Supplementary Information

Assessment of the efficacy of membrane filtration processes to remove human enteric viruses and the suitability of bacteriophages and a plant virus as surrogates for those viruses

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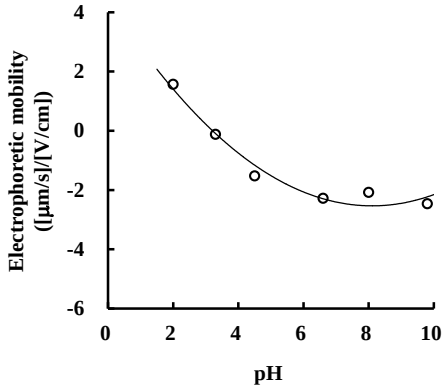

Figure S1. Electrophoretic mobility of PMMoV in prepared Milli-Q water. Values are means and error bars (too small to be visible in the Figure) indicate standard deviations (n = 9).

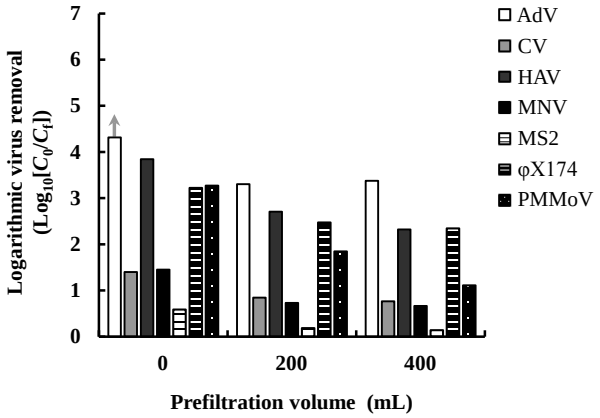

Figure S2. Effect of prefiltration with unspiked river water on virus removal by means of direct MF at pH 7. The PVDF-0.1-HP membrane was used. Arrows indicate that the virus concentrations were below the quantification limit.

