APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2008, p. 3315–3318 Vol. 74, No. 10
0099-2240/08/$08.00+0 doi:10.1128/AEM.02148-07

Use of Murine Norovirus as a Surrogate To Evaluate Resistance of Human Norovirus to Disinfectants[▽]

Gaël Belliot,* Amandine Lavaux, Donya Souihel, Davide Agnello, and Pierre Pothier

Laboratoire de Virologie, Centre National de Référence des Virus Entériques, Centre Hospitalier Universitaire de Dijon, 7 boulevard Jeanne d'Arc, 21079 Dijon Cedex, France

Received 19 September 2007/Accepted 23 March 2008

Murine norovirus (MNV) was used as a surrogate to study resistance of human norovirus to disinfectants used in hospitals. MNV was sensitive to alcohol, alcohol hand rubs, bleach, and povidone iodine-based disinfectant. Real-time reverse transcription-PCR results indicated that the presence of viral RNA did not correlate with the presence of infectious virus.

Human noroviruses (NoVs) are causative agents of gastroenteritis in all age classes (14). Human NoV is responsible for large outbreaks in community settings like nursing homes or hospitals, in which nosocomial infections have also been reported (11, 15, 29). Person-to-person, food-borne, and waterborne transmissions of NoV have been documented (11). Previous studies showed that NoVs were resistant in the environment (7, 20) and suggested the risk of NoV transmission via contaminated surfaces (3, 13).

The study of human NoV has been hampered by the lack of a cell culture system, as reviewed previously (9). To study resistance of human NoV to environmental factors or virucidal agents, cultivatable feline calicivirus (FCV) from the genus *Vesivirus* has been used as a surrogate until now (27). FCV is sensitive to ethanol, 1-propanol, and isopropanol (12, 21). FCV is also inactivated in the presence of sodium hypochlorite, chlorine dioxide, iodine, or glutaraldehyde (6, 8, 10, 28, 30). Alcohol-based hand rubs induced 1 to 2 $\log_{10}$ 50%-tissue-culture-infective-dose reductions in the FCV titer in the presence of organic soil (16).

Recently the first murine NoV (MNV) was characterized (18) and adapted to cell culture on murine macrophage-related cells (31). The MNV capsid structure, genomic organization, and replication cycle are very similar to those of human NoVs (18, 26). Recently Cannon et al. showed that MNV and FCV were resistant to organic solvents and their inactivation rates were similar at 63 and 72°C (2). However, MNV was more resistant than FCV to basic and acidic pHs. Long-term resistance was also higher for MNV than for FCV at room temperature, once resuspended in stool material. Additionally, MNV could be effectively inactivated by liquid- and fog-based hypochlorous acid solutions on porous and nonporous surfaces (24). In this study, we determined whether alcohols (ethanol and isopropanol), alcohol-based hand rubs (Stérillium, Aniosgel 85NPC, and Purell), and commercial disinfectants (Asphène381, bleach, and Betadine), which are commonly used in French hospitals, could reduce the viral titer of MNV by 4 $\log_{10}$ as required by European standards for virucidal efficacy, as reviewed previously (27). Plaque assay and real-time reverse transcription (RT)-PCR procedures were evaluated for the detection and the quantification of MNV.

The RAW cells were maintained as described previously (31). MNV was propagated RAW cells in fetal bovine serum (FBS)-free Dulbecco's modified Eagle medium (DMEM) and harvested at 2 days postinfection. High-titer MNV stocks were prepared by ultracentrifugation of precleared MNV-infected cell lysate. For MNV titration, the cells were inoculated with 10-fold dilutions of MNV in DMEM containing 10% FBS (DMEM-FBS). A plaque assay was then performed as described previously (31). The alcohols and disinfectants were prepared in a final volume of 1 ml prior to adding 111 μl of MNV-infected cell lysate. The virucidal assays were performed in triplicate in the absence of interfering substances for 0.5, 1, and 3 min at room temperature. To overcome problems of cellular toxicity and stop the effect of the compounds on the MNV, a 111-μl aliquot of the assay was 10-fold diluted with DMEM-FBS at each time point (alcohol, alcohol-based hand rubs, and Asphène381). For chlorine- and iodine-based disinfectants, the compounds were reduced by adding sodium thiosulfate prior to titration. For the neutralization control, the compounds were first diluted with DMEM-FBS or reduced with sodium thiosulfate before addition of the virus. Finally, we set up a real-time RT-PCR to determine whether the MNV RNA genome is resistant to the disinfectants that produced a 4-$\log_{10}$ drop in the viral titer. The virucidal assays and the neutralization procedures have been described above, except for the alcoholic hand rubs, where DMEM without FBS was used for the dilution. The primers and probe are given in Table 1. The RT-PCR assays were performed with an ABI Prism 7000 sequence detection system (Applied Biosystems) according to the manufacturer's instructions. The copy number of the MNV genomic RNA was determined from a standard curve generated with serial dilutions of the full-length cDNA of the MNV strain CW1 (26).

