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Study of *human norovirus* proliferation method using commercial human iPS cell-derived small intestinal epithelial like cell

市販ヒトiPS細胞由来腸管上皮細胞を用いた ヒトノロウイルスの増殖法に関する研究

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利益相反開示

演題名：Study of *human norovirus* proliferation method using commercial human iPS cell-derived small intestinal epithelial like cell

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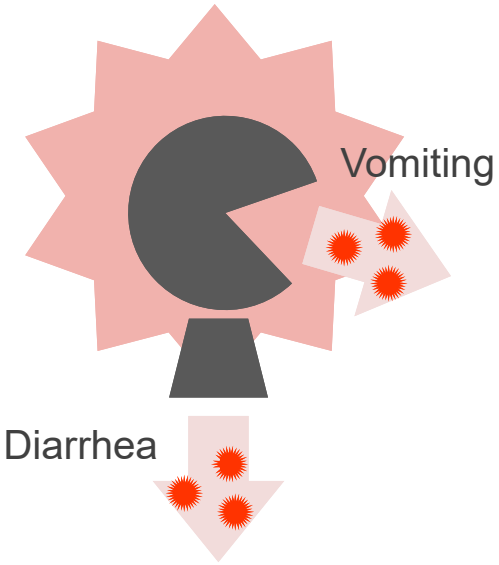
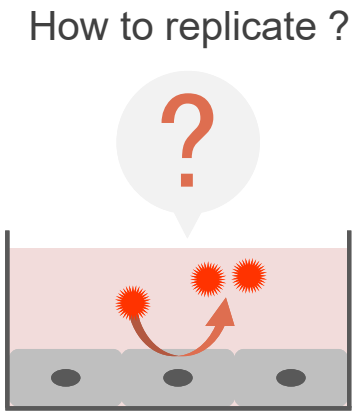
筆頭発表者 冨瀬 彩加は、富士フイルム株式会社の社員である

Introduction

◆ *Human Norovirus (HuNoV)*

- Causes acute gastroenteritis.
- *In vitro HuNoV* culture system has been developed.

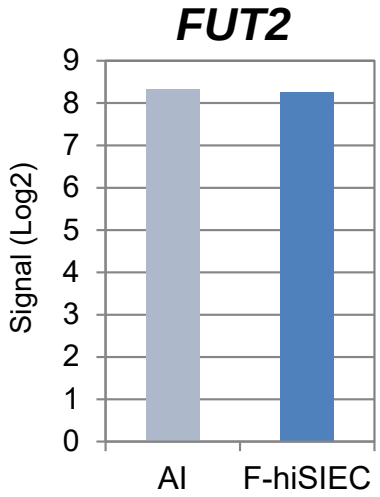




◆ FUJIFILM human iPS cell-derived Small Intestinal Epithelial like Cell (F-hiSIEC™)

- Similar characteristics as *in vivo* human small intestine.
- Equal Expression of the *FUT2* gene as an adult small intestine.

Fucosyltransferase 2 (FUT2) gene expression of F-hiSIEC™ was measured by microarray analysis and compared to adult small intestine (AI). Y-axis: relative mRNA expression.