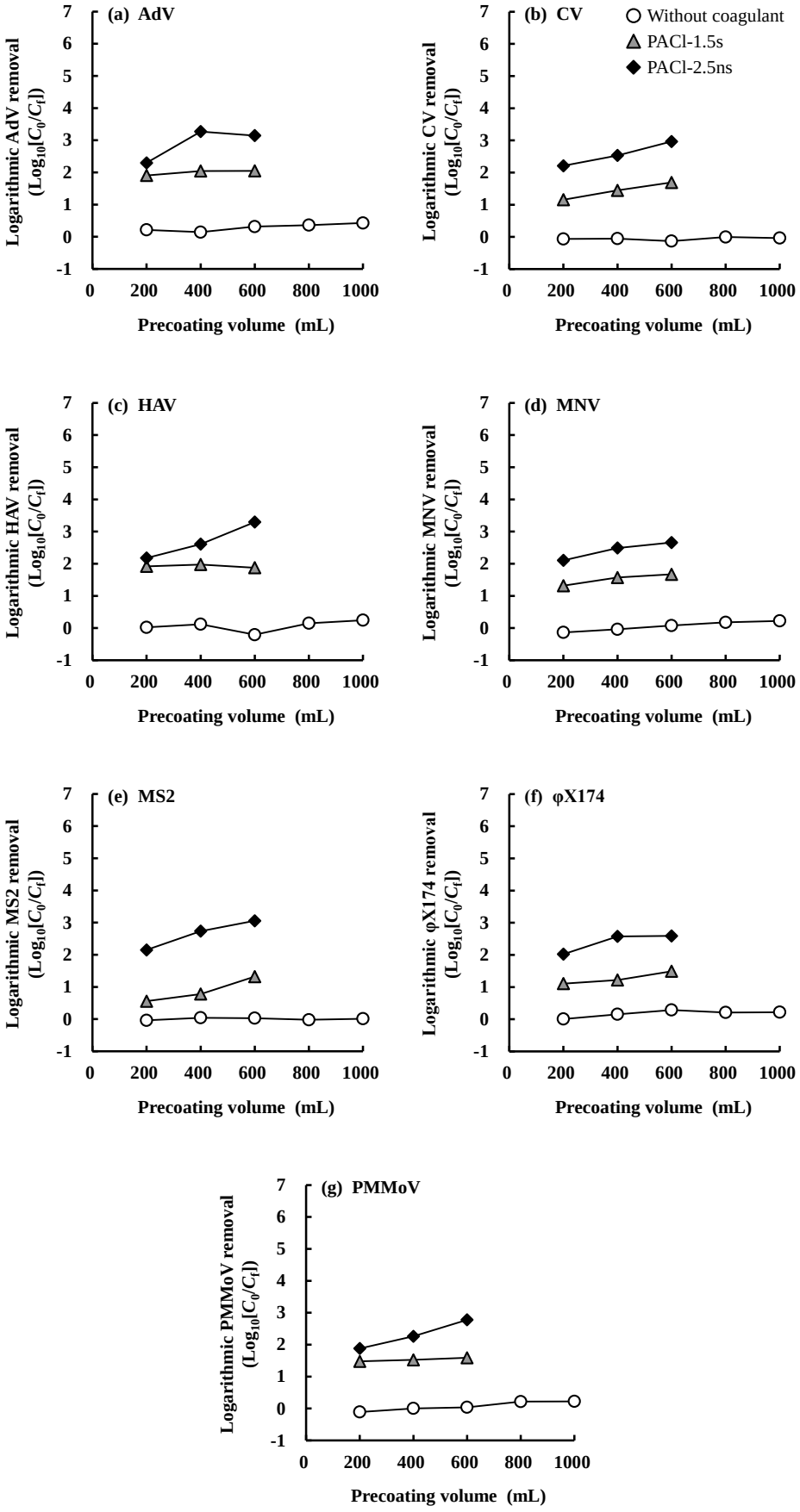
(a) AdV
(b) CV
(c) HAV
(d) MNV
(e) MS2
(f) φX174
(g) PMMoV
Without coagulant
PACl-1.5s
PACl-2.5ns
Logarithmic AdV removal (Log10[C0/Cf])
Logarithmic CV removal (Log10[C0/Cf])
Logarithmic HAV removal (Log10[C0/Cf])
Logarithmic MNV removal (Log10[C0/Cf])
Logarithmic MS2 removal (Log10[C0/Cf])
Logarithmic φX174 removal (Log10[C0/Cf])
Logarithmic PMMoV removal (Log10[C0/Cf])
Precoating volume (mL)
7
6
5
4
3
2
1
0
-1
0
200
400
600
800
1000

Figure S3. Effect of cake layer formation on virus removal by means of direct MF at pH 7. The PVDF-0.1-LP membrane was used. Precoating was conducted with a coagulant dosage of 1.08 mg-Al/L at pH 7.

