

**PROJECT TITLE:** Building the Evidence Base for Vomit Clean-Up Procedures in Long-term Care Facilities

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## STRUCTURED ABSTRACT

**Purpose:** Translational research was conducted to fill key knowledge gaps impeding development of detailed infection control procedures for vomitus.

**Scope:** Long-term care facilities, home for nearly 2.5 million people who are mostly older adults

**Methods:** Epidemiological and laboratory studies were employed to complete four aims – (1) quantify role of vomiting as a route of transmission in LTCF norovirus outbreaks; (2) measure dispersion of vomitus into the environment; determine the efficacy of broad-spectrum disinfection strategies on carpet; and determine norovirus persistence in the environment.

**Results:** Two epidemiological studies support control measures that limit exposure to vomitus during HuNoV outbreaks in long-term care facilities. The average extent of two-dimensional dispersion of simulated vomitus was 44.8 m<sup>2</sup>, spanning a width of 3.6 m and a length of 7.7 m from the source, confirming current recommendations for cleaning after vomiting. Although hydrogen peroxide-based disinfectants can serve as an alternative to chlorine-based disinfectants on both stainless steel and carpets, steam vapor emerged as the most effective disinfectant against norovirus and *C. difficile* endospores on carpets, albeit with a few minor limitations.

**Key Words:** Vomiting, Human Norovirus, Porous Surfaces, Carpet, Disinfectants, Dispersion

## **PURPOSE**

Human norovirus (HuNoV) is the leading cause of acute gastroenteritis outbreaks in the United States. Long-term care facilities (LTCFs), home for nearly 2.5 million people, mostly older adults, are the most common setting for such outbreaks. Epidemiologic studies have identified vomiting, which occurs among more than 50% of symptomatic norovirus cases, to be a strong risk factor for norovirus transmission. Vomitus can contain over 30,000,000 million viral particles per vomitus event, resulting in contamination of environmental surfaces via aerosolization and/or improper clean up. Vomitus clean-up guidelines are available, but the evidence base informing procedures is limited, resulting in procedural steps that are insufficiently detailed (e.g., radius of clean up and disinfection procedures for porous surfaces, such as carpet). This lack of detail could result in multiple interpretations of how to execute a clean-up step, possibly resulting in the ineffective removal of HuNoV. In this multidisciplinary study, we conducted translational research to fill key knowledge gaps impeding development of detailed infection control procedures for vomitus. Specifically, four research aims were completed:

- Aim 1: Quantify the role of vomiting as a route of transmission in LTCF norovirus outbreaks.
- Aim 2: Measure dispersion of vomitus into the environment.
- Aim 3: Determine the efficacy of broad-spectrum disinfection strategies on carpet.
- Aim 4: Determine norovirus persistence in the environment.

## **SCOPE**

Epidemiological and laboratory studies were employed to complete our four aims. These findings can be used to populate the evidence base to inform procedural changes to clean-up protocols so that we can ultimately decrease disease and death attributed to HuNoV transmission in LTCFs. Long-term care settings are the number one site for HuNoV outbreaks. Presently there are >2.5 million residents in LTCFs, many are older adults, who are highly vulnerable to developing more severe, serious symptoms of HuNoV.

## **METHODS**

A variety of methodologies were used to complete our four aims. Methods used for each aim are briefly described below. Results for each aim are reported in the Results section of this report.

*Aim 1: Quantify the role of vomiting as a route of transmission in LTCF norovirus outbreaks.*

Sophisticated epidemiological analytical methods were employed to complete two studies in which existing outbreak data were analyzed to quantify the role of vomiting in HuNoV transmission in LTCFs. In the first study, six nursing home HuNoV outbreaks occurring in South Carolina from 2014 to 2016 were examined. The contribution of symptoms and other case characteristics in HuNoV transmission was determined using the reproduction number ( $RE_i$ ) as an estimate of individual case infectivity in order to examine how transmission changes over the course of an outbreak. Individual estimates of  $RE_i$  were calculated using a maximum likelihood procedure to infer the average number of secondary cases generated by each case. Associations between case characteristics and  $RE_i$  were estimated using a multivariate mixed linear model. In the second study, line lists for 107 norovirus outbreaks that took place in LTCFs in five U.S. states from 2015 to 2019 were analyzed. The individual effective reproduction number,  $R_i$ , was

estimated to quantify individual case infectiousness in order to examine the contribution of vomiting, diarrhea, and being a resident (vs. staff) to case infectiousness. Associations between case characteristics and  $R_i$  were estimated using a multivariable, log-linear mixed model with inverse variance weighting.