Virus
***HuNoV* (+) stool samples**
Toyama Institute of Health

+

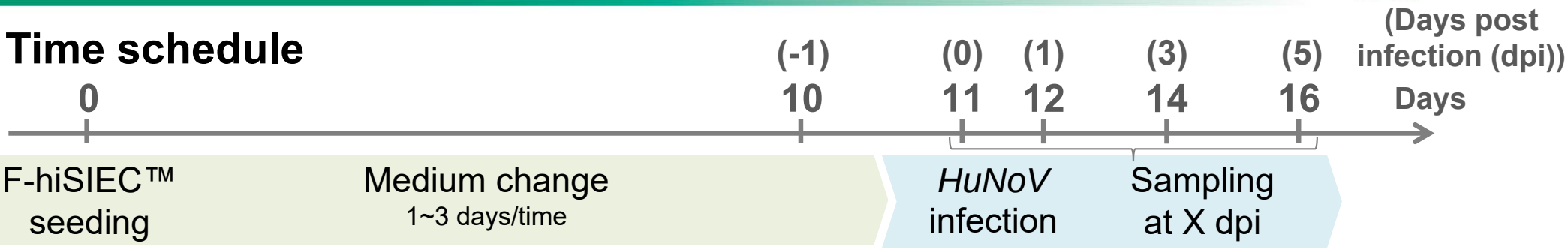
Host
F-hiSIEC™
FUJIFILM Corporation

→

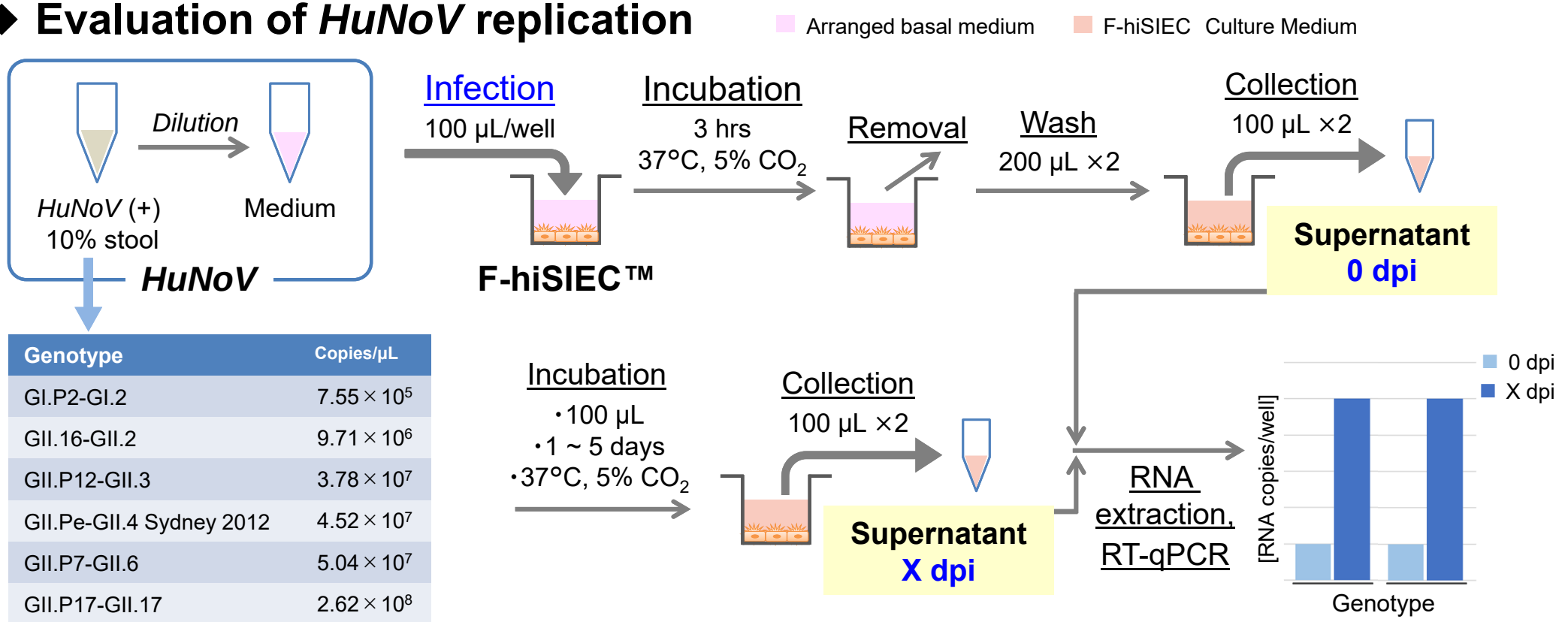
Study of *human norovirus* proliferation method by using F-hiSIEC™