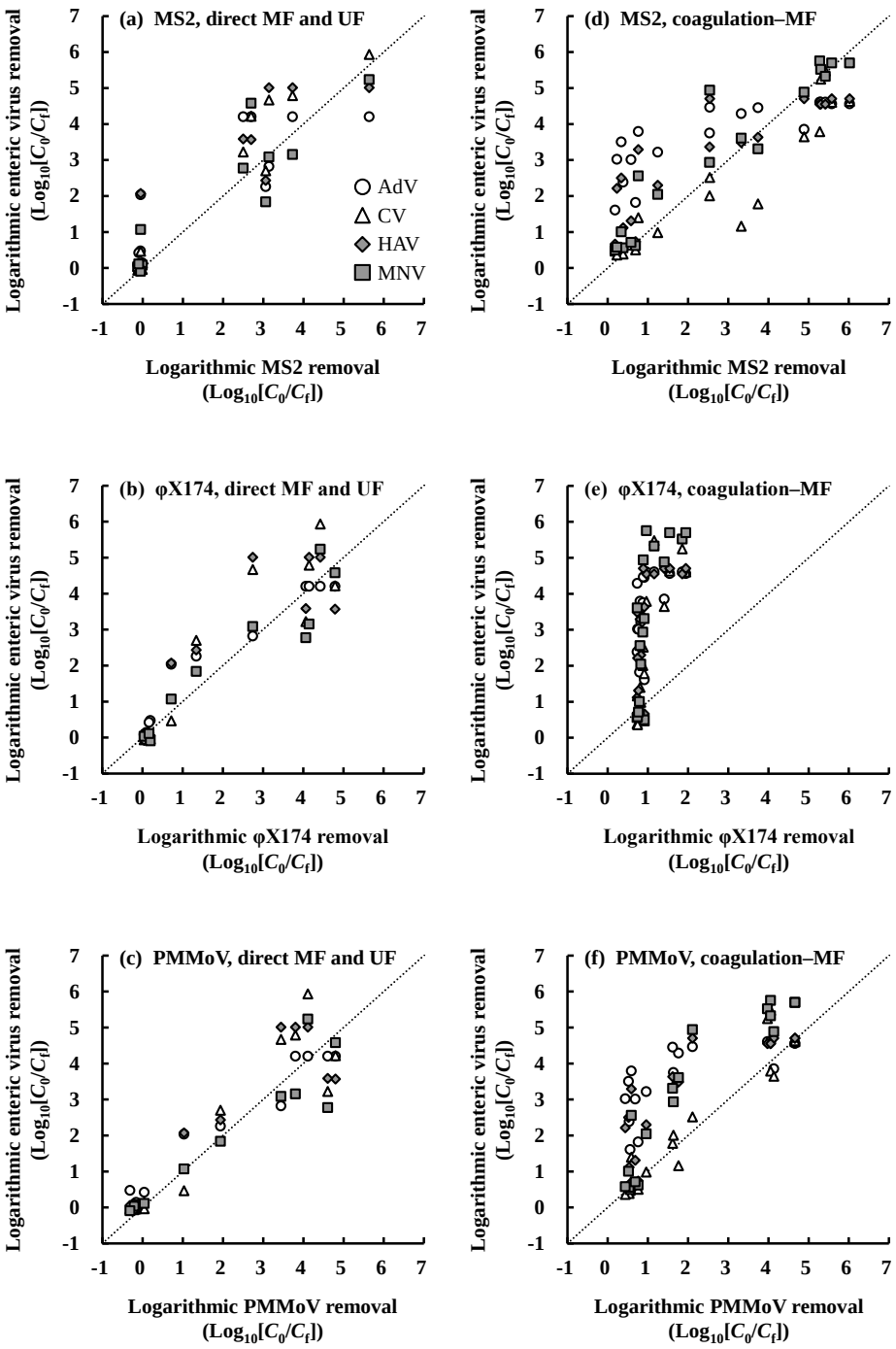

Figure S4. Relationships between the removal ratios of enteric viruses and those of the bacteriophages MS2 or φX174, or PMMoV, during direct MF and UF (a–c) or in-line coagulation–MF (d–f).

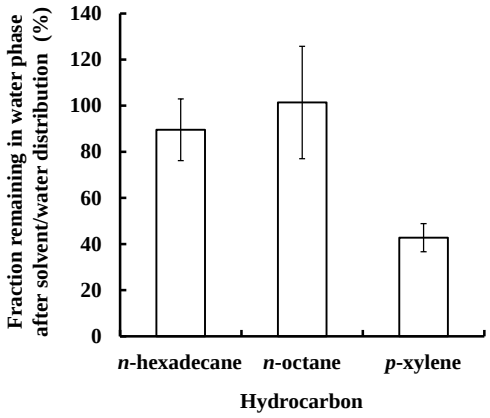

Figure S5. Hydrophobicity of PMMoV. Values are means and error bars indicate standard deviations (n = 6).