*Aim 2: Measure dispersion of vomitus into the environment.* A scoping review and a series of experimental simulations were conducted to measure the dispersion of vomitus into the environment. First, a scoping literature review was conducted to describe the extent of knowledge related to the two research questions: (1) What are the determinants of post-doffing contamination? (2) Where is post-doffing contamination most likely to occur after PPE doffing? Two databases were searched (PubMed and sciVerse Scopus), and search terms and inclusion criteria were defined. Data extraction was based on the matrix method, with templates constructed in Microsoft Excel. Extraction was performed by one investigator, and a second investigator reviewed the findings. Second, an experimental simulation study was performed to quantify the magnitude and extent of environmental contamination due to vomiting in order to inform the spatial area for cleaning after a vomitus event. The dispersion of fluorescein-containing simulated vomitus was measured on surfaces and in air using a life-sized vomiting simulator. Four experimental design factors believed to influence vomiting dispersion – head angle, vomitus volume, ejection pressure, and vomitus viscosity – were tested using a blocked randomized design. Fluorescein was measured on 36-floor samplers and with four air samplers.

*Aim 3: Determine the efficacy of broad-spectrum disinfection strategies on carpet.* The efficacy of chemical- and non-chemical-based disinfection strategies was tested on seeded carpet using two norovirus surrogates and *C. difficile*. Nine disinfectants identified from List G: EPA's Registered Antimicrobial Products Effective against Norovirus were tested against feline calicivirus (FCV), tulane virus (TuV), and *Clostridioides difficile* endospores in suspension on stainless-steel surface and carpet. Additionally, steam vapor and photoClO<sub>2</sub> were explored as alternatives to chemical disinfectants for surface disinfection. In all efficacy tests, 'efficacious' was defined as a 4-log reduction of FCV and a 6-log reduction of *C. difficile* endospores on hard, nonporous surfaces. As EPA does not recognize TuV as a target agent, 'efficacious' was defined as a 3-log reduction of general viral surrogates. First, suspension tests were conducted to screen disinfectants against FCV, TuV, and *C. difficile* endospores. Next, a carrier test on stainless steel was conducted to confirm efficacy on nonporous hard surfaces. Additionally, two nylon carpets constructed with water-permeable or waterproofing backings for the carpet test were tested for difference in efficacy. All results from the suspension and stainless steel carrier test were confirmed with TuV by co-investigators at CDC. Only two of the nine chemical candidates were tested on carpets. To test against FCV, TuV, and *C. difficile* endospores on the carpet mentioned above, we selected two hydrogen peroxide-based disinfectants and bleach as the positive control. Additionally, we evaluated the efficacy of steam vapor on both carpets. Carpet damage due to repeated disinfection was evaluated by examining color change, surface damage of carpet fiber, and tensile strength of carpet backings. PhotoClO<sub>2</sub>, a novel photocatalyzed disinfectant producing chlorine dioxide, was tested against FCV, TuV, and *C. difficile* endospores on stainless steel and nylon carpets with two different backings. In addition, indoor lighting conditions were tested against FCV and TuV to determine feasibility for application of this novel disinfectant.

