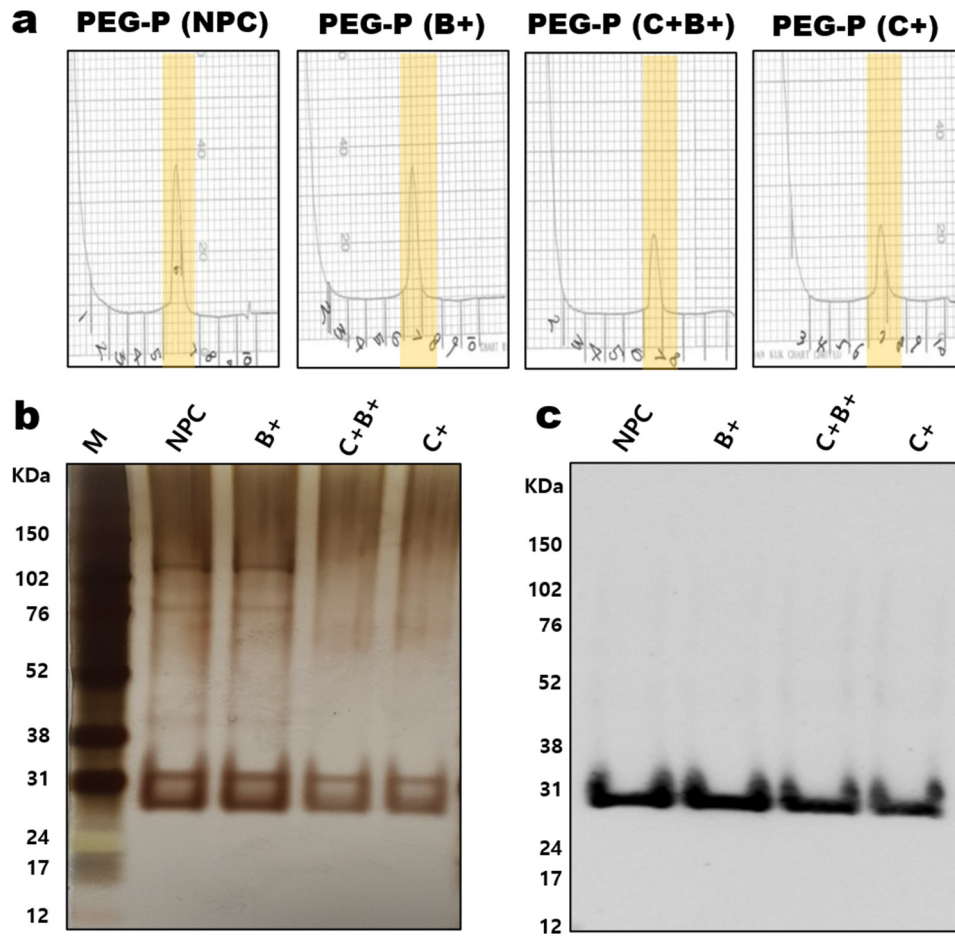


Fig. S2. Purity of 146S antigen peak fractions collected by SDG fractionation from the 10× concentrates for PEG precipitate (PEG-P) of FMDV O SKR/Boeun/2017.

(a) Original chromatograms from SDG fractionation of PEG-P (10×) dependent on each pretreatment method. Yellow backgrounds indicate the collected target peak fractions for further purity tests in B and C. (b) A silver-stained gel after SDS-PAGE of SDG ultracentrifugation target peak fraction dependent on each pretreatment method. (c) Western blot result against FMDV type O VP1 on SDG ultracentrifugation target peak fraction dependent on each pretreatment method.

Abbreviations: SDG, sucrose density gradient; NPC, non-pretreated control; B+, benzonase digestion; C+, chloroform extraction; C+B+, combinational pretreatment of chloroform and benzonase; M, marker.