Coagulants

Two commercially available sulfated PACls, one with basicity 1.5 (PACl-1.5s; product name, PACl 250A) and one with basicity 2.1 (PACl-2.1s; product name, PACl 700A); a noncommercial, nonsulfated PACl with basicity 2.1 (PACl-2.1ns); a commercially available, nonsulfated aluminum chlorohydroxide solution with basicity 2.5 (PACl-2.5ns, Takibain #1500); and commercially available alum (aluminum sulfate) were provided by Taki Chemical Co. (Kakogawa, Japan). Although PACl-2.5ns is not generally used in water treatment processes (it is mainly used as a ceramic binder and auxiliary agent in papermaking), we used this solution as a coagulant in the present study. Immediately prior to use, the coagulants were diluted with Milli-Q water (Milli-Q Advantage, Millipore Corp., Billerica, MA, USA).

Determination of membrane hydrophobicity

The contact angle between the water and membrane surface was obtained by using a contact angle meter (DMe-211; Kyowa Interface Science Co., Ltd., Saitama, Japan). Two microliters of distilled water was used as the probe liquid in all of the analyses. The average value of the contact angles measured at three random locations per membrane was considered the contact angle for that membrane surface.

Determination of membrane zeta potential

A section of membrane with the prescribed dimensions was attached to a sample holder by using double-sided adhesive tape. The sample holder was then inserted into a disposable, plastic, square cuvette containing Milli-Q water (the alkalinity of which had been brought to 20 mg $CaCO_3$/L by the addition of 0.4 mM sodium hydrogen carbonate, and the pH had been adjusted to 7 with HCl) and a 0.01% (v/v) suspension of latex microspheres (mean diameter 0.5 μm; 5050A; Thermo Fisher Scientific Inc., Waltham, MA, USA) as tracer particles for the measurements. Measurements taken

at 25 °C and a 17° measurement angle at five different distances from the sample surface were used to calculate the zeta potential of the membrane.

Propagation of PMMoV

The seeds and seedlings of *Nicotiana benthamiana* were grown in 200 mL pods filled with gardening soil (Kumiai gardening soil; Hokusan Co., Kitahiroshima, Japan) and cultured in a growth chamber at 25 °C under a long-day photoperiod (16-h light, 8-h dark) for approximately 1 month. Milli-Q water was added to the pods every 1 or 2 days during cultivation. After cultivation, one of the leaves of each plant was covered with 600-mesh carborundum, and then 100 μL of PMMoV stock solution, prepared as described below, was rubbed into the leaf with a gloved finger. The virus-inoculated plant was incubated at 20 °C for 5 min, and then the inoculum mixed with carborundum was washed away with Milli-Q water. After washing, the plant was further incubated in the growth chamber at 25 °C under the long-day photoperiod for 7–10 days. At the end of the incubation period, approximately 1 g of leaf was excised and crushed with a pestle and mortar into 10 mL of phosphate-buffered saline. The PMMoV-containing solution was then centrifuged (2000 × *g*, 10 min) and passed through a hydrophilic cellulose acetate membrane filter (nominal pore size, 0.45 μm; Dismic-25cs, Toyo Roshi Kaisha, Tokyo, Japan) to prepare the PMMoV stock solution.

Direct membrane filtration experimental procedure

Purified solutions of three human enteric viruses AdV, CV, and HAV, MNV, and two bacteriophages (MS2 and φX174), and stock solution of PMMoV were added to the river water at initial concentrations (C_0) of approximately $10^{2–3}$ PFU/mL for AdV, CV, HAV, and MNV, 10^7 PFU/mL for MS2, 10^5 PFU/mL for φX174, and 10^3 lesions/mL for PMMoV. Because the purified solutions and stock solution of viruses were diluted by their addition to the source water, the addition of virus contributed less than 0.3 mg/L of unintentional carry-over of dissolved organic carbon. HCl or

NaOH was added to the spiked water to adjust the pH to 7. The hydrophobic filtration membranes (i.e., PVDF-0.1-HP and PVDF-0.22-HP) were soaked in 400 mL of 50% (v/v) ethanol for 10 min to wet the membrane pores (van Voorthuizen *et al.,* 2001), and then soaked in 400 mL of Milli-Q water for 60 min to rinse the membrane prior to use. The contact angle and the surface charge of PVDF-0.1-HP were almost the same before (contact angle, 112.2 ± 2.3 °; zeta potential, −38.4 ± 0.9 mV) and after (contact angle, 136.5 ± 4.6 °; zeta potential, −33.6 ± 4.2 mV; Table 3) soaking.

