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# Enteric Viruses And Aquatic Environment

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## Abstract

Enteric viruses are mostly found in a variety of aquatic reservoirs including ground water, sewage water, streams, rivers and marine water. They are associated with health risks and responsible for variety of infections in humans. Enteric viruses existence varied greatly in the aquatic environment and also depends upon human activities. Traditional water quality indicators like bacterial coliforms provide no information regarding enteric viruses load in different types of water samples. Environmental water samples are analyzed by a variety of analytical methods for the detection and monitoring of enteric viruses. The virus particles in environment samples are concentrated by adsorption/elution protocols, ultrafiltration or ultra-centrifugation prior to isolation or detection. In spite of the limitations for each method traditional cell culture infectivity assay and molecular biology tools like nucleic acid amplification, microarray based assay or combination of cell culture and polymerase chain reaction are used as valuable informative tool to detect enteric viruses in aquatic environment.

## INTRODUCTION

Water borne diseases have great socioeconomic impacts on public health all around the world. Natural water is a complex mixtures of inorganic, organic compounds and macro-organisms. Swage water comprises of different substance originates from industrial, commercial and residential activities. It also carries different chemicals toxic wastes and biological infectious agents of fungal, bacterial and viral origins. Enteric viral pathogens presence in waste water is important with public health perspective(Bosch, 1998). Enteric viruses commonly cause gastroenteritis and mostly transmitted through the fecaloral route. They include different group of viruses present in human intestinal tract and responsible for different illnesses like fever, meningitis, hepatitis, rashes and respiratory diseases. They are divided in to a number of different families including Picornaviridae (polioviruses, coxsackieviruses, echoviruses, enteroviruses and hepatitis A virus), Adenoviridae (adenoviruses), Reoviridae (reoviruses and rotaviruses), and Caliciviridae (noroviruses, caliciviruses, and astroviruses) (Wilhelmi et al., 2003).

Although some enteric viruses have been found in the aquatic environments there is still a lack of comprehensive indicator system for monitoring of these organisms (Wyn Jones et al., 2011). These viruses have the ability to survive for a longer time (130) days and remain infectious at lower doses. They are discharged continuously to environment from their hosts (Fong and Lipp, 2005).The monitoring of enteric viruses in different aquatic environments would be

helpful to assess the risk of human exposures. In aquatic environment the quantification of these viruses is difficult technically, requires a long time and is expensive. In fact, the viruses of primary concern are difficult or impossible to quantify. Therefore, many public agencies rely on bacterial indicator organisms to establish a quality However, they are not correlated with the occurrence of enteric viruses (Parkin et al., 2003). The main problems with bacterial indicators are, they do not reflect the presence for other pathogens, such as viruses and parasites, they do not depict the fact that many pathogens reproduce in the environment after being excreted by their host (Fong and Lipp, 2005). In some cases, where assays have been within the limits recommended for bacterial indicators in water, human enteric viruses have been found at dangerously high concentrations. Current microbial indicators (e.g. enterococci, fecal streptococci and fecal coliform bacteria) simply do not give information on the viral quality of water (Ehlers et al., 2005). Other studies suggest the use of alternative indicators of viral pathogens, such as bacteriophages, (Ogorzaly et al., 2009), or adenoviruses (Wyn Jones et al., 2011). But still there is no consensus on the best indicator for viral pathogens in the environment. Different types of enteric viruses and diseases caused by are grouped in (Table 1).

**Table 1**

Human enteric viruses

Family	Example	Genome	Envelope	Diseases
Adenoviridae	Adenoviruses	ds DNA linear	No	Respiratory diseases conjunctivitis gastroenteritis
Caliciviridae	Hepatitis E Human calicivirus Norwalk virus noroviruses	SS RNA(+) nonsegmented	No	Gastroenteritis Hepatitis Respiratory disease
Picornaviridae	Poliovirus Coxsackievirus A,B Echovirus Hepatitis A	SS RNA(+) nonsegmented	No	Paralysis, meningitis, fever, herpangina, respiratory diseases, Hand-foot and mouth disease, rash, myocarditis, gastroenteritis
Reoviridae	Reovirus Totavirus	Ds RNA Segmented	No	Gastroenteritis
Astroviridae	Astrovirus	SS RNA(+) nonsegmented	No	Gastroenteritis
Coronaviridae	Coronaviruses Torovirus	SS RNA(+) nonsegmented	Yes	Gastroenteritis respiratory diseases
Parvoviridae	Parvoviruses	SS DNA linear	No	Gastroenteritis
Circoviridae	Torgue tenovirus Torgus tenovirus-like virus	SS DNA circular	No	Respiratory diseases Liver diseases
Picobirnaviridae	Picobirnavirus	ds RNA segmented	No	Gastroenteritis
Polyomaviridae	JC virus BK virus Simian virus 40	ds DNA circular	No	Leukoencephalopathy Hemorrhagic cvstitis