Ethanol (Prolabo, Fontenay sous Bois, France) and isopropanol (Sigma-Aldrich, France) were diluted with sterile water to reach a final concentration of 60%, 30%, or 10% in the presence of the virus. Of note, the 60%-isopropanol prepara-

---

* Corresponding author. Mailing address: Laboratoire de Virologie, Centre National de Référence des Virus Entériques, Centre Hospitalier Universitaire de Dijon, 7 boulevard Jeanne d'Arc, 21079 Dijon Cedex, France. Phone: (33) 3-80-29-31-70. Fax: (33) 3-80-29-36-04. E-mail: gael.belliot@u-bourgogne.fr.

[▽] Published ahead of print on 31 March 2008.

TABLE 1. Primers and probe for real-time RT-PCR

Oligonucleotide	Sequence[a]	Polarity	Location[a]
MNV1-RT	5′-CCATATCCAACTCGA GGTTGGT-3′	−	4590–4611
MNV1-FW	5′-CCTGACATTGTGATG CAAGAATC-3′	+	4530–4552
MNV1-probe[b]	5′-CGTCATCACCATAGA AG-3′	−	4562–4578

[a] Oligonucleotide sequences and locations were based on the nucleotidic sequence of the MNV CW1 strain (GenBank accession number AY228235).

[b] The probe was linked to the fluorophor label 6-carboxyfluorescein and the quencher dye MGBNFQ at the 5′ and 3′ ends, respectively.

TABLE 2. Virucidal activities of ethanol and isopropanol for MNV

Concn of alcohol (%)	Time (min)	Reduction in titer[a] [$\log_{10}$ PFU/ml ± SD (% reduction)]	Reduction in titer [$\log_{10}$ copy no./ml ± SD (% reduction)]
Ethanol			
60	0.5	>4 (99.99)	1.22 ± 0.23 (93.97)
	1.0	>4 (99.99)	ND[b]
	3.0	>4 (99.99)	ND
30	0.5	0.11 ± 0.06 (22.38)	ND
	1.0	0.16 ± 0.20 (30.82)	ND
	3.0	0.29 ± 0.11 (48.71)	ND
10	0.5	None	ND
	1.0	None	ND
	3.0	None	ND
Isopropanol			
60	0.5	**3.86 (99.98)**	1.08 ± 0.57 (91.68)
	1.0	**>4 (99.99)**	ND
	3.0	**>4 (99.99)**	ND
30	0.5	0.63 ± 0.18 (76.56)	ND
	1.0	0.78 ± 0.08 (83.40)	ND
	3.0	1.62 ± 0.39 (97.60)	ND
10	0.5	None	ND
	1.0	0.08 ± 0.05 (16.82)	ND
	3.0	0.06 ± 0.02 (12.90)	ND

[a] High-titer stock of MNV was used for the control and the infectivity assays (bold case).

[b] ND, not determined.

tions were still cytotoxic at the dilution 100 and required the use of a high-titer stock of MNV to document a 4-$\log_{10}$ drop in the viral titer. Sixty percent ethanol and isopropanol showed 4- and 3.5-$\log_{10}$ reductions in the viral titer, respectively, within a 0.5-min exposure (Table 2). The efficacies of the two alcohols rapidly dropped at lower concentrations. Both alcohols are commonly used in nucleic acid purification procedures, and degradation of the nucleic acids was not expected. Indeed, the copy number of viral RNA was reduced only by around 1 $\log_{10}$ (Table 2) in the presence of alcohols. Aggregation of the virions might explain this reduction. The loss of infectivity coupled with the continued presence of genomic RNA suggests that protein alteration was the mechanism by which the MNV was inactivated.