In the filtration experiments, 50 mL of virus-spiked river water was directly filtered either through a MF membrane attached to a plastic filter holder (Sterifil 500; Millipore Corp.) by using a suction vessel and a chemical duty pump at a constant vacuum pressure of –50 kPa, or through an UF membrane attached to a stirred plastic filter cell (Model 8050, Millipore Corp.) with nitrogen gas at a constant pressure of 450 kPa and constant stirring at 250 rpm. In each experiment, the first 10 mL of filtrate was discarded, and the remaining 40 mL was sampled for quantification of virus concentration (C_f).

To investigate the contribution of adsorption of the virus onto the membrane surface to virus removal during filtration, 50 or 200 mL of the virus-spiked river water (C_0) was directly filtered through a MF membrane as described above. This filtration was repeated several times with another 50 or 200 mL of spiked water without changing the membrane. In each experiment, the first 10 mL (when 50 mL was added) or 160 mL (when 200 mL was added) of filtrate was discarded, and the remaining 40 mL was sampled for quantification of virus concentration (C_f).

To investigate the competitive adsorption onto the membrane surface between the virus particles and natural organic substances in the source water, PVDF-0.1-HP was prefiltrated with 200 or 400 mL of unspiked river water, and then 200 mL of virus-spiked river water (C_0) was directly filtered through the same membrane. The first 160 mL of filtrate was discarded, and the remaining 40 mL was sampled for quantification of virus concentration (C_f).

Coagulation–MF experimental procedures

The coagulation–MF experiments were conducted with virus-spiked river water at 20 °C, as described in the previous section. To maintain the pH of the filtrate at 7 or 8, HCl or NaOH was added to the spiked water. The virus-spiked river water (C_0) was fed into the in-line coagulation system at a constant flow rate (59 mL/min) by a peristaltic pump. Because 1 to 2 mg-Al/L of PACl is the dose used to treat the Edo River water by the Kanamachi Water Purification Plant (Tokyo, Japan), coagulant was injected before the in-line static mixer (G value, 260 s^{-1}; hydraulic retention time, 1.8 s; 1/4-N40-172-0, Noritake Co., Ltd., Nagoya, Japan) at a constant flow rate (1 mL/min) by a peristaltic pump to obtain a coagulant dose of 0.54, 1.08, or 2.16 mg-Al/L. A combination of an in-line static mixer followed by a Tygon tube reactor (internal diameter, 6.4 mm; total hydraulic retention time, 1 min) was used to obtain a coagulation time of 1 min. After the in-line coagulation, 50 mL of the coagulated water was immediately filtered through PVDF-0.1-LP, as described above. The first 10 mL of filtrate was discarded, and the remaining 40 mL was sampled for quantification of virus concentration (C_f).

To investigate the effect of filter cake accumulation on virus removal, a PVDF-0.1-LP membrane filter was precoated with aluminum floc particles by continuously feeding 200, 400 or 600 mL of unspiked coagulated river water through the in-line coagulation system at pH 7 and a coagulant dose of 1.08 mg-Al/L, as described above. After precoating, 50 mL of virus-spiked river water (C_0) without coagulation was directly filtered through the precoated membrane, as described above. The first 10 mL of filtrate was discarded, and the remaining 40 mL was sampled for quantification of virus concentration (C_f).

