P0405

Development of a novel *in vitro* culture system for human norovirus

: a robust platform for drug discovery, vaccine development, and disinfectant evaluation

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Introduction

Human Norovirus (HuNoV) is the principal cause of epidemic gastroenteritis globally. Research has been hindered by the lack of a robust and reproducible cell culture system, necessitating the use of surrogate viruses such as feline calicivirus (FCV) for disinfectant and drug testing. Recent advances have shown that human intestinal organoids derived from small intestinal tissue can support HuNoV replication, enabling direct investigation.
Herein, we report the establishment of an in vitro culture system for HuNoV using human iPSC-derived small intestinal epithelial cells (hiSIECs : Manufactured by Fujifilm)[1]. And development of an efficacy test for disinfectant solutions, antiviral compounds and antibody against HuNoV.

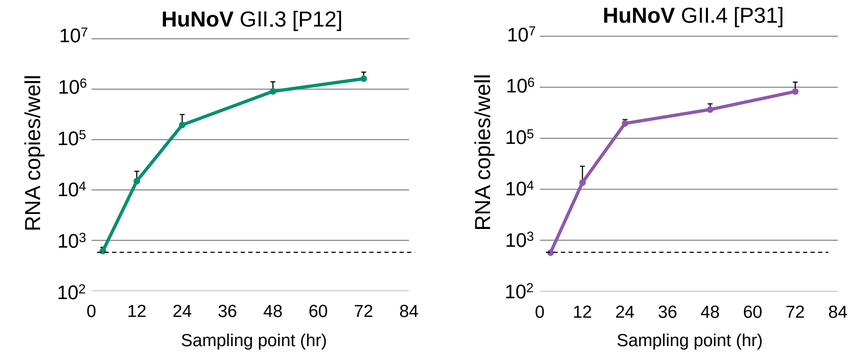
[1] Yuki Imakura et al., Biochem Biophys Res Commun. 2024 Jan;692(149356).

Results

1. Confirm of replication by the growth kinetics

Human iPSC-derived small intestinal epithelial cells (hiSIECs) were inoculated with HuNoV-positive stool filtrates, Culture supernatants were collected, and HuNoV RNA copy numbers were quantified by RT-qPCR.

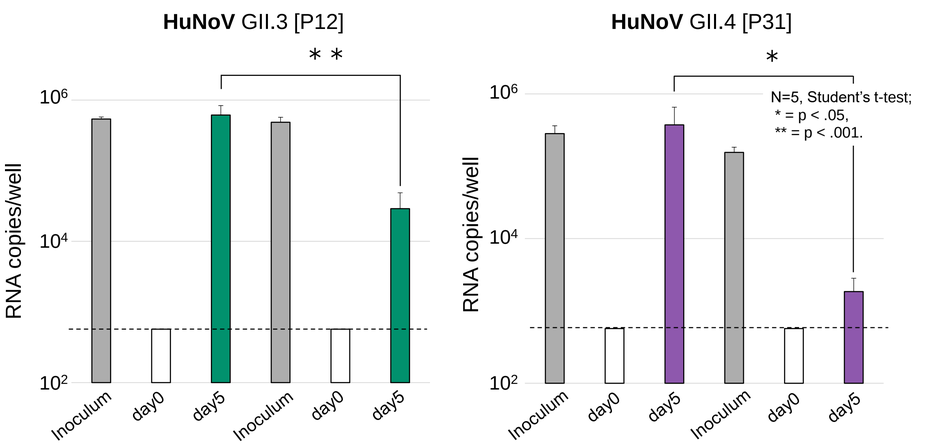
HuNoV: Stool specimens from HuNoV-positive patients, collected at domestic healthcare facilities, were utilized.



N=3,Error bars represent standard deviation (SD).
The lower limit : 571 copies/well

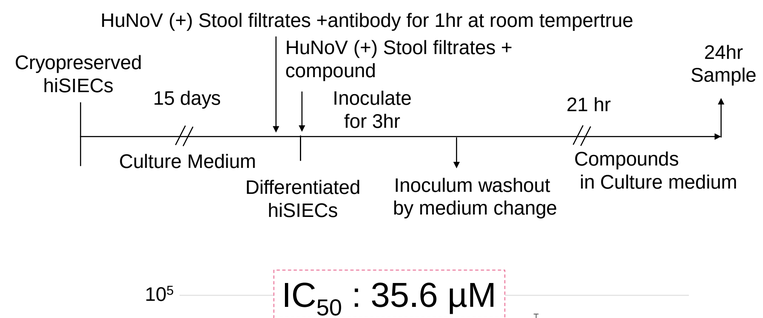
2. Inactivation of HuNoV infectivity by heat treatment

hiSIECs were inoculated with HuNoV-positive stool filtrates, either heat-treated at 60°C for 10 minutes or untreated.