Methods

◆ Time schedule



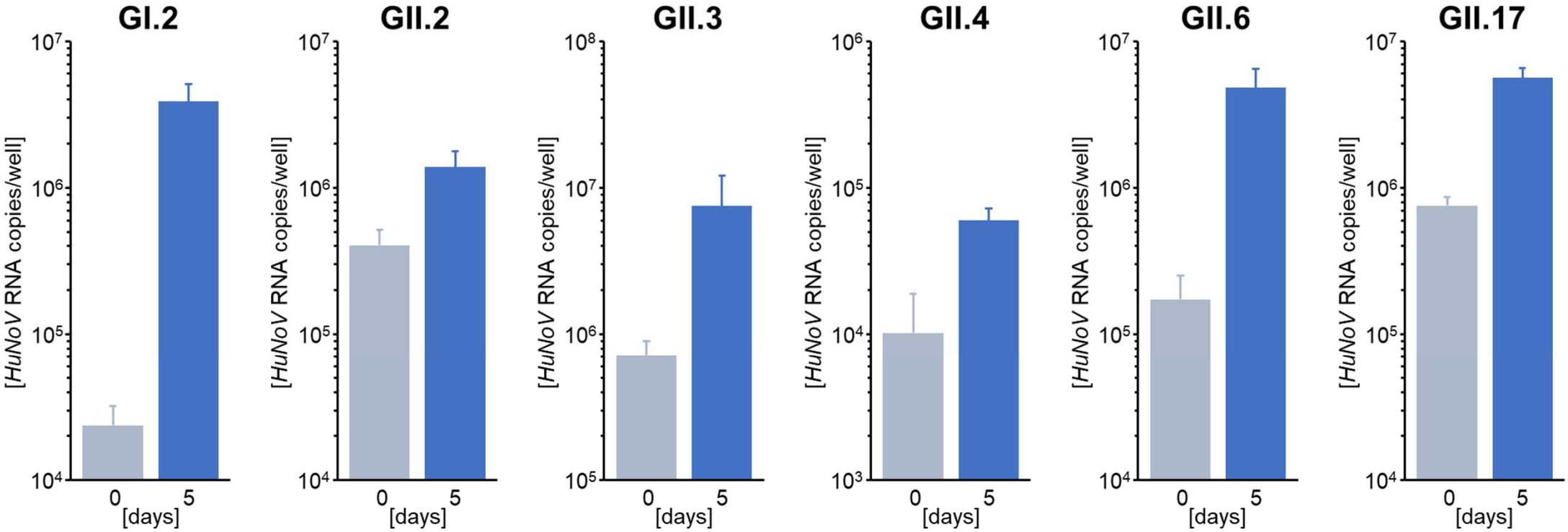
◆ Evaluation of *HuNoV* replication



Genotype	Copies/µL
GI.P2-GI.2	7.55×10^5
GII.16-GII.2	9.71×10^6
GII.P12-GII.3	3.78×10^7
GII.Pe-GII.4 Sydney 2012	4.52×10^7
GII.P7-GII.6	5.04×10^7
GII.P17-GII.17	2.62×10^8