Commercial preparations of hypochlorite-based bleach (Avix; Coldis, Morières-les-Avignon, France) and povidone iodine ("Betadine dermique"; Viatris, Merignac, France) were 10-fold diluted to obtain 0.26% active chlorine (36.4 mM sodium hypochlorite) and 1% (wt/vol) povidone iodine, respectively. The sodium hypochlorite and the povidone iodine were reduced by adding 100 μl and 20 μl of 1 M sodium thiosulfate (Sigma-Aldrich, France), respectively. The bleach preparation was then buffered with 100 μl of 1 M HEPES (pH 7.4) (Invitrogen, France) prior to titration. The tested concentrations of povidone iodine and sodium hypochlorite produced at least a 4-$\log_{10}$ drop of the MNV infectious titer within a 0.5-min exposure (Table 3). These data suggest that diluted chlorine- and iodine-based disinfectants effectively abolished MNV infectivity. However, the viral RNA was still detectable after the povidone iodine treatment, whereas it was not detected after chlorine treatment (Table 3).

Asphène381 (Laboratoires Anios, Lille-Hellemmes, France) is a surface disinfectant which is commonly used for cleaning contaminated surfaces. The disinfectant was tested at a concentration of 0.25% as recommended by the manufacturer. One volume of the compound was diluted in water at 0.5% (vol:vol) and mixed with an equal volume of MNV-infected cell lysate. At each time point, one aliquot was immediately diluted and titrated as described for the assay using alcohols. The infectious titer of the MNV was reduced by 1 $\log_{10}$ after a 3-min exposure. The compound was then tested for 15, 30, and 60 min, and a similar reduction of the viral titer was observed after a 1-h exposure (Table 3).

The alcohol-based hand rubs Sterillium gel (Bode Chemie, Hamburg, Germany), Purell hygienic hand rub (Gojo, Rueil-Malmaison, France), and Aniosgel 85 NPC (Laboratoires Anios, Lille-Hellemes, France) were tested pure. The addition of 1 volume of viral suspension in DMEM to 10 volumes of hydroalcoholic solution liquefied the gel, with the presence of a white precipitate. The three alcohol-based hand rubs were toxic for the cells at dilutions up to 100 (data not shown), and it was necessary to use a high-titer MNV to test Sterillium and Aniosgel 85NPC for the infectivity assay, as described above for isopropanol. Sterillium and Aniosgel 85NPC were able to produce at least a 4-$\log_{10}$ drop in the MNV titer after only a 0.5-min exposure (Table 4). The Purell hand rub was somewhat less effective, and a 60-min exposure was necessary to reduce the MNV viral titer by 4 $\log_{10}$. The detection of genomic RNA by real-time RT-PCR required the use of high-titer MNV stock and showed that the Sterillium and Purell hand rubs have minor effects on the viral RNA (Table 4). The data from the infectivity assays and the real-time RT-PCR suggested that alteration of the capsid was the mechanism by which the MNV became inactivated and that MNV genomic RNA or part of it was still present after the assay. We couldn't evaluate the effects of Aniosgel 85NPC because of the presence of strong PCR inhibitors (data not shown).

FCV has often been used as a surrogate to study human NoVs. However, vesiviruses are structurally different from NoV (4). Additionally, FCV and NoVs show respiratory and enteric tropisms, respectively, and these characteristics might explain the relative acid lability of FCV compared to human or murine NoVs (2, 5). For this study, MNV was used as a surrogate of human NoVs since both belong to the same genus and very likely share similar physical and chemical properties.

Person-to-person contact and contaminated surfaces are the cause of large NoV outbreaks of gastroenteritis. These observations underlined the need for good hygiene standards to prevent nosocomial outbreaks in clinical institutions (e.g., hos-

TABLE 3. Virucidal activities of diluted preparations of bleach, Betadine, and Asphène381 on MNV

Type of disinfectant	Active molecule[a]	Time (min)	Reduction in titer [$\log_{10}$ PFU/ml ± SD (% reduction)]	Reduction in titer [$\log_{10}$ copy no./ml ± SD (% reduction)]
Bleach[b]	Chlorine (0.26)	0.5	>4 (99.99)	>4 (99.99)
		1	>4 (99.99)	ND
		3	>4 (99.99)	ND
Betadine[b]	Povidone iodine (1)	0.5	>4 (99.99)	0.41 ± 0.51 (61.10)
		1	>4 (99.99)	ND
		3	>4 (99.99)	ND
Asphène 381	Quaternary ammonia, alkylamin, non-ionic detergent	0.5	0.75 ± 0.09 (82.22)	ND
		1	1.01 ± 0.01 (90.23)	ND
		3	0.96 ± 0.15 (89.04)	ND
		15	0.59 ± 0.09 (74.30)	ND
		30	0.35 ± 0.14 (55.33)	ND
		60	0.55 ± 0.13 (71.82)	ND

[a] Concentrations of active molecule in percentages are indicated in parentheses.