Error bars represent standard deviation (SD).
The lower limit:571 copies/well

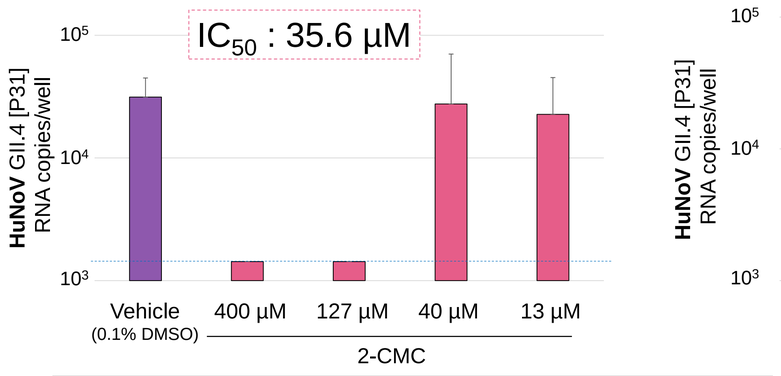
3. Efficacy of antiviral compounds and antibody against HuNoV.

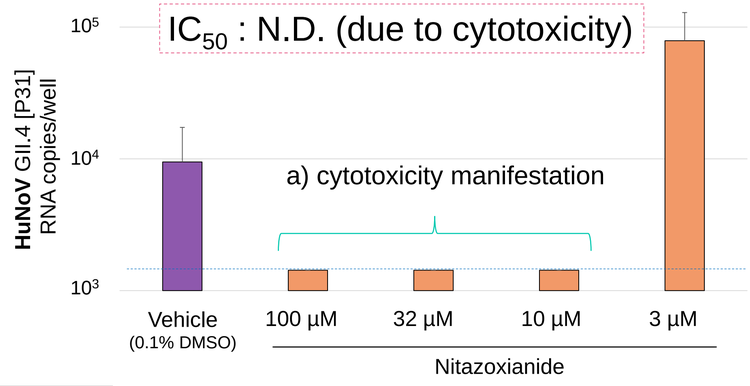


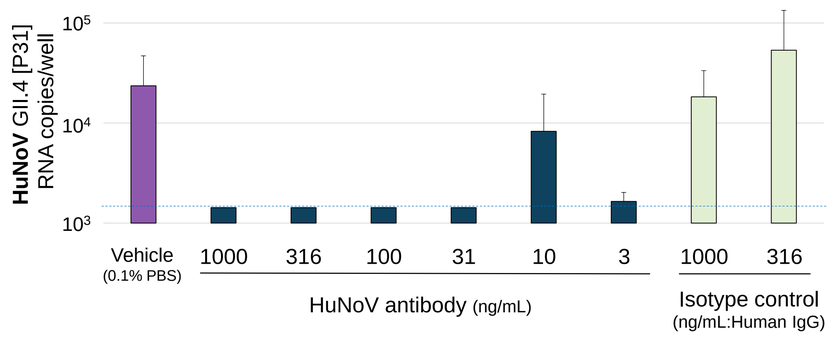
Antiviral activity was assessed by RNA copy number reduction in the culture supernatant on 24hr.

N=3,Error bars represent standard deviation (SD).

The lower limit：1429 copies/well (dash line)





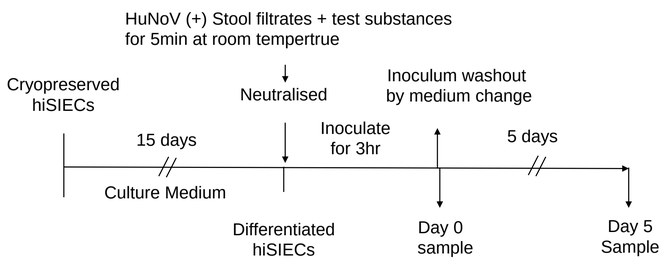


As reported previously, our culture system also confirmed inhibition of viral replication by 2-CMC (2'-C-Methylcytidine)[2] and demonstrated the neutralizing activity of the HuNoV antibody (Anti-Norovirus GII.4 P domain [clone A1431]) [3] . Nitazoxanide exhibited cytotoxicity, preventing confirmation of its antiviral activity.

