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Removal of Astrovirus from Water and Sewage Treatment Plants, Evaluated by a Competitive Reverse Transcription-PCR▽

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Quantification of human astrovirus genogroups A and B was undertaken with sewage and water samples, collected from the Greater Cairo area in Egypt from November 1998 to October 1999, by a competitive reverse transcription (RT)-PCR with an internal control. The number of RNA copies of genogroup A/liter in quantifiable samples ranged from 3.4×10^3 to 5.6×10^6 in raw sewage and from 3.4×10^3 to 1.1×10^4 in treated effluents, while the number of infectious units per liter in these samples as determined by cell culture RT-PCR (CC-RT-PCR U/liter) ranged from 3.3×10^1 to 3.3×10^3 in raw sewage and was 3.3×10^0 in treated effluents. On the other hand, the number of RNA copies/liter in quantifiable genogroup B samples ranged from 1.1×10^4 to 8.7×10^6 in raw sewage and from 1.1×10^3 to 6.2×10^5 in treated effluents, while the number of infectious units ranged from 3.3×10^1 to 3.3×10^5 CC-RT-PCR U/liter in raw sewage and from 3.3×10^1 to 3.3×10^2 CC-RT-PCR U/liter in treated effluents. These higher numbers of both RNA copies/liter and infectious particles of genogroup B may indicate the emergence of genogroup B in the area. Additionally, genogroup B astrovirus exhibited a higher resistance to removal treatments with regard to the number of RNA copies per ml. When the equipment for real-time approaches is unavailable, a competitive PCR or RT-PCR with an internal control may be employed for virus quantification in validations of the efficiency of virus removal treatments.

The World Health Organization (WHO) website (http://www.who.int/water_sanitation_health/diseases/en/index.html) reports that 1.8 million people, 90% of whom are children younger than 5 years old, die each year from gastroenteritis diseases. Nearly 90% of diarrheal infections are waterborne or water related, and improved sanitation may reduce diarrhea morbidity by 37.5%.

Among the viral agents responsible for gastroenteritis are human astroviruses (HAstV), which were originally described in 1975 in association with outbreaks of gastroenteritis in newborns (11) and which constitute a family of nonenveloped, positive single-stranded RNA viruses, the *Astroviridae* (13). Astrovirus infections occur worldwide and are most frequent in young children, although illness rates increase again in the elderly (12). Astroviruses are transmitted by the fecal-oral route, and outbreaks have been associated with consumption of sewage-polluted water and food (16, 17).

Phylogenetic analyses based on the well-conserved partial sequence close to the protease motif coding region of astrovirus results in two clearly differentiated genogroups, genogroup A (HAstV-1 to HAstV-5 and HAstV-8) and genogroup B (HAstV-6 and HAstV-7) (3). Data on the occurrence and epidemiology of astrovirus genogroup B are scarce.

Nucleic acid amplification-based techniques are a major step forward in virus monitoring in water samples, in particular when fastidious viruses are the target for detection. Real-time PCR approaches enable not only qualitative determination but also, and particularly, quantitative diagnostic assays. The possibility of quantifying virus agents by PCR represents a seminal refinement in monitoring virology, since it enables the determination of removal efficiencies for nonculturable viruses. However, real-time equipment may not be available in some circumstances. In this study, an internal control is employed to quantify RNA copies of astrovirus in sewage and water samples using both competitive reverse transcription-PCR (RT-PCR) and competitive multiplex RT-PCR. Infectious astrovirus was quantified in parallel in the same samples using an integrated cell culture RT-PCR (CC-RT-PCR) procedure.

MATERIALS AND METHODS

Samples. Three liters of both raw sewage and treated effluent samples were collected monthly over a 1-year period (November 1998 to October 1999) from three sewage treatment plants (Balaks, El-Berka, and Zenin in Cairo, Egypt). Sewage treatment at Balaks consisted only of primary sedimentation, while an activated sludge treatment was performed at El-Berka and Zenin. A final chlorination step (10 mg/liter) was performed year round at El-Berka, while chlorination at Zenin (0.5 mg/liter) was performed from May 1999 to October 1999.

Forty liters of both Nile water and final drinking water samples were collected quarterly over the same year (December 1998 to September 1999) from three drinking water treatment plants (El-Giza, El-Maadi, and Mostorod). Treatment consisted of prechlorination, coagulation, sedimentation, rapid sand filtration, and final chlorination.