[b] Bleach and Betadine products were reduced by sodium thiosulfate.

pitals and nursing homes). In this study, we evaluated surface disinfectants (bleach and Asphène381) and hand cleaning products (alcohol-based hand rubs and Betadine), which are routinely used at the hospital. It is noteworthy that the disinfectants we tested were primarily selected by the hospital because they were well tolerated by the users (e.g., health care professionals and patients) (19) and because of their efficacy against microbial pathogens and enveloped viruses.

For example, alcohol-based hand rubs are widely used in hospitals. They are recommended by the French public health system for health care workers (25). Previous studies showed that hygienic hand rubs were very effective against enveloped viruses (17). In our study, we showed that two alcohol-based hand rubs could reduce the MNV titer by 4 $\log_{10}$. The efficacy of the third hand rub was somewhat lower. Additionally, a study conducted with volunteers showed that ethanol-based hand rubs with a higher alcohol concentration were the most effective against FCV (16). Certain compounds present in the gel could protect the virus from the alcohol, which may explain why a higher concentration of alcohol is required for an optimal virucidal activity as suggested previously (16). Chlorine-based disinfectants are among the most effective virucidal disinfectants. Previous studies showed that 1,000 to 3,000 ppm of chlorine was necessary to inactivate FCV (6, 8). Our data showed that MNV is also sensitive to 0.26% of active chlorine (2,600 ppm [wt/vol]). In our study, it was very likely that the MNV capsid was denatured by the chlorine, as shown previously for FCV (22). However, further studies will be required to determine whether the MNV capsid could withstand lower concentrations of chlorine and protect the genomic RNA. The incubation of the treated virions with proteinase K prior to RT-PCR, as described previously for FCV (23), or the transfection of viral RNA from treated virus (1) should help to determine whether viral RNA is still present in the sample.

TABLE 4. Virucidal activities of alcohol-based hand rubs for MNV

Type of disinfectant	Time (min)	Reduction in titer[a] [$\log_{10}$ PFU/ml ± SD (% reduction)]	Reduction in titer[b] [$\log_{10}$ copy no./ml ± SD (% reduction)]
Sterillium gel	0.5	>**4 (99.99)**	0.30 ± 0.18 (49.88)
	1	>**4 (99.99)**	ND
	3	>**4 (99.99)**	ND
Aniosgel 85NPC	0.5	>**4 (99.99)**	ND
	1	>**4 (99.99)**	ND
	3	>**4 (99.99)**	ND
Purell	0.5	1.85 ± 0.05 (98.59)	ND
	1	2.07 ± 0.11 (99.15)	ND
	3	2.89 ± 0.13 (99.87)	ND
	15	2.26 ± 0.09 (99.45)	ND
	30	2.87 ± 0.12 (99.87)	ND
	60	>4 (99.99)	0.63 ± 0.19 (76.56)

[a] High-titer stock of MNV was used for the control and infectivity assays (boldface type).

[b] High-titer stock of MNV was used for real time RT-PCR.

Finally, we performed real-time RT-PCR to assess whether the presence of viral RNA could be related to the presence of infectious particles after exposure to disinfectants. For noncultivatable human NoV, the presence of viral RNA determined by real-time RT-PCR is usually associated with a risk of infection. However, our data showed the MNV viral titer could be effectively reduced by the disinfectants and that the viral genome or a fragment of it was still detectable by RT-PCR (e.g., alcohols, povidone iodine preparations, and gel hand rubs), and thus, the detection of viral RNA could not be related to the presence of infectious virus during the virucidal assay. Of note, a similar conclusion was reached for MNV after exposure to heat (1). Our data suggest that MNV will be a useful tool to predict physical and chemical properties of human NoVs. Cultivatable MNV should help in assessing the risk of infection with human NoVs, which are present in food and in clinical and environmental samples.