Result 1

◆ Replication of *HuNoV*s with different genotypes

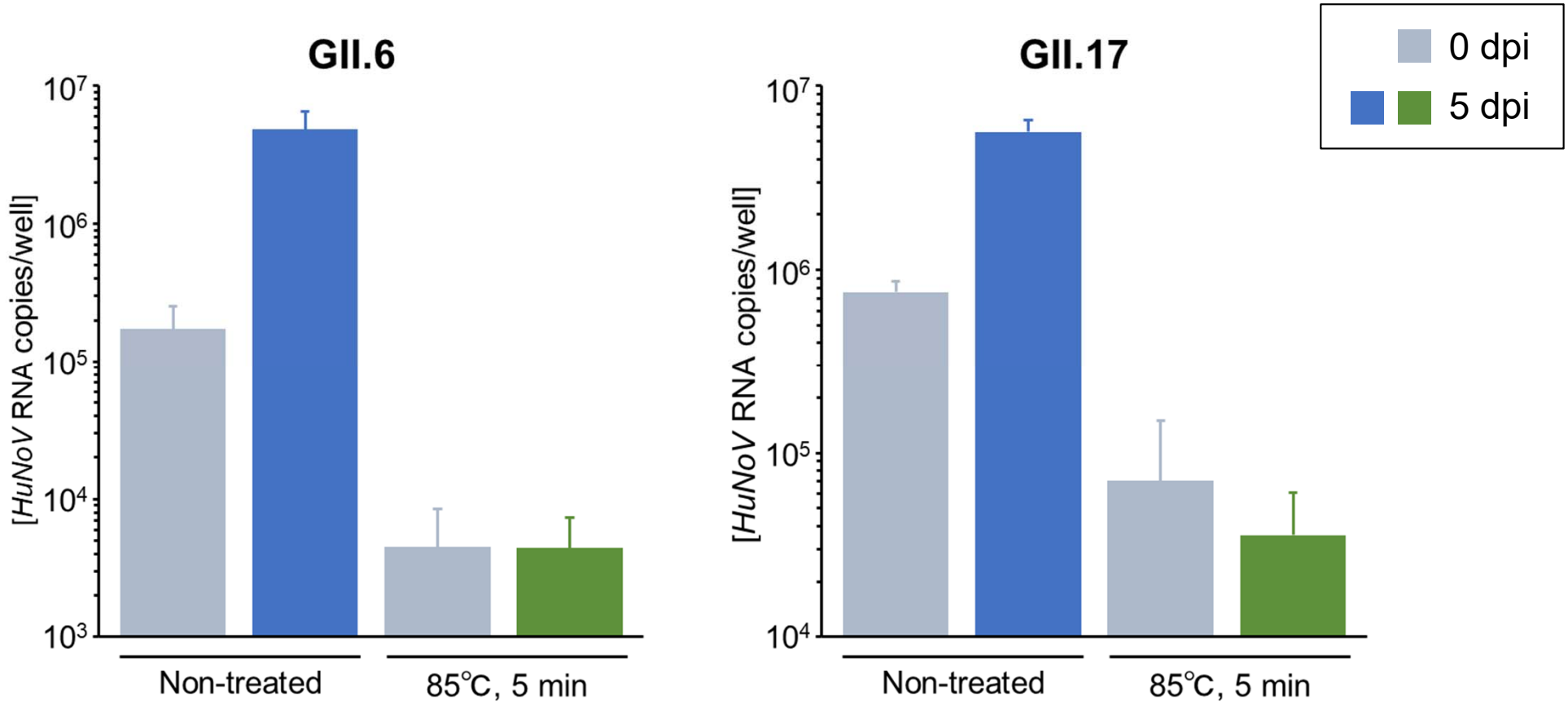


F-hiSIEC™ was inoculated with the indicated *HuNoV* genotypes in the presence of 0.03% porcine bile. Inoculum titers (*HuNoV* RNA copies/well) were as follows: GI.2, 2.29×10^7; GII.2, 9.71×10^7; GII.3, 3.78×10^8; GII.4, 4.52×10^6; GII.6, 5.04×10^7; GII.17, 2.62×10^8. The values represent the mean and standard deviation (SD) (n=3).

F-hiSIEC™ was inoculated with *HuNoV*s of a variety of genotypes. *HuNoV* RNA in supernatants were increased at 5 dpi.