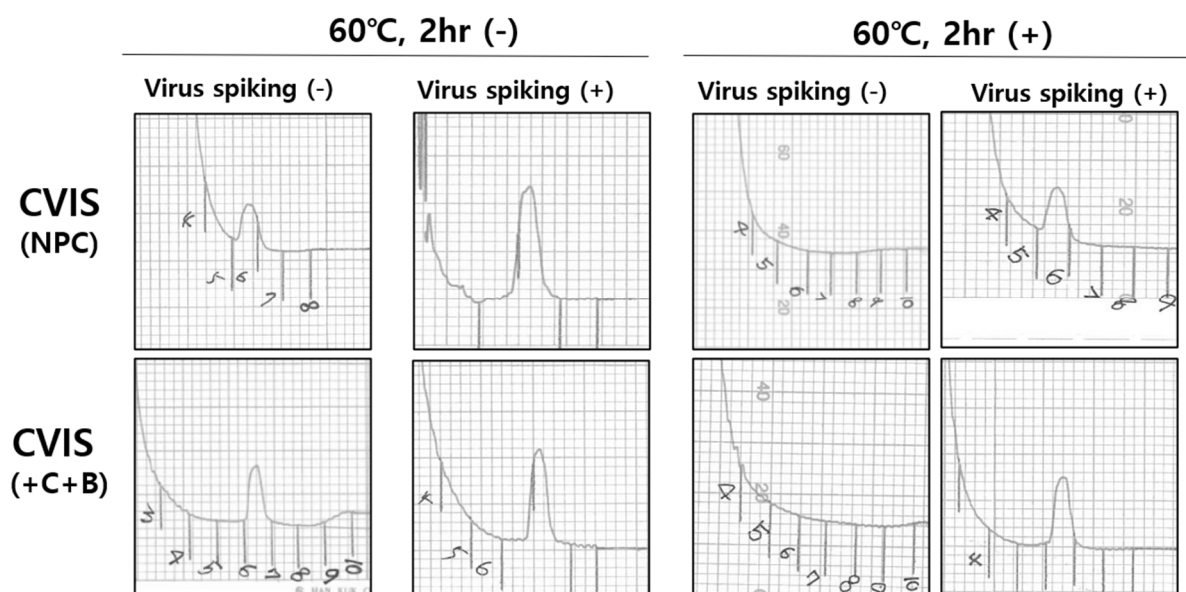


Fig. S3. Representative original chromatograms drawn by SDG fractionation of CVIS (1×) with or without spiked 146S antigens.

Abbreviations: CVIS, crude virus infection supernatant; SDG, sucrose density gradient; NPC, non-pretreated control; C+B+, combinational pretreatment of chloroform and benzonase.

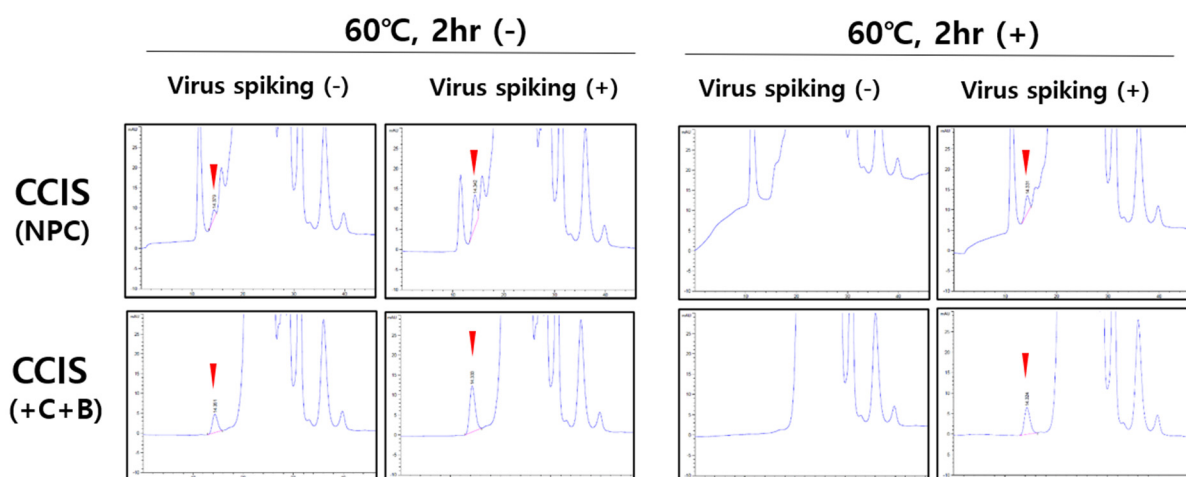


Fig. S4. Representative original chromatograms drawn by SE- HPLC of CVIS (1×) with or without spiked 146S antigens. Red arrowheads indicate the FMDV 146S antigen peaks.

Abbreviations: CVIS, crude virus infection supernatant; SE-HPLC, size-exclusion high-performance liquid chromatography; NPC, non-pretreated control; C+B+, combinational pretreatment of chloroform and benzonase.