All drinking water and wastewater treatment plants are inside the Greater Cairo area.

Concentration of samples. Sewage samples were concentrated by filtration through nitrocellulose membranes (0.45-μm pore size and 142-mm diameter; Schleicher & Schuell) and elution with 75 ml of 0.05 M glycine buffer, pH 9.5, containing 3% beef extract (19, 21). Water samples were concentrated by direct filtration through 1-MDS filters (Cuno) without preconditioning (22). Adsorbed viruses were eluted with 1 liter of 0.05 M glycine buffer, pH 9.5, containing 3% beef extract (20). All samples were reconcentrated by organic flocculation (8).

Viral nucleic acid extraction. Viral nucleic acids were extracted from 50-μl sample concentrates by guanidine thiocyanate lysis, adsorption to silica particles, and elution with an aqueous low-salt buffer (4).

Viruses and cells. A cell-adapted strain (p23795) of human astrovirus serotype 4 (kindly provided by W. D. Cubitt, Great Ormond Street Hospital for Children,

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TABLE 1. Numbers of RNA copies and numbers of infectious particles per liter in raw sewage and treated effluent samples positive for astrovirus genogroup A[a]

Sample location, date	No. of RNA copies/liter of raw sewage	CC-RT-PCR U/liter of raw sewage[b]	No. of RNA copies/liter of treated effluent	CC-RT-PCR U/liter of treated effluent
Balaks, Dec. 1998	2.4×10^5	3.3×10^1	1.1×10^4	3.3×10^0
Balaks, Jan. 1999	2.3×10^6	3.3×10^1	3.4×10^3	3.3×10^0
Balaks, May 1999	1.1×10^4	$<3.3 \times 10^0$	NQ	$<3.3 \times 10^0$
Zenin, Nov. 1998	5.6×10^6	3.3×10^3	NQ	$<3.3 \times 10^0$
Zenin, Dec. 1998	1.1×10^5	3.3×10^2	NQ	$<3.3 \times 10^0$
Zenin, Jan. 1999	3.4×10^3	3.3×10^1	NQ	$<3.3 \times 10^0$
Zenin, Oct. 1999	NQ	3.3×10^1	NQ	$<3.3 \times 10^0$

[a] Reported values were determined by taking into consideration that 3-liter samples were concentrated to a final volume of 1 ml (3,000× concentration factor). NQ, nonquantifiable; the sample was positive only after Southern blot hybridization of the RT-PCR product.
[b] The reciprocal endpoint dilution detected by CC-RT-PCR.

London, United Kingdom) was propagated in CaCo-2 cells, as previously described (18). Human astrovirus serotype 6 prototype strain was kindly provided by M. Koopmans (RIVM, The Netherlands).

Molecular quantification of the astrovirus genogroup A genomes by a competitive RT-PCR with an internal control. Samples were screened for HAstV by RT-PCR and Southern blot hybridization using previously published A1 and A2 primers specific for genogroup A (7). Positive samples showing RT-PCR product bands in agarose gels were quantified by a competitive RT-PCR with primers A1/A2 in the presence of an internal RNA standard (internal control [IC]). This IC is a 511-mer RNA molecule containing a region complementary to the A2 primer and, at a distance of 135 nucleotides, a region identical to the A1 primer, thus establishing competitive conditions (5). The 135-bp IC amplimer is easily distinguishable from the viral amplimers, whose lengths range from 192 to 237 bp, depending on the strain (7). The sensitivity of detection for the RNA IC in this RT-PCR, in the absence of viral RNA, was 17 molecules per reaction.

The competitive RT-PCR was performed in the same way as the standard RT-PCR (7), but a known number of molecules of the RNA IC was added to each reaction tube. Samples with an abundant PCR product in the HAstV screening RT-PCR were quantified using serial sample dilutions, each of which received a fixed number of molecules of the RNA IC. In contrast, samples with a scarce PCR product were quantified using serial dilutions of the RNA IC mixed with the undiluted sample. The detection limit for this competitive RT-PCR is 3.4×10^3 RNA copies/ml. The number of HAstV contained in a given sample was estimated from the dilution where the viral PCR amplimer (192- to 237-bp length) showed fluorescence intensity comparable to that of the IC PCR product (135-bp length). In this way, it may be assumed that, for a given dilution, there are approximately equal numbers of molecules of the RNA IC and of the viral RNA, which enables the estimation of the number of genome copies for a given volume.