Result 2

◆ Inoculation of heat-treated *HuNoV*

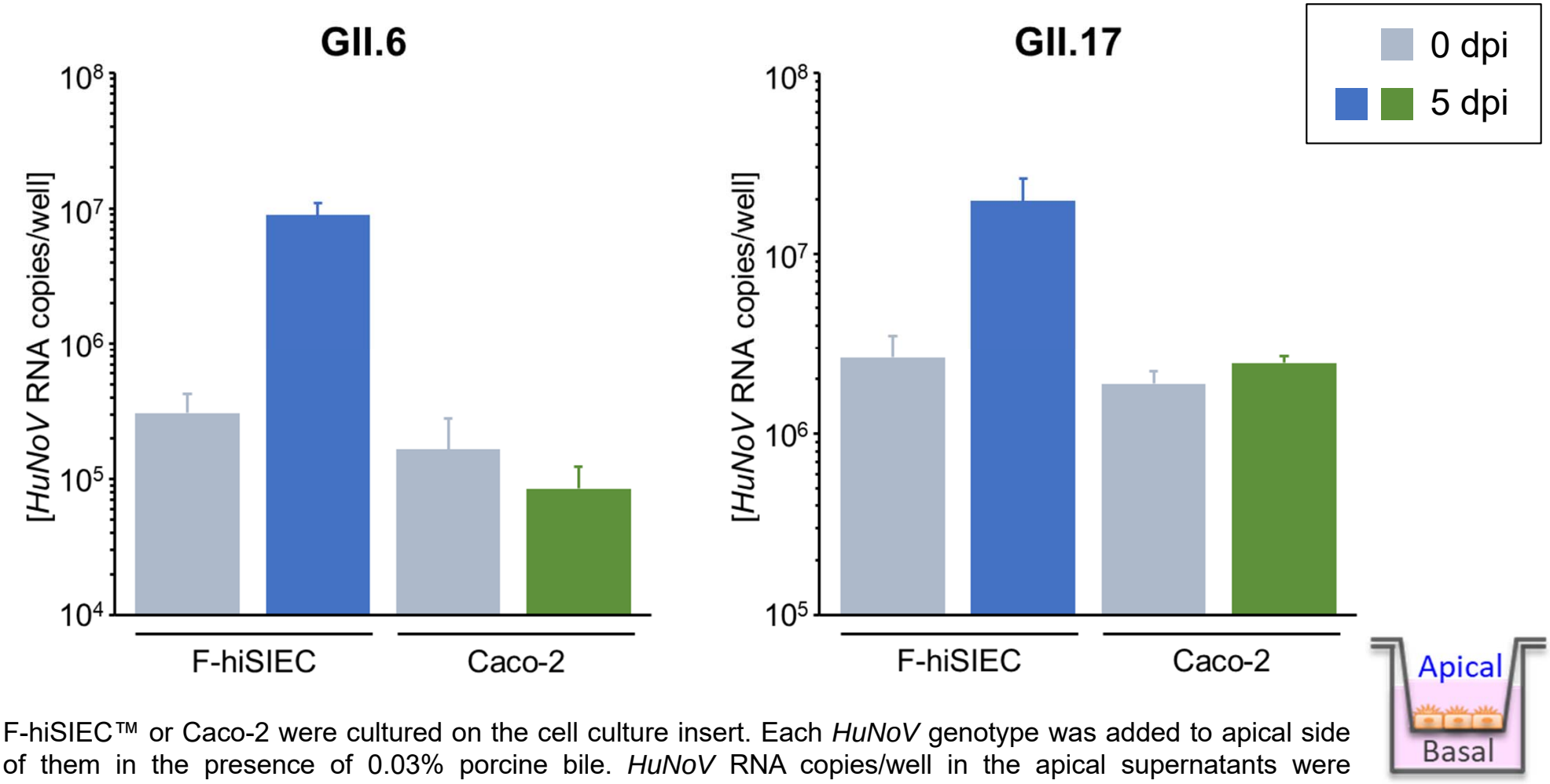


HuNoV positive 10% stool samples were heated at 85°C for 5 min and diluted with the medium. F-hiSIEC™ was inoculated with heated or non-treated samples in the presence of 0.03% porcine bile. The values represent the mean and SD (*n*=3).

No increase of *HuNoV* RNA was observed after inoculating of heat-treated viruses.