This work was partly funded by the Institut National de Veille Sanitaire (InVS) and the Public Hospital of Dijon.

We thank Christiane Wobus and Herbert Virgin (Washington University, St. Louis, MO) for providing us with the MNV CW1 strain and the full-length construct of its genome. We thank Pia Villa (Mario Negri Institute, Milan, Italy) for providing us with the RAW cells. We extend our thanks to Jean-Baptiste Bour and Serge Aho (Public Hospital of Dijon, France) for helpful discussion and Philip Bastable (Université de Bourgogne, Dijon, France) for editorial assistance.

REFERENCES

1. **Baert, L., C. E. Wobus, E. Van Coillie, L. B. Thackray, J. Debevere, and M. Uyttendaele.** 2008. Detection of murine norovirus 1 by using plaque assay, transfection assay, and real-time reverse transcription-PCR before and after heat exposure. Appl. Environ. Microbiol. **74:**543–546.
2. **Cannon, J. L., E. Papafragkou, G. W. Park, J. Osborne, L. A. Jaykus, and J. Vinje.** 2006. Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. J. Food Prot. **69:**2761–2765.
3. **Cheesbrough, J. S., L. Barkess-Jones, and D. W. Brown.** 1997. Possible prolonged environmental survival of small round structured viruses. J. Hosp. Infect. **35:**325–326.
4. **Chen, R., J. D. Neill, J. S. Noel, A. M. Hutson, R. I. Glass, M. K. Estes, and B. V. Prasad.** 2004. Inter- and intragenus structural variations in caliciviruses and their functional implications. J. Virol. **78:**6469–6479.
5. **Dolin, R., N. R. Blacklow, H. DuPont, R. F. Buscho, R. G. Wyatt, J. A. Kasel, R. Hornick, and R. M. Chanock.** 1972. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. Proc. Soc. Exp. Biol. Med. **140:**578–583.
6. **Doultree, J. C., J. D. Druce, C. J. Birch, D. S. Bowden, and J. A. Marshall.** 1999. Inactivation of feline calicivirus, a Norwalk virus surrogate. J. Hosp. Infect. **41:**51–57.
7. **D'Souza, D. H., A. Sair, K. Williams, E. Papafragkou, J. Jean, C. Moore, and L. Jaykus.** 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. Int. J. Food Microbiol. **108:**84–91.
8. **Duizer, E., P. Bijkerk, B. Rockx, A. De Groot, F. Twisk, and M. Koopmans.** 2004. Inactivation of caliciviruses. Appl. Environ. Microbiol. **70:**4538–4543.
9. **Duizer, E., K. J. Schwab, F. H. Neill, R. L. Atmar, M. P. Koopmans, and M. K. Estes.** 2004. Laboratory efforts to cultivate noroviruses. J. Gen. Virol. **85:**79–87.
10. **Eleraky, N. Z., L. N. Potgieter, and M. A. Kennedy.** 2002. Virucidal efficacy of four new disinfectants. J. Am. Anim. Hosp. Assoc. **38:**231–234.
11. **Fankhauser, R. L., S. S. Monroe, J. S. Noel, C. D. Humphrey, J. S. Bresee, U. D. Parashar, T. Ando, and R. I. Glass.** 2002. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. J. Infect. Dis. **186:**1–7.
12. **Gehrke, C., J. Steinmann, and P. Goroncy-Bermes.** 2004. Inactivation of feline calicivirus, a surrogate of norovirus (formerly Norwalk-like viruses), by different types of alcohol in vitro and in vivo. J. Hosp. Infect. **56:**49–55.
13. **Green, J., P. A. Wright, C. I. Gallimore, O. Mitchell, P. Morgan-Capner, and D. W. Brown.** 1998. The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse-transcriptase polymerase chain reaction assay. J. Hosp. Infect. **39:**39–45.
14. **Green, K. Y.** 2007. *Caliciviridae*: the noroviruses, p. 949–980. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, 5th ed., vol. 1. Lippincott, Williams & Wilkins, Philadelphia, PA.
15. **Green, K. Y., G. Belliot, J. L. Taylor, J. Valdesuso, J. F. Lew, A. Z. Kapikian, and F. Y. Lin.** 2002. A predominant role for Norwalk-like viruses as agents of epidemic gastroenteritis in Maryland nursing homes for the elderly. J. Infect. Dis. **185:**133–146.