Quantification of HAstV genogroup B genome copies by a competitive multiplex RT-PCR with an IC. Samples were screened for HAstV genogroup B by RT-PCR with the specific primers A1bis (5′-CCTGCCCCCCGTATAATTAAA C-3′) and A2bis (5′-ATAGGACTCCCATATAGGTGC-3′). HAstV quantification in the positive samples showing RT-PCR product bands in agarose gels was performed by a competitive RT-PCR with primers A1 and A1bis and a newly designed primer, A2 internal (5′-CCATACGTTTGTTTGTGAGTATGG-3′). Primers A1 and A2 internal were used for the amplification of the RNA IC with a predicted product size of 110 bp, while primers A1bis and A2 internal were used for the amplification of a genogroup B, 167-bp genome fragment. Primer A2 internal was used for the RT, and a known number of molecules of the RNA IC were added to each tube. Samples with an abundant PCR product in the previous screening were quantified using serial sample dilutions, each of which received a fixed number of RNA IC molecules. In contrast, samples with a faint PCR product were quantified using serial dilutions of the RNA IC mixed with the undiluted samples. All primers—A1, A1bis, and A2 internal—were added to the PCR mix containing 5 μl of Expand buffer (Roche), 2 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.5 μM of each primer, and 0.525 U of the Expand enzyme (Roche). After a 3-min denaturation step at 95°C, 40 cycles of amplification at 94°C for 30 s, 50°C for 1 min, and 72°C for 30 s were performed, with a final extension of 7 min at 72°C. The sensitivity of detection of the RNA IC in this RT-PCR, in the absence of viral RNA, was found to be 17 molecules per reaction, the same sensitivity as the RT-PCR with primers A1/A2. The set of primers employed for astrovirus quantification in samples positive for HAstV genogroup B amplifies the numbers of astroviruses of both genogroups A and B. In consequence, to determine the actual numbers of HAstV copies of genogroup B in samples that also contained HAstV genogroup A, the number of genome copies estimated with primers A1/A2 must be subtracted from the number of genome copies estimated with primers A1, A1bis, and A2 internal.

Quantification of infectious astrovirus. Infectious HAstV copy numbers were calculated as numbers of cell culture RT-PCR units by following a previously described procedure (1) based on combined infection of cultured CaCo-2 cell monolayers and RT-PCR with sets of primers A1/A2 and A1bis/A2bis for genogroups A and B, respectively. An RT-PCR unit is defined as the reciprocal endpoint dilution detectable by CC-RT-PCR. The detection limit of this assay is 1×10^1 CC-RT-PCR U/ml. Prior to cell monolayer infection, samples were decontaminated with chloroform as described elsewhere (2).

RESULTS

Eight out of 35 (23%) raw sewage samples were positive for HAstV genogroup A by RT-PCR and Southern blot hybridization, while 16 of these samples were positive for HAstV genogroup B (46%). After treatment, 2 (6%) and 10 (29%) treated effluent samples were positive for HAstV genogroups A and B, respectively. With regard to drinking water, 1 out of 12 untreated Nile water samples (8%; Mostorod, June 1999) and 1 out of 12 treated water samples (8%; Mostorod, June 1999) were positive for genogroup B HAstV. These higher percentages of samples positive for genogroup B also correlated with higher numbers of RNA copies/ml (Tables 1 and 2). With regard to infectious virus, numbers were also higher for genogroup B, mainly in raw sewage.

In the two positive drinking water samples (Mostorod, June 1999; before and after treatment), the same number of 2.3×10^3 RNA copies/liter was detected; however, the Nile water sample contained 1.2×10^0 CC-RT-PCR U/liter, while no infectivity was detected in the drinking water sample (data not shown).

In an attempt to elucidate whether genogroups A and B HAstV exhibited differential resistance to the virus removal treatments applied to sewage, log reductions were estimated for each genogroup and treatment (Table 3). While no differences could be observed at the infectivity level, significant ($P < 0.05$) differences were seen in numbers of RNA copies, with HAstV genogroup B being more persistent than genogroup A. As expected, activated sludge treatment and/or chlorination in combination with primary sedimentation were more effective for HAstV removal, irrespective of the genogroup, than primary sedimentation alone.