Result 3

◆ *HuNoV* cultured in Caco-2 cells

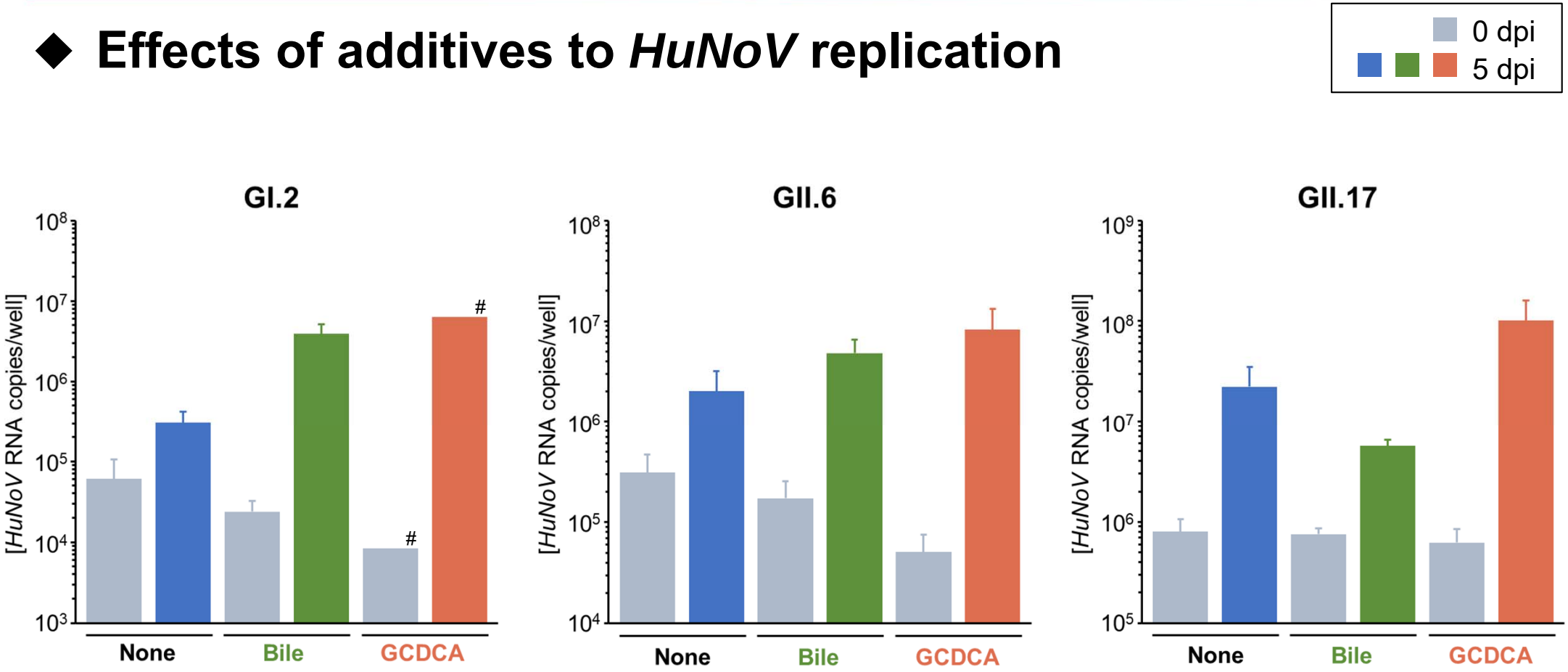


F-hiSIEC™ or Caco-2 were cultured on the cell culture insert. Each *HuNoV* genotype was added to apical side of them in the presence of 0.03% porcine bile. *HuNoV* RNA copies/well in the apical supernatants were quantified. The values represent the mean and SD (*n*=3).

No increase of *HuNoV* RNA was observed in Caco-2, unlike in F-hiSIEC™.

Result 4

◆ Effects of additives to *HuNoV* replication

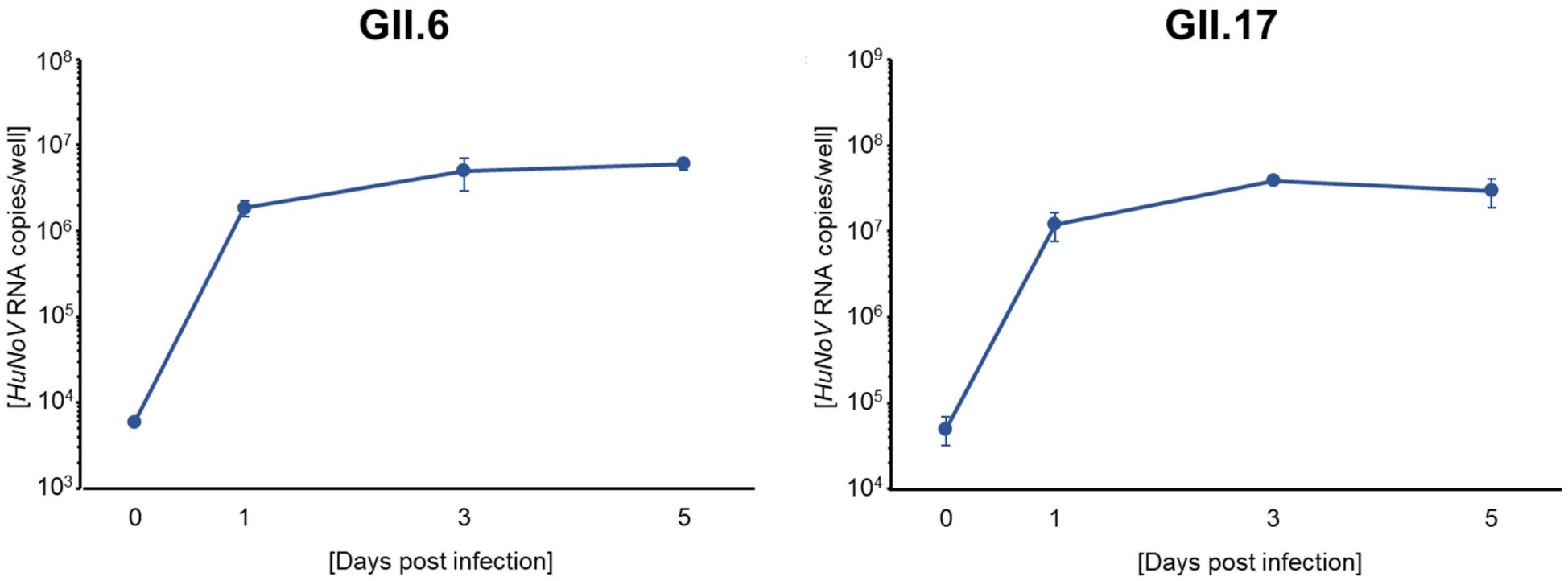


F-hiSIEC™ was inoculated with each *HuNoV* genotype in the presence of 0.03% porcine bile, 500 μM GCDCA or no additives. The values represent the mean and SD (*n*=3, #; *n*=2).

HuNoV RNA were increased more in the presence of GCDCA than in the presence of bile or no additives.