16. **Kampf, G., D. Grotheer, and J. Steinmann.** 2005. Efficacy of three ethanol-based hand rubs against feline calicivirus, a surrogate virus for norovirus. J. Hosp. Infect. **60:**144–149.
17. **Kampf, G., J. Steinmann, and H. Rabenau.** 2007. Suitability of vaccinia virus and bovine viral diarrhea virus (BVDV) for determining activities of three commonly-used alcohol-based hand rubs against enveloped viruses. BMC Infect. Dis. **7:**5.
18. **Karst, S. M., C. E. Wobus, M. Lay, J. Davidson, and H. W. Virgin IV.** 2003. STAT1-dependent innate immunity to a Norwalk-like virus. Science **299:**1575–1578.
19. **Labadie, J. C., G. Kampf, B. Lejeune, M. Exner, O. Cottron, R. Girard, M. Orlick, M. L. Goetz, J. C. Darbord, and A. Kramer.** 2002. Recommendations for surgical hand disinfection—requirements, implementation and need for research. A proposal by representatives of the SFHH, DGHM and DGKH for a European discussion. J. Hosp. Infect. **51:**312–315.
20. **Malik, Y. S., P. B. Allwood, C. W. Hedberg, and S. M. Goyal.** 2006. Disinfection of fabrics and carpets artificially contaminated with calicivirus: relevance in institutional and healthcare centres. J. Hosp. Infect. **63:**205–210.
21. **Malik, Y. S., S. Maherchandani, and S. M. Goyal.** 2006. Comparative efficacy of ethanol and isopropanol against feline calicivirus, a norovirus surrogate. Am. J. Infect Control **34:**31–35.
22. **Nuanualsuwan, S., and D. O. Cliver.** 2003. Capsid functions of inactivated human picornaviruses and feline calicivirus. Appl. Environ. Microbiol. **69:**350–357.
23. **Nuanualsuwan, S., and D. O. Cliver.** 2002. Pretreatment to avoid positive RT-PCR results with inactivated viruses. J. Virol. Methods **104:**217–225.
24. **Park, G. W., D. M. Boston, J. A. Kase, M. N. Sampson, and M. D. Sobsey.** 2007. Evaluation of liquid- and fog-based application of Sterilox hypochlorous acid solution for surface inactivation of human norovirus. Appl. Environ. Microbiol. **73:**4463–4468.
25. **Société Française d'Hygiène Hospitalière.** 2002. Recommandations pour la désinfection des mains. Collection hygiènes. Health & Co., Rillieux-Crepieux, France.
26. **Sosnovtsev, S. V., G. Belliot, K. O. Chang, V. G. Prikhodko, L. B. Thackray, C. E. Wobus, S. M. Karst, H. W. Virgin, and K. Y. Green.** 2006. Cleavage map and proteolytic processing of the murine norovirus nonstructural polyprotein in infected cells. J. Virol. **80:**7816–7831.
27. **Steinmann, J.** 2004. Surrogate viruses for testing virucidal efficacy of chemical disinfectants. J. Hosp. Infect **56**(Suppl. 2)**:**S49–S54.
28. **Thurston-Enriquez, J. A., C. N. Haas, J. Jacangelo, and C. P. Gerba.** 2005. Inactivation of enteric adenovirus and feline calicivirus by chlorine dioxide. Appl. Environ. Microbiol. **71:**3100–3105.
29. **Traore, O., G. Belliot, C. Mollat, H. Piloquet, C. Chamoux, H. Laveran, S. S. Monroe, and S. Billaudel.** 2000. RT-PCR identification and typing of astroviruses and Norwalk-like viruses in hospitalized patients with gastroenteritis: evidence of nosocomial infections. J. Clin. Virol. **17:**151–158.
30. **Urakami, H., K. Ikarashi, K. Okamoto, Y. Abe, T. Ikarashi, T. Kono, Y. Konagaya, and N. Tanaka.** 2007. Chlorine sensitivity of feline calicivirus, a norovirus surrogate. Appl. Environ. Microbiol. **73:**5679–5682.
31. **Wobus, C. E., S. M. Karst, L. B. Thackray, K. O. Chang, S. V. Sosnovtsev, G. Belliot, A. Krug, J. M. Mackenzie, K. Y. Green, and H. W. Virgin.** 2004. Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol. **2:**e432.