*Aim 4: Determine norovirus persistence in the environment.* Persistence of HuNoV on porous and nonporous surfaces was determined using a very new culture system for HuNoV available only in two laboratories (Baylor College of Medicine and the U.S. Centers for Disease Control and Prevention). The system is based on a novel human-intestinal organoid (mini-gut) cell culture system. First, the persistence of HuNoV over time was determined after seeding onto carpet coupons followed by the identification of the efficacy of chemical-based disinfectants other than chlorine against HuNoV replication on stainless steel and carpet coupons. Specifically, in a biosafety cabinet, 10- $\mu$ L aliquots (replicates) of a pooled virus inoculum containing FCV ( $4 \times 10^3$  virus particles) and TuV ( $6 \times 10^4$  virus particles) were deposited on the membranes of Amicon filter units and dried for 1 hour. Next, 100  $\mu$ L of test disinfectant, either Virasept or OxivirTB, was added onto the virus-inoculated areas of the membranes and incubated at a specific contact time of either 10 or 30 minutes. The membranes were then washed twice with 0.5 mL of infection media: Minimal Essential Medium (MEM), OPTI-MEM™, with a 2% fetal bovine serum (FBS). This was followed by centrifugation at 13,000 revolutions per minute (rpm) for 10 minutes to remove the disinfectant. The concentrates (15  $\mu$ L) in the filter units were then transferred into a clean Eppendorf tube, and the volume was adjusted to 1 mL with infection media. The samples were 10-fold serially diluted in infection media, and each dilution (100, 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup>) was assayed in quadruplicate to determine the viral infectivity using the tissue culture infectious dose (TCID)<sub>50</sub> method.

## RESULTS

Results are presented for each aim.

*Aim 1: Quantify the role of vomiting as a route of transmission in LTCF norovirus outbreaks.*

In the first study, outbreak data from six SC long-term care facilities were used to determine the role of individual case characteristics (e.g., symptoms or demographics) in HuNoV transmissibility. Outbreaks began with one to three index case(s) with large estimated  $RE_i$ 's (range: 1.48 to 8.70) relative to subsequent cases. Of the 209 cases, 155 (75%) vomited, 164 (79%) had diarrhea, and 158 (76%) were LTC residents (vs. staff). Cases who vomited infected 2.74 (95% CI: 1.90, 3.94) more individuals than did nonvomiters; cases with diarrhea infected 1.62 (95% CI: 1.09, 2.41) more individuals than did cases without diarrhea; and resident-cases infected 1.69 (95% CI: 1.18, 2.42) more individuals than did staff-cases. Index cases tended to be residents (vs. staff) who vomited and infected considerably more secondary cases compared with nonindex cases. Results suggested that individuals, particularly residents, who vomit are more infectious and tend to drive norovirus transmission in U.S. LTCF norovirus outbreaks. Though diarrhea also plays a role in HuNoV transmission, it is to a lesser degree than vomiting in these settings. Results lend support for prevention and control measures that focus on cases who vomit, particularly if those cases are residents. In the second study, lists for 107 norovirus outbreaks that took place in LTCFs in five U.S. states from 2015 to 2019 were analyzed to quantify individual case infectiousness and examined the contribution of vomiting, diarrhea, and being a resident (vs. staff) to case infectiousness. The associations between case characteristics, and  $R_i$  were estimated using a multivariable, log-linear mixed model with inverse variance weighting. We found that cases with vomiting infected 1.28 (95% CI: 1.11, 1.48) times the number of secondary cases compared with cases without vomiting, and LTCF residents infected 1.31 (95% CI: 1.15, 1.50) times the number of secondary cases compared with staff. There was no difference in

infectiousness between cases with and without diarrhea (1.07; 95% CI: 0.90, 1.29). Similar to the findings from our first study, these findings also suggest that vomiting, particularly by LTCF residents, was a primary driver of norovirus transmission. These results support control measures that limit exposure to vomitus during HuNoV outbreaks in LTCFs.

*Aim 2: Measure dispersion of vomitus into the environment.* Nineteen studies were included in the scoping literature review to determine the role of PPE doffing in the spread of infectious agents. All studies reported some post-doffing contamination, most frequently observed on the hands, wrist, face, and neck. Reviewed studies used a variety of tracer contaminants, PPE ensembles, doffing protocols, tracer assessment locations, and methods, making it difficult to identify patterns across studies. In sum, the PPE doffing process was likely to play a role in body contamination. However, it was impossible to assess the key determinants of the magnitude and location of post-doffing contamination from the existing literature. Moreover, there is a need to improve and standardize experimental protocols to obtain generalizable information about the determinants of post-doffing contamination. In the experimental simulation study the design factors were found to not affect the dispersion meaningfully. The average extent of two-dimensional dispersion was 44.8 m<sup>2</sup>, spanning a width of 3.6 m and a length of 7.7 m from the source. These results confirm current recommendations for cleaning after vomiting.