Result 5

◆ Time kinetics of *HuNoV* replication

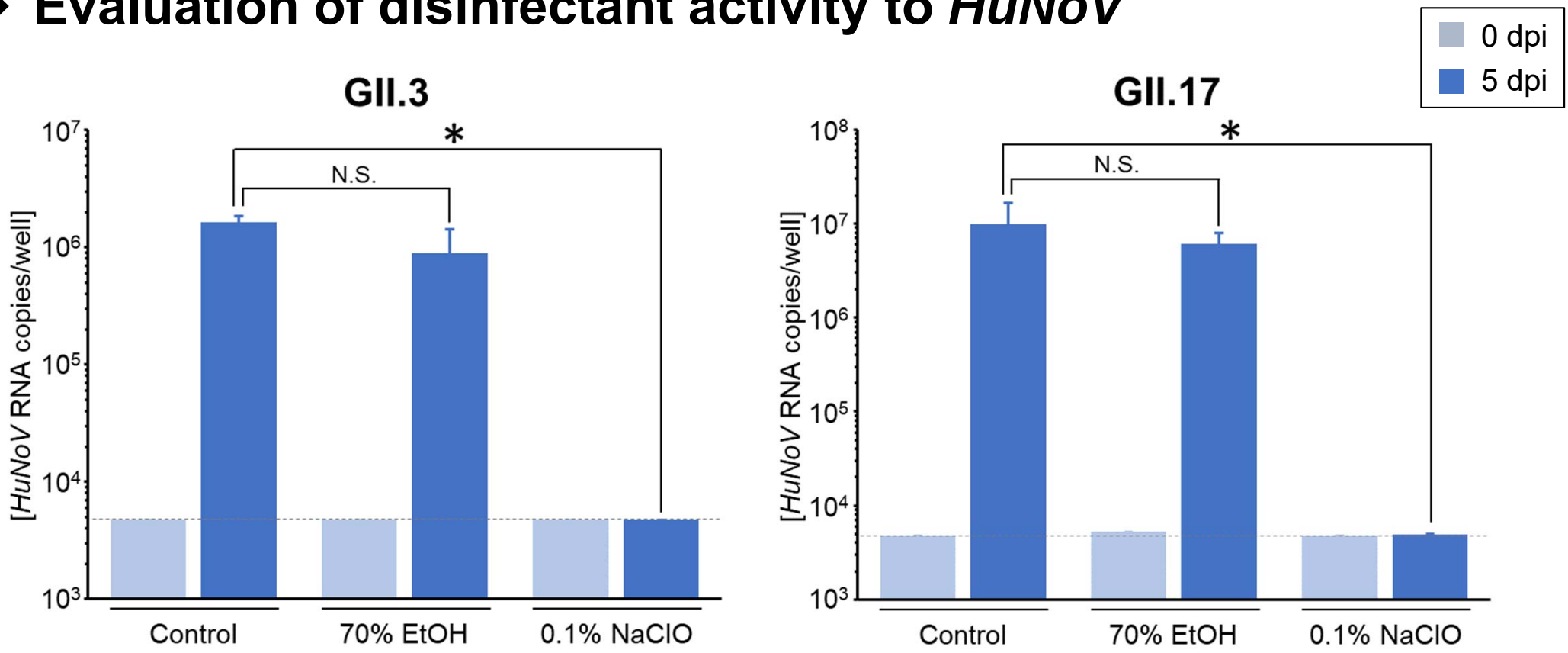


F-hiSIEC™ was inoculated with each *HuNoV* genotype in the presence of 500 µmol/L GCDCA. Cell culture supernatants were taken at 0, 1, 3 and 5 dpi. The values represent the mean and SD (*n*=3). The plot of GII.6 at 0 dpi includes the value calculated using the lowest value of standard curve and its SD isn't determined.