Quantification of viruses with real-time PCR or real-time RT-PCR

Viral DNA or RNA was extracted from 200 μL of sample by using a QIAamp MinElute Virus Spin Kit (Qiagen, Tokyo, Japan) to obtain a final volume of DNA or RNA of 50 μL. Extracted RNA

solution was added to a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems Japan, Tokyo, Japan) for the reverse-transcription reaction, which was conducted at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s with subsequent cooling to 4 °C in a thermal cycler (Thermal Cycler Dice Model TP600; Takara Bio Inc., Otsu, Japan). Extracted DNA solution, or the cDNA solution resulting from reverse transcription of the extracted RNA, was then amplified with TaqMan Universal PCR Master Mix, No AmpErase UNG reagent solution (Applied Biosystems Japan) together with 400 nM of primers (HQ-SEQ grade, Takara Bio Inc.) and 250 nM TaqMan probe (Applied Biosystems Japan). The oligonucleotide sequences of the primers and the probes used for the quantification of the three human enteric viruses, MNV, and the two bacteriophages are described in our previous reports (Shirasaki *et al.*, 2016; Shirasaki *et al.*, 2017), and those used for the quantification of PMMoV were taken from previous reports (Zhang *et al.*, 2006; Haramoto *et al.*, 2013). Amplification was conducted at 50 °C for 2 min, 95 °C for 10 min, and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min in an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems Japan). A standard curve for the real-time PCR method was constructed based on the relationship between the plaque assay-measured concentration of infectious viruses in a freshly prepared purified solution of viruses or the local lesion count assay-measured concentration of infectious viruses in a freshly prepared stock solution of viruses and the number of PCR amplification cycles (the C_t value). The quantification limit of the PCR assay was approximately 0.1 PFU/mL or lesions/mL. When the virus concentration in the membrane filtrate was less than the quantification limit of the PCR assay, 12 mL of filtrate was further concentrated to 200 μL by using a centrifugal filter device (nominal molecular weight cutoff, 100 kDa; RC membrane, Amicon Ultra-15; Millipore Corp.) prior to the quantification of virus concentration.

Effect of filter cake accumulation on virus removal

The formation of a filter cake comprising materials such as kaolinite (Jacangelo *et al.,* 1995), constituents of natural water (Tanneru and Chellam 2012), or floc particles generated by the coagulation pretreatment (Shirasaki *et al.,* 2009; Tanneru and Chellam 2012; Matsushita *et al.,* 2013; Tanneru *et al.,* 2013) on the membrane surface is known to affect virus removal during membrane filtration. We investigated the effect of the accumulation of a filter cake consisting of natural constituents of the river water used in the present study and aluminum floc particles generated by coagulation of the river water with PACl-1.5s or PACl-2.5ns on virus removal (Figure S3). Whereas almost no removal of the three human enteric viruses or MNV was observed when PVDF-0.1-LP was precoated with natural constituents of the river water, direct MF with PVDF-0.1-LP precoated with aluminum floc particles was able to remove those viruses (1–3-$\log_{10}$ virus removal; Figure S3a–d). A similar trend was observed for the removal of MS2, φX174, and PMMoV (Figure S3e–g). These results indicated that the cake layer consisting of aluminum floc particles contributed to the removal of nonaggregated virus particles by reducing the effective pore size of the membrane. Additionally, because the isoelectric point of aluminum floc particles (i.e., $Al[OH]_3$) is within the pH range 7.0 to 9.0 (Pontius, 1990), aluminum floc particles tend to be positively charged at pH 7 and would therefore adsorb negatively charged viruses, which likely further contributed to the removal of nonaggregated virus particles by the filter cake.

The virus removal performances of the filter cake formed with PACl-2.5ns increased with precoating volume and were approximately 1-$\log_{10}$ higher than those obtained with filters precoated with PACl-1.5s. These results indicate that the characteristics of the filter cake differed depending on the coagulant used, and that the filter cake formed with PACl-2.5ns was more effective than that formed with PACl-1.5s for the removal of human enteric viruses.

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