TABLE 2. Numbers of RNA copies and numbers of infectious particles per liter in raw sewage and treated effluent samples positive for astrovirus genogroup B[a]

Sample location, date	No. of RNA copies/liter of raw sewage[b]	CC-RT-PCR U/liter of raw sewage[c]	No. of RNA copies/liter of treated effluent	CC-RT-PCR U/liter of treated effluent
Balaks, Nov. 1998	3.8×10^5	3.3×10^3	9.9×10^4	3.3×10^2
Balaks, Jan. 1999	8.7×10^6	3.3×10^3	1.1×10^5	3.3×10^2
Balaks, May 1999	8.7×10^5	3.3×10^2	6.2×10^5	3.3×10^1
El-Berka, Nov. 1998	1.1×10^5	3.3×10^3	1.1×10^3	$<3.3 \times 10^0$
El-Berka, July 1999	1.1×10^4	$<3.3 \times 10^0$	NQ	$<3.3 \times 10^0$
El-Berka, Aug. 1999	1.1×10^4	$<3.3 \times 10^0$	NQ	$<3.3 \times 10^0$
El-Berka, Sept. 1999	1.1×10^5	3.3×10^2	NQ	$<3.3 \times 10^0$
Zenin, Nov. 1998	9.9×10^5	3.3×10^3	1.1×10^3	$<3.3 \times 10^0$
Zenin, Dec. 1998	5.4×10^6	3.3×10^5	NQ	$<3.3 \times 10^0$
Zenin, Jan. 1999	6.2×10^5	3.3×10^2	1.1×10^4	$<3.3 \times 10^0$
Zenin, Feb. 1999	3.7×10^5	3.3×10^2	1.1×10^4	$<3.3 \times 10^0$
Zenin, Apr. 1999	1.1×10^5	3.3×10^1	NQ	$<3.3 \times 10^0$
Zenin, May 1999	1.1×10^5	3.3×10^1	NQ	$<3.3 \times 10^0$
Zenin, June 1999	1.1×10^4	$<3.3 \times 10^0$	NQ	$<3.3 \times 10^0$
Zenin, July 1999	1.1×10^4	$<3.3 \times 10^0$	NQ	$<3.3 \times 10^0$
Zenin, Oct. 1999	1.1×10^5	3.3×10^2	1.1×10^3	$<3.3 \times 10^0$

[a] Reported values were determined by taking into consideration that 3-liter samples were concentrated to a final volume of 1 ml (3,000× concentration factor). NQ, nonquantifiable; the sample was positive only after Southern blot hybridization of the RT-PCR product.
[b] The actual numbers of HAstV genogroup B copies in samples that also contained HAstV genogroup A were obtained by subtracting the number of genome copies estimated with primers A1/A2 (Table 1) from the numbers of genome copies estimated with primers A1, A1bis, and A2 internal.
[c] The reciprocal endpoint dilution detected by CC-RT-PCR.

DISCUSSION

Methods for virus quantification are required to evaluate the efficiencies of treatments for virus removal in wastewater and drinking water treatment plants. In the present study, a competitive RT-PCR using an RNA IC was employed to quantify HAstV copy numbers in sewage and water samples, before and after treatment, from Greater Cairo. In this way, HAstV genogroups A and B were quantified in raw and treated wastewater and in river and drinking water samples that were previously screened for genogroup A and B HAstV. Furthermore, the addition of the RNA IC in the RT mix enabled dismissal of the possibility of the presence in RT–PCR-negative samples of substances interfering with the enzymes when the IC amplimer was visualized.

In a previous study with sewage samples collected from treatment plants during 1995 in France (9), the one sample from which enteroviruses could be quantified had a concentration of about 10^3 viral copies/ml. In this French study, 400 copies of the IC were introduced into the RT mix, and the sensitivity threshold was 300 viral copies/ml of sample. Consequently, it could be assumed that the rest of the samples may have contained a number of copies of RNA below the sensitivity threshold. However, although the detection limit in our competitive RT-PCR is 3.4×10^3 viral RNA copies/ml, we could quantify HAstV in most sewage samples which were positive by our standard RT-PCR and also in the two positive water samples, which provides an indication that high numbers of RNA copies were present in sewage and water in the Egyptian samples in comparison with the French sewage samples analyzed for enteroviruses.