***HuNoV* RNA were increased remarkably during 0 and 1 dpi.**

Result 6

◆ Evaluation of disinfectant activity to *HuNoV*

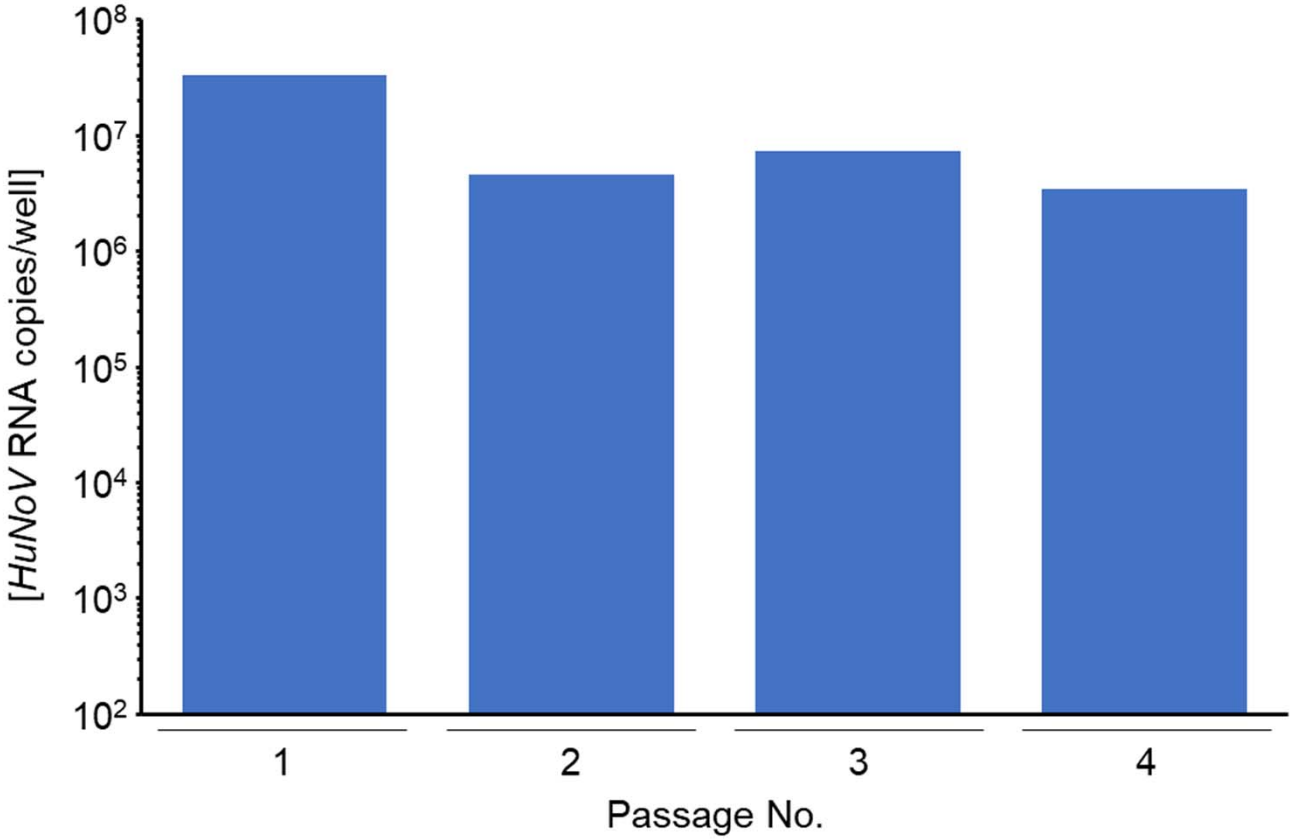


HuNoV positive 10% stool samples were suspended with threefold volumes of water as control, 70% ethanol (EtOH) or 0.1% sodium hypochlorite (NaClO) for 5 min at room temperature. F-hiSIEC™ was inoculated with pre-treated *HuNoVs* in the presence of 500 µmol/L GCDCA. *HuNoV* RNA copies/well at 5 dpi were compared using Dunnett's multiple comparison test. Statistical significance was established at $p<0.05$. The values represent the mean and SD ($n=3$). Dashed line; limit of quantity. *; $p<0.05$. N.S.; not significance.

0.1% NaClO suppressed the increase of *HuNoV* RNA, while 70% EtOH did not.

Result 7

◆ Serial passage of *HuNoV* GII.17



F-hiSIEC™ was cultured for 15 or 16 days before *HuNoV* infection. The cells were inoculated with *HuNoV* in the presence of 500 µmol/L GCDCA and the cells were cultured in arranged basal medium. Cell culture supernatants were taken at 1 dpi and those from 2 wells of each day were mixed (*n*=1).

***HuNoV* was able to be passaged serially in F-hiSIEC™.**

Summary

- *HuNoV*s with different genotypes (GI.2, GII.2, GII.3, GII.4, GII.6 and GII.17) were increased in F-hiSIEC™.
- GCDCA promoted increase of *HuNoV* in F-hiSIEC™.
- Disinfectant activity against *HuNoV* was successfully evaluated using F-hiSIEC™.
- *HuNoV* was able to be passaged serially in F-hiSIEC™

F-hiSIEC™, a commercially available human iPS cell-derived small intestinal epithelial like cell, is a tool for studying *HuNoV* replication.

Please contact us by e-mail using the contact information below for any questions.

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