*Aim 3. Determine the efficacy of broad-spectrum disinfection strategies on carpet.* In suspension tests, five disinfectants were shown to reduce FCV to >4 logs in 1 min, and two reduced *C. difficile* endospores to 6 logs in 10 min (Table 1). Hydrogen peroxide and ethanol were the main active ingredients in validated disinfectants. The efficacy of chlorine- and quaternary ammonium compound-based products was not confirmed in the suspension test, resulting in <3 log reduction of FCV and 1 log reduction of *C. difficile*. In a confirmatory test on stainless steel carriers, five disinfectants that showed efficacy in the suspension tests reduced >4 logs of FCV on stainless steel coupons in 5 min, but only one product with hydrogen peroxide and peracetic acid reduced >6 logs of *C. difficile* endospores in 10 min. Another with only hydrogen peroxide reduced 3.4 logs of *C. difficile* endospores on the carrier. For the carpet disinfection, only one hydrogen peroxide-based disinfectant was efficacious, showing a >5 log reduction of FCV and >4 logs of TuV on the carpet with waterproof backing in 30 min, but it was not efficacious on another carpet with water-permeable backing (Table 2). None achieved >6 log reduction of *C. difficile* endospores on carpets in 30 min, but a hydrogen peroxide-based disinfectant reduced 5.8 and 4.9 logs of *C. difficile* endospores on carpets with water-permeable and waterproof backings, respectively. Overall, carpet backing affected disinfection efficacy, and one hydrogen peroxide-based disinfectant was deemed a better alternative to bleach for carpet disinfection, though it changed carpet fiber properties and backings slightly after repeated application. Importantly, steam vapor was found to be efficacious against FCV and TuV on both carpets with a 15-s contact time, though it was only efficacious against *C. difficile* endospores on the nylon carpet with waterproof backing after 120 s (Table 3). Moreover, steam vapor did not significantly change carpet fiber properties and backings. On stainless steel carriers, photoClO<sub>2</sub> reduced >5.3 logs of FCV and 3.3 logs of TuV in 60 min as well as 0.5 logs of *C. difficile* endospores in 120 min (Table 4). In addition, under an indoor lighting condition, photoClO<sub>2</sub> inactivated 4.3 logs of FCV and 1.4 logs of TuV on stainless steel after 12 min. Besides, photoClO<sub>2</sub> achieved a 2.9 log- and

2.5 log-reduction of FCV and TuV on the nylon carpet with waterproof backings in 60 min, respectively---a higher efficacy than on the carpet with water-permeable backings (1.3- and 1.1-reduction, respectively).

*Aim 4. Determine norovirus persistence in the environment.* Results: During previous experiments, the CDC had shown that two hydrogen peroxide-based products (Virasept and OxivirTB) reduced the infectivity of both FCV ( $>5.1$  log) and TuV ( $\geq 2.4$  log) after 5 minutes of contact time on stainless steel carriers. In this study, the CDC confirmed that the Amicon column-based gel filtration method could recover 100% of infectious FCV and 60% of infectious TuV (Table 5). Specifically, Virasept inactivated the infectious titer of TuV by  $\geq 2.3$  and FCV by  $\geq 3.7$  log after 10 minutes of contact time on the Amicon filter membranes. It was also observed that 10 minutes of contact time did not result in complete inactivation of FCV and TuV for OxivirTB, which required longer contact time (30 minutes) (Table 6). Both disinfectants resulted in cytotoxicity on their host cells when tested undiluted (Supplementary Table). In summary, on porous surfaces, FCV and TuV were more resistant to the Virasept and OxivirTB disinfectants than they were on stainless steel coupons. Virasept was more efficacious against both viruses than OxivirTB was and should be considered to decontaminate soft surfaces, including carpets, although it may need longer contact times there compared with nonporous surfaces to completely inactivate infectious virus.