TABLE 3. Mean levels of differential astrovirus genogroup removal ($\log_{10}$) by different treatments over the study period[a]

Genogroup	Primary sedimentation		Primary sedimentation activated sludge		Primary sedimentation activated sludge chlorination	
	No. of RNA copies	CC-RT-PCR U	No. of RNA copies	CC-RT-PCR U	No. of RNA copies	CC-RT-PCR U
A	2.7 ± 1.3	1.0 ± 0.0	5.1 ± 1.6	2.5 ± 1.0	NA	NA
B	0.9 ± 0.9	1.0 ± 0.0	3.6 ± 2.2	2.5 ± 0.8	3.8 ± 1.2	2.5 ± 0.8

[a] Values are means ± standard deviations. NA, not available.

High numbers of RNA copies/liter were observed at the end of autumn and during the winter months and tended to decrease as temperatures rose. The lowest numbers of RNA copies/liter were observed in the summer months. These data on HAstV occurrence in environmental samples are in concordance with reports on the seasonality observed for HAstV infections among the population (7).

Interestingly, the numbers of both RNA copies/liter and infectious particles of genogroup B, which includes serotypes 6 and 7, are higher than the numbers of RNA copies/liter and infectious particles of genogroup A, which includes serotypes 1, 2, 3, 4, 5, and 8. In a previous study in Egypt using clinical samples, HAstV-1 appeared to be the most common genotype, followed by HAstV-5, HAstV-6, and HAstV-7 (14). The high number of RNA copies and infectious units of genogroup B detected in the present study may indicate the emergence of new HAstV strains. HAstV genogroup B-positive samples were further analyzed in the capsid region (15) and were revealed to contain serotype 6 strains (data not shown).

The 4-log reduction recommended by the USEPA (23) as a result of water treatment could never be observed after primary sedimentation alone. When activated sludge treatment was added, a 4-log reduction could be documented for genogroup A but not for genogroup B. This difference, however, was evident only in the number of RNA copies and not for infectivity. This finding could reflect that molecular quantification alone may fail to indicate the actual threat posed by the detected virus, i.e., its infectivity. Additional evidence that mo-

lecular quantification does not provide sufficient indication of this threat is provided by the Nile water sample and the corresponding treated drinking water sample, which showed the same number of genome copies (2.3×10^3 copies/liter), although infectious virus could be detected only in the untreated sample. Thus, residual amplifiable RNA can be detected in the absence of infectivity. Nevertheless, for viruses that grow poorly or not at all in cell cultures, only molecular assays can be applied. However, although HAstV may replicate in CaCo-2 cells, different RNA replication patterns are observed between isolates, both in vivo (5) and in vitro (6), leading to strain-specific sensitivities in an assay based on an integrated cell culture-molecular analysis procedure. Considering the evidence and the complex mixture of naturally occurring HAstV strains in sewage, we believe that the best estimation of the efficiency of astrovirus removal is provided by RNA copy decay.

Our approach to quantify HAstV through a competitive RT-PCR with an RNA IC can be employed for the quantification of other health-significant enteric viruses that may not be quantifiable by infectivity assays. Obviously, when quantification is pursued, the gold standard is real-time PCR. However, the equipment for this type of assay may not always be available in some parts of the world; in such cases, the procedure described in this work may be useful to quantify viruses and to evaluate virus removal through water treatments.

Although the Milwaukee cryptosporidiosis outbreak (10) reminded us that waterborne diseases may also occur in developed countries, the burden of waterborne illnesses is more severe in developing societies. The procedures described in this work will enable valuable determinations of the level of environmental virus contamination in developing community scenarios.