[2] Tsuyoshi Hayashi et al., Antimicrobial Chemotherapy. 2021 Nov; Vol. 6 Issue 6 e00623-21. [3] Lisa C et al., Immunity 2019; 50_1530-1541.e8

The antiviral activity of 2-CMC and the neutralizing activity of antibody were detected, demonstrating the capability of our system to evaluate antiviral compounds and antibodies.

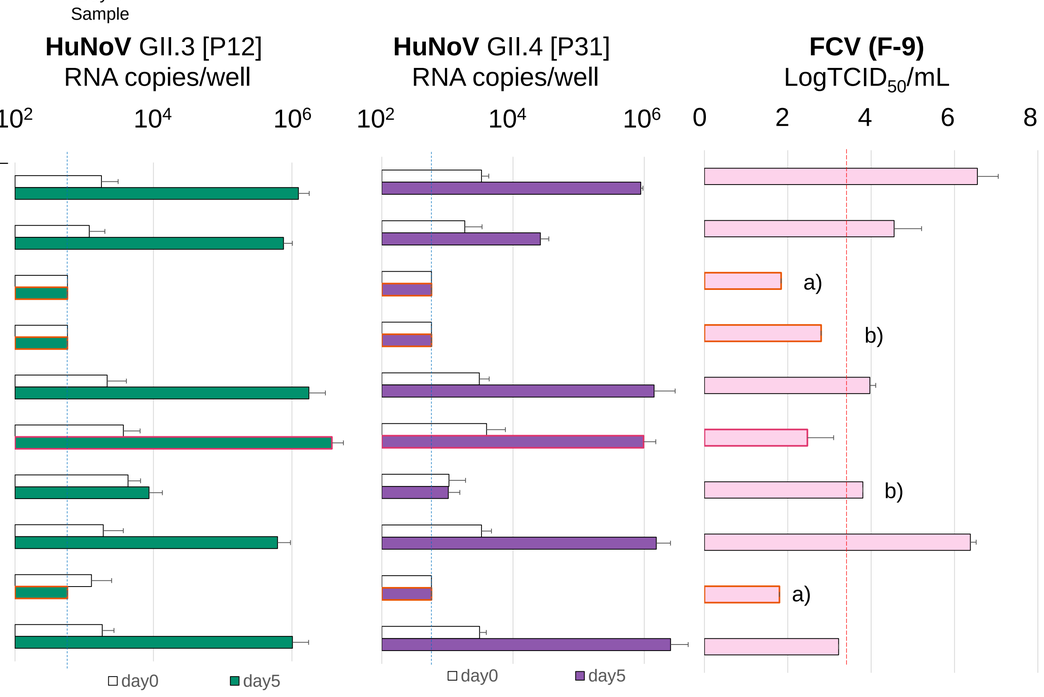
4. Efficacy of disinfectant solutions against HuNoV and FCV.



Disinfection efficacy was determined by RNA copy number reduction in the culture supernatant on Day5.

The efficacy test against FCV was conducted in accordance with the EN standard (EN14476:2013+A2:2019).

Test substance	pH	No.
Control（dH_2O）	7	1
75%Ethanol solution	7	2
0.1%Sodium hypochlorite solution	–	3
10%Povidone Iodine solution	–	4
0.1%Benzalkonium chloride solution	7	5
Bicarbonate buffer solution	11	6
0.1%Benzalkonium chloride bicarbonate buffer solution	11	7
1%Citrate solution	1	8
1%Citrate and 75%Ethanol solution	1	9
1%Citrate and 0.1%Zinc solution	1	10



N=3, error bars represent SD, the lower limit : 571 copies/well (Blue dash line)

The dashed line indicates 3-log reduction in viral titre compared to the control (dH_2O). N=3, error bars represent SD.

Annotation a): Detection limit in virus titre measurement,
Annotation b): Lower detection limit due to cytotoxicity manifestation.

FCV infectivity was reduced by basic conditions (pH 11), but HuNoV infectivity was not affected (No.6). Disinfectants effective against HuNoV, including sodium hypochlorite, povidone-iodine, and citric acid-containing ethanol solutions, were also effective against FCV (No.3, 4, 9).

Conclusions

✓ This novel in vitro culture system using hiSIECs enables robust and reproducible replication of HuNoV.
✓ Consistent reductions in viral RNA copy numbers following heat inactivation confirm the platform's reliability in assessing viral infectivity.
✓ Our platform allows reliable and quantitative evaluation of viral infectivity, antiviral agents, antibodies, and disinfectants.
✓ Evaluation of disinfectant efficacy reveals critical differences between HuNoV and surrogate virus.

Our platform represents a significant breakthrough for drug discovery, thereby offering unprecedented opportunities for developing targeted therapeutics against HuNoV.

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