**Table 1.** Efficacy against feline calicivirus (FCV) and Tulane virus (TuV) on carpets with different backings <sup>a</sup>

Disinfectant	Active ingredient	Concentration Reduction <sup>b</sup>					
		TuV		FCV		<i>C. difficile</i>	
		Suspension	Steel	Suspension	Steel	Suspension	Steel
A	0.5 % Hydrogen peroxide	3.8±0.6 <sup>AB</sup>	0.8	5.1±0.6 <sup>A</sup>	>5.1 <sup>A</sup>	>6.0 <sup>A</sup>	3.4±0.4 <sup>B</sup>
B	0.88 % Hydrogen peroxide	3.4±0.7 <sup>AB</sup>	1.1	4.1±1.4 <sup>AB</sup>	>5.1 <sup>A</sup>	0.1±0.1 <sup>B</sup>	0.2±0.1 <sup>D</sup>
C	1.4 % Hydrogen peroxide	3.8±0.8 <sup>A</sup>	2.4	5.0±0.4 <sup>A</sup>	>5.1 <sup>A</sup>	1.4±0.1 <sup>C</sup>	1.0±0.3 <sup>CD</sup>
D	3.13 % Hydrogen peroxide/0.05 % Peracetic acid	3.9±0.5 <sup>A</sup>	>3.1	>5.4 <sup>A</sup>	>5.1 <sup>A</sup>	>6.0 <sup>A</sup>	>6.0 <sup>A</sup>
E	5 % Hydrogen peroxide/0.01 % Silver	2.5±0.0 <sup>B</sup>	0.8	2.7±0.2 <sup>B</sup>	2.2±0.7 <sup>C</sup>	1.3±0.2 <sup>C</sup>	1.3±0.1 <sup>C</sup>
F	Citric acid/Silver	0.2±0.2 <sup>C</sup>	1.0	2.2±0.2 <sup>B</sup>	>5.1 <sup>A</sup>	0.0±0.1 <sup>B</sup>	0.1±0.2 <sup>D</sup>
G	Chlorine dioxide/QACs/	1.8±0.8 <sup>B</sup>	0.9	0.3±0.1 <sup>C</sup>	0.2±0.2 <sup>B</sup>	0.4±0.3 <sup>B</sup>	0.4±0.3 <sup>D</sup>
H	QACs/Ethanol/Isopropanol	3.8±0.6 <sup>AB</sup>	1.0	1.9±1.3 <sup>B</sup>	>5.1 <sup>A</sup>	0.4±0.4 <sup>B</sup>	0.3±0.2 <sup>D</sup>
I	Ethyl and isopropyl alcohols	4.3±0.2 <sup>A</sup>	1.9	5.2±0.8 <sup>A</sup>	>5.1 <sup>A</sup>	0.0±0.1 <sup>B</sup>	0.1±0.1 <sup>D</sup>

<sup>a</sup> Contact time for FCV and TuV was 1 min in suspension and 5 min on stainless steel coupons, and the contact time for *C. difficile* endospores was 10 min in suspension and on stainless steel.

<sup>b</sup> The data are expressed as means ± standard deviations (SDs) from duplicates in each of two trials of experiments. Different letters (i.e., A and B) indicate significant differences ( $p < 0.05$ ) within the same columns.



**Table 2.** Efficacy of chemical disinfectants against feline calicivirus (FCV), Tulane virurs (TuV), and *C. difficile* endospore on carpets with different backings.<sup>a</sup>

Disinfectant	Reduction (logs) <sup>b</sup>					
	Color Accent® (water-permeable)			Highlight® (waterproof)		
	FCV	TuV	<i>C. difficile</i>	FCV	TuV	<i>C. difficile</i>
A	0.8±0.2	0.3±0.2	0.9±0.3	2.4±0.4	1.2±0.3	0.7±0.1
D	3.1±0.3	2.5±0.4	5.8±0.3	5.0±0.3	>4.0	4.9±0.5
Bleach	0.9±0.2	0.4±0.4	1.2±0.4	1.6±0.4	1.2±0.4	0.4±0.3

<sup>a</sup> The contact time was 30 min.

<sup>b</sup> The data are expressed as means ± standard deviations (SDs) from five replicates in each of two trials of experiments.

**Table 3.** Efficacy of steam vapor against FCV, TuV, and *C. difficile* endospore on nylon carpets with different backings.

