Supplemental Information

Effects of transfecting Carrier RNA into BGMK cells

Carrier RNA (cRNA, small pieces of RNA with random sequences) is used during the extraction of RNA to increase recovery efficiency. It was suspected during testing of the transfection method that transfection of carrier RNA into BGMK cells could potentially lead to CPE. To verify this, blank samples consisting of only PBS without viral RNA were subjected to the same RNA extraction process as regular samples (including the manufacturer recommended amount of cRNA). The concentration of cRNA was estimated to be approx. 69.3 ng/µL in the final extracted sample based on the manufacturer recommended addition of 5.54 µg of cRNA for each sample mix during extraction and the final elution of 80 µL from the RNA extraction column. These blank samples were then serially diluted (1:2), and the blanks with their dilutions were transfected onto BGMK cells as described above. Cells were checked for CPE over 5-10 days in order to determine if CPE occurred in the presence of cRNA alone without viral RNA, and if so, to determine the cutoff concentration of cRNA below which CPE would not occur.

Table S1: Transfection of carrier RNA onto BGMK cells. The number of wells positive for cytopathic effect (CPE) per the total number of transfected wells is shown for different estimated concentrations of carrier RNA.

RNA Extract per well (µL)	Estimated Amount of cRNA (µg)	CPE Positive Wells/ Total Wells
2	1.39E-01	10/10
1	6.93E-02	10/10
0.5	3.47E-02	0/5
0.25	1.73E-02	0/5
0.125	8.66E-03	0/5
0.0625	4.33E-03	0/5

CPE caused by cRNA transfection

Transfection of blank samples containing no viral RNA but containing cRNA used during the RNA extraction process was found to result in significant CPE in BGMKs (Table S1). Amounts of cRNA > 34.7 ng per well were able to produce CPE in 10/10 (100%) of wells transfected. When media from these transfected wells was inoculated onto fresh BGMK cells, CPE was not observed, indicating that CPE was not due to the presence of contaminating infectious agents. At amounts of cRNA ≤ 34.7 ng, no CPE was observed in any of the 10 wells (0%), indicating a cutoff of transfection induced cRNA associated CPE in BGMKs somewhere between 34.7 and 69.3 ng per well. Due to the results of this experiment, MPN positives from the transfection assay were not used for the purpose of calculating MPN values when estimated cRNA content was >34.7 ng per well.

Detection of Transfectable RNA with and without cRNA

The addition of cRNA is usually only indicated for in samples that have low concentrations of RNA, in which case it can improve chances of detection. Given our observations of CPE caused by cRNA, we performed a simple test to observe the effect of extracting RNA from serially diluted virus samples with a and without cRNA to determine the effect on the transfection assay detection limit (Table S2). In samples with the lowest concentration of IV/ml, transfectable RNA was not detected in samples extracted without cRNA (Table S2). Extraction with cRNA thus allowed us to detect lower concentrations of transfectable RNA. This lower detection limit was partly offset by the occurrence of CPE due to cRNA. However, as long as we confirmed the presence of replicable E11 in these wells by culturing or PCR to exclude false-positives (see section 2.1), the wells affected by cRNA could nevertheless be included to determine the TGU/mL. Overall, including cRNA in the extraction procedure would allow us to maintain a larger window of observable transfection reductions and we decided against removing cRNA from the extraction step.

Table S2. Observed concentrations of transfected samples of RNA extracted from serially diluted E11 virus samples with and without cRNA.

IV / mL (log10)	TGU / mL (log_{10}), RNA extracted without cRNA	TGU / mL (log_{10}), RNA extracted with cRNA
5.7	Not detected	2.8
6.7	3.8	4.2
8.5	5.0	4.7

Loss of intact virus and viral RNA is minimal during Amicon concentration step

To test the loss of virus due to the centrifugal (Amicon) concentration step in our procedure, we compared the total number of genome copies (GC) in duplicate samples taken from our heat inactivation experiment before and after subjecting them to centrifugal concentration. We detected only slight reductions in GC (0.33-log_{10}) in control samples (prior to heating).. Similarly, minimal reductions in detected GC (0.06-log_{10}) were observed for samples treated with heat (55°C) for 50 sec. From these results we concluded that loss of intact virus and naked viral genome due to the concentration step was minimal in our experiments and thus did not play a significant role in low transfection efficiencies.

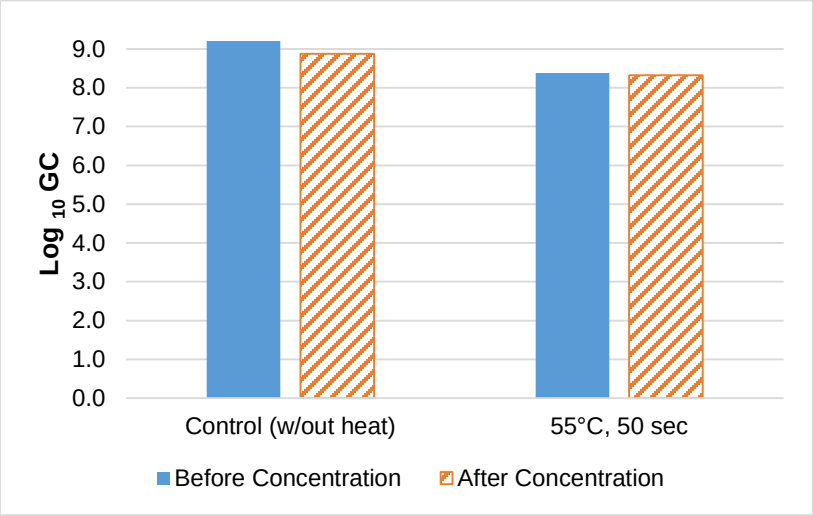

Figure S1: Effects of concentration step on detection of viral RNA from intact untreated virus (control) and viruses treated with heat (55°C) for 50 sec. The bars represent total GC (back calculated to the amount present in the original sample) detected in the sample before concentration (Blue bars) and after concentration (orange dashed bars).