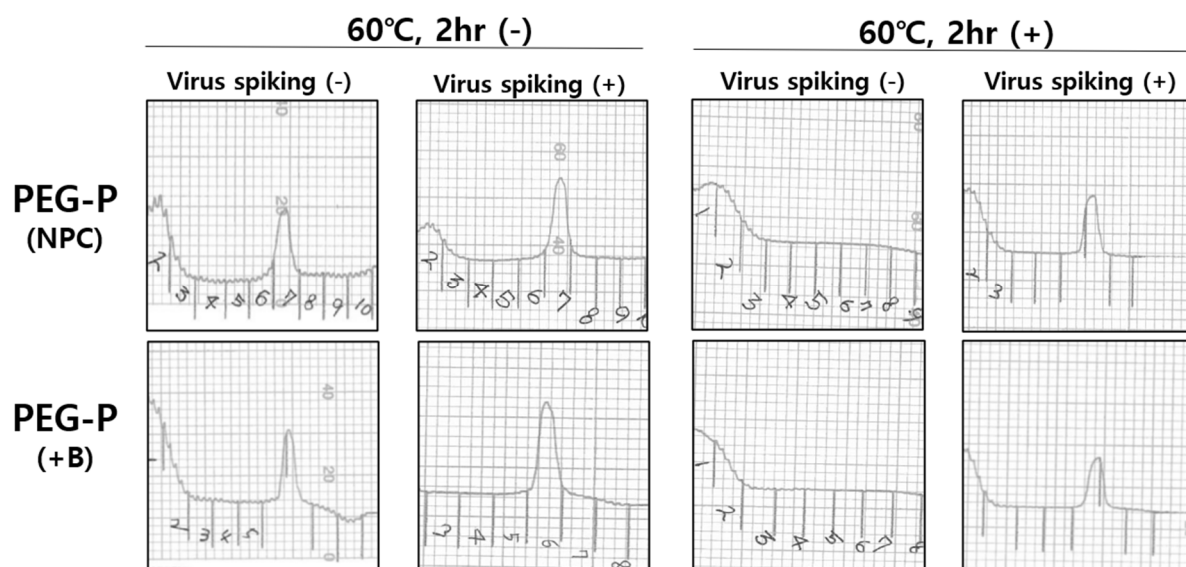


Fig. S5. Representative original chromatograms drawn by SDG fractionation of PEG-P (1×) with or without spiked 146S antigens.

Abbreviations: PEG-P, polyethylene glycol-precipitate; SDG, sucrose density gradient; NPC, non-pretreated control; B+, benzonase digestion.

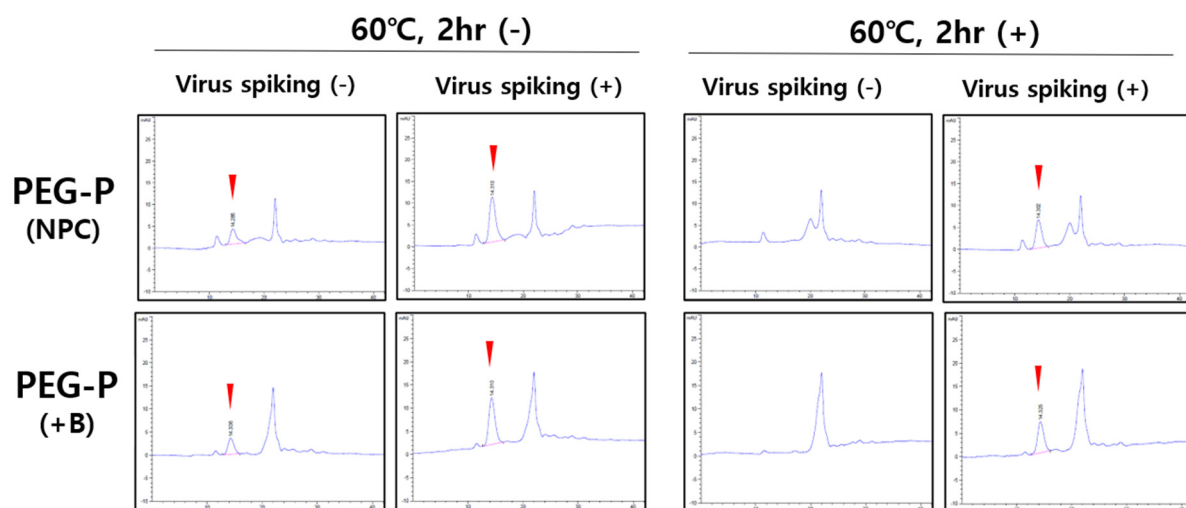


Fig. S6. Representative original chromatograms drawn by SE- HPLC of PEG-P (1×) with or without spiked 146S antigens. Red arrowheads indicate the FMDV 146S antigen peaks.

Abbreviations: PEG-P, polyethylene glycol-precipitate; SE-HPLC, size-exclusion high-performance liquid chromatography; NPC, non-pretreated control; B+, benzonase digestion.