Contact time	Reduction (logs) <sup>a</sup>					
	Color Accent® (water-permeable)			Highlight® (waterproof)		
	FCV	TuV	<i>C. difficile</i>	FCV	TuV	<i>C. difficile</i>
15 s	5.1±0.1	>3.0	n.a.	>5.4	>3.5	n.a.
30 s	>5.2	>3.0	3.4±0.1	>5.4	>3.5	2.5±0.1
60 s	>5.2	>3.0	4.0±0.1	>5.4	>3.5	3.6±0.2
120 s	n.a.	n.a.	4.9±0.6	n.a.	n.a.	6.1±0.1

<sup>a</sup> The data are expressed as means ± standard deviations (SDs) from five replicates in each of two trials of experiments. “n.a.” indicates data not available.

**Table 4.** Efficacy of photoClO<sub>2</sub> against FCV, TuV, and *C. difficile* endospore on different surfaces.

Microorganism	Surface	Lighting	Reduction (logs) <sup>a</sup>	
			60 min	120 min
FCV	Stainless steel	Controlled light	>5.3	n.a.
	Stainless steel	Indoor lighting	2.4±0.3	4.3±0.2
	Color Accent® (water-permeable)	Controlled light	1.3±0.3	n.a.
	Highlight® (waterproof)	Controlled light	2.9±0.5	n.a.
TuV	Stainless steel	Controlled light	3.3±0.3	n.a.
	Stainless steel	Indoor Lighting	0.8±0.1	1.4±0.1
	Color Accent® (water-permeable)	Controlled light	1.1±0.5	n.a.
	Highlight® (waterproof)	Controlled light	2.5±0.7	n.a.
<i>C. difficile</i>	Stainless steel	Controlled light	0.0±0.1	0.5±0.2
	Color Accent® (water-permeable)	Controlled light	n.a.	0.3±0.2
	Highlight® (waterproof)	Controlled light	n.a.	0.3±0.2

<sup>a</sup> The data are expressed as means ± standard deviations (SDs) from three replicates for stainless steel coupons and five replicates for carpet coupons in each of two trials of experiments. "n.a." indicates data not available.

**Table 5.** Viral recovery of Feline calicivirus (FCV) and Tulane virus (TuV) on Amicon column units

	FCV	TuV
Viral titers in initial inoculum (log <sub>10</sub> TCID <sub>50</sub> /mL)	3.6	5.0
Viral titers in column eluant (log <sub>10</sub> TCID <sub>50</sub> /mL)	3.6	4.8
Recovery (%)	100	60

**Table 6.** Disinfectant efficacy against Feline calicivirus (FCV) and Tulane virus (TuV)

<b>Mean reduction (log<sub>10</sub> TCID<sub>50</sub>/mL)<sup>1</sup></b>				
	<b>FCV</b>		<b>TuV</b>	
	10 min	30 min	10 min	30 min
Virasept	≥3.7	≥3.7	≥2.3	≥2.3
OxivirTB	3.4	≥3.7	2.0	≥2.3

<sup>1</sup> Reduction =  $-\log_{10}(CT/C_0)$ , where C<sub>0</sub> is virus titer from negative filter unit and CT is virus titer from test filter unit

**Supplementary Table.** Disinfection efficacy

	<b>Remaining infectious virus titers (log<sub>10</sub> TCID<sub>50</sub>/mL)</b>			
	<b>FCV</b>		<b>TuV</b>	
	10 min	30 min	10 min	30 min
Virasept	≥ 1.3, ≥1.3 <sup>1</sup>	≥1.3, ≥1.3	≥1.3, ≥1.3	≥1.3, ≥1.3
OxivirTB	1.6, 1.6	≥1.3, ≥1.3	1.6, 1.6	≥1.3, ≥1.3

\* Infectious titers of untreated viruses recovered by Amicon columns were 3.6 log<sub>10</sub> TCID<sub>50</sub>/mL for Tulane virus and 5.0 log<sub>10</sub> for Feline calicivirus and were used as basal levels.

<sup>1</sup> 1.3 log<sub>10</sub> TCID<sub>50</sub>/mL were the lowest titers to be measured because of the residual disinfectant induced cytotoxicity effect on cells.

## List of Publications and Products Use

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