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REFERENCES

1. **Abad, F. X., R. M. Pintó, C. Villena, R. Gajardo, and A. Bosch.** 1997. Astrovirus survival in drinking water. Appl. Environ. Microbiol. **63:**3119–3122.
2. **American Public Health Association.** 1989. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, DC.
3. **Belliot, G., H. Laveran, and S. S. Monroe.** 1997. Detection and genetic differentiation of human astroviruses: phylogenetic grouping varies by coding region. Arch. Virol. **142:**1323–1334.
4. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
5. **Caballero, S., S. Guix, W. M. El-Senousy, I. Calico, R. M. Pintó, and A. Bosch.** 2003. Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains. J. Med. Virol. **71:**245–250.
6. **Guix, S., S. Caballero, A. Bosch, and R. M. Pintó.** 2005. Human astrovirus C-terminal nsP1a protein is involved in RNA replication. Virology **333:**124–131.
7. **Guix, S., S. Caballero, C. Villena, R. Bartolomé, C. Latorre, N. Rabella, M. Simó, A. Bosch, and R. M. Pintó.** 2002. Molecular epidemiology of astrovirus infection in Barcelona, Spain. J. Clin. Microbiol. **40:**133–139.
8. **Katzenelson, E., B. Fattal, and T. Hostovesky.** 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. **32:**638–639.
9. **Le Guyader, F., D. Menard, E. Dubois, L. Haugarreau, H. Kopecka, and M. Pommepuy.** 1997. Use of an RT-PCR internal control to evaluate viral removal. Water Sci. Technol. **35:**461–465.
10. **Mac Kenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierczak, D. G. Addiss, K. R. Fox, J. B. Rose, and J. P. Davis.** 1994. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. N. Engl. J. Med. **331:**161–167.
11. **Madeley, C. R., and B. P. Cosgrove.** 1975. 28 nm particles in faeces in infantile gastroenteritis. Lancet **ii:**451–452.
12. **Matsui, S. M., and H. B. Greenberg.** 1996. Astroviruses, p. 811–824. *In* B. N. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman, and S. E. Straus (ed.), Fields virology, 3rd ed. Lippincott-Raven, Philadelphia, PA.
13. **Monroe, S. S., M. J. Carter, J. Herrmann, D. K. Mitchel, and A. Sanchez-Fauquier.** 2005. Family Astroviridue, p. 859–864. *In* C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy. 8th Report of the International Committee on Taxonomy of Viruses. Elsevier, Amsterdam, The Netherlands.
14. **Naficy, A. B., M. R. Rao, J. L. Holmes, R. Abu-Elyazeed, S. J. Savarino, T. F. Wierzba, R. W. Frenck, S. S. Monroe, R. I. Glass, and J. D. Clemens.** 2000. Astrovirus diarrhea in Egyptian children. J. Infect. Dis. **182:**685–690.
15. **Noel, J. S., T. W. Lee, J. B. Kurtz, R. I. Glass, and S. S. Monroe.** 1995. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. J. Clin. Microbiol. **33:**797–801.
16. **Oishi, I., K. Yamazaki, T. Kimoto, Y. Minekawa, E. Utagawa, S. Yamazaki, S. Inouye, G. S. Grohmann, S. S. Monroe, S. E. Stine, C. Carcamo, T. Ando, and R. I. Glass.** 1994. A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. J. Infect. Dis. **170:**439–443.
17. **Pintó, R. M., F. X. Abad, R. Gajardo, and A. Bosch.** 1996. Detection of infectious astroviruses in water. Appl. Environ. Microbiol. **62:**1811–1813.
18. **Pintó, R. M., J. M. Diez, and A. Bosch.** 1994. Use of the colonic carcinoma cell line CaCo-2 for in vivo amplification and detection of enteric viruses. J. Med. Virol. **44:**310–315.
19. **Rose, J. B., S. N. Singh, C. P. Gerba, and L. M. Kelley.** 1984. Comparison of microporous filters for concentration of viruses from wastewater. Appl. Environ. Microbiol. **47:**989–992.
20. **Schwab, K. J., R. De Leon, and M. D. Sobsey.** 1996. Immunoaffinity concentration and purification of waterborne enteric viruses for detection by reverse transcriptase PCR. Appl. Environ. Microbiol. **62:**2086–2094.
21. **Smith, E. M., and C. P. Gerba.** 1982. Development of a method for detection of human rotavirus in water and sewage. Appl. Environ. Microbiol. **43:**1440–1450.
22. **Sobsey, M. D., S. E. Oglesbee, and D. A. Wait.** 1985. Evaluation of methods for concentrating hepatitis A virus from drinking water. Appl. Environ. Microbiol. **50:**1457–1463.
23. **U.S. Environmental Protection Agency.** 1989. Guidance manual for compliance with the filtration and disinfection requirements for public water systems using surface water sources. Office of Water, U.S. Environmental Protection Agency, Washington, DC.