Human enteroviruses are members of the family Picornaviridae, which consist of non-enveloped virus particles containing a single-stranded positive sense RNA genome protected by an icosahedral capsid (Okoh et al., 2010). Enteroviruses can be transmitted by the fecal–oral route from any surface or ground water such as estuarine water, seawater, rivers, springs and drinking water that directly or indirectly receives treated or untreated wastewater (Chen et al., 2007; Vivier et al., 2004). Polioviruses are transmitted via faecal-oral route. Thus raising some concerns not to underestimate the infection risk by exposure to the viruses in water more so in rural communities which use sewage polluted river water for domestic purposes (Pavlov et al., 2005). Noroviruses are a major cause of acute viral gastroenteritis that impact on people of all ages worldwide (Okoh et al., 2010). They are estimated to cause up to 66% of all food-borne illnesses attributable to known causes in the United States (Allwood et al., 2003). Adenovirus is frequently found in urban rivers and polluted coastal waters (Jiang et al., 2005). It has been observed that human adenoviruses are present at a higher frequency in sewage than any other enteric virus (54) and that are excreted in high concentrations from infected patients (Fong et al., 2010). Rotaviruses are the most important causes of acute diarrhea in young children worldwide (Gutiérrez-Aguirre et al., 2008).

Hepatitis is a collective term for several diseases of the liver caused by viruses, five of which have been reasonably characterized to come from a wide range of families (Hunt and Saab 2009). Hepatitis A virus is a significant cause of morbidity, life-threatening and attendant socio-economic losses in many parts of the world especially in developing countries (Kittigul et al., 2000). Coxsackievirus A is the common cause of hand, foot and mouth disease in infants and children which is characterized by fever and vesicles in the mouth and on distal extremities (Frydenberg and Starr, 2003).

**SOURCES OF VIRAL POLLUTION**

The point source of enteric viruses to the aquatic environment includes the human waste like sewage, industrial waste, vessel discharges are transferred to the marine environment continuously. Nonpoint source like land runoff can affect the volume of microbial discharge in the aquatic environment after rain fall (Lipp et al., 2001; Gerba et al. 2002).

Upon the discharge of enteric viruses in aquatic environment the risk of disease outbreaks would depend on the duration of survival and high concentrations. These viruses have the ability to survive and remain infectious for long time in aquatic environment (Rzezutka and Cook, 2004). However different environmental factors affect the persistence and survival of these viruses in aquatic reservoirs.

**ENVIRONMENTAL EFFECTS**

The environmental factors like temperature, pH, ultraviolet light and salinity have great effects on survival in aquatic environment. Higher temperatures cause inactivation of viruses by denaturing the proteins and nucleic acid while lower temperature support the viral survival for longer times (Lipp et al., 2001). The slow inactivation rate of enteroviruses was shown at 2°C as compare to 30° C but at acidic pH and it was found that pH along with salinity act as a major factor for inactivation (Wetz et al.2004). Enteric viruses can be inactivated by ultraviolet light by damaging the nucleotides (Lamont et al., 2007).

**WATER AND VIRUS CONCENTRATION METHODS FOR DETECTION**

Enteric viruses are found in a variety of aquatic reservoirs like Sewage, ground water, drinking water, rivers and oceans. Due to the small number of these viruses in aquatic environment virus concentration and purification methods are important for isolation or detection by molecular methods. Optimization of viral particle concentration methods in

aquatic water samples is a critical step for the detection enteric viruses. Each of these methods has advantages and disadvantages. Most methods for detection and quantification of waterborne viruses from environmental samples require concentration from large volumes (100 to 1,000 L) of water. Methods for concentration and detection of enteric viruses from environment water samples are reviewed by Griffin et al. (2003), Wyn-Jones et al. (2011), Rajtar et al. (2008) and Hamza et al.(2011).A good viral concentration method has the following characteristics: it would be simple technically, fast, provides high recoveries, capable of recovering a high percentage from wide range of viruses, produces a small volume of concentrate and inexpensive (Bosch, 1998). A variety of methods have been used to concentrate viruses from different aquatic environments waters. These include ultrafiltration (Wommack et al., 2010), ultracentrifugation (Lawrence and Steward, 2010), and adsorption-elution techniques (Katayama et al., 2002). Ultrafiltration and ultracentrifugation have been used to concentrate viruses from large sample volumes (100L) of natural water (Lawrence and Steward, 2010; Steward and Culley, 2010). Adsorption-elution methods have been used for water quality assessments and detection of human viral pathogens from small sample volume (1L) by simple equipment and procedures.

### DETECTION METHODS

Environmental virology has been evolving as a scientific field for more than 60 years and variety of sampling and analytical methods are available for detection of enteric viruses in aquatic environment (lee et al. 2005). Initial virus detection methods relied on cell cultures and those are still in widespread use today. Newer, polymerase chain reaction (PCR)-based molecular methods have been developed to increase the sensitivity of virus detection in the environment. These methods are efficient for the detection of enteric viruses but confirmation infectivity requires the presence of the viral host. Cell culture and plaque assays are still the preferred techniques to measure infectivity (li et al. 2010). The viral strains require specific host cell for cell culture and it is a limitation to use this technology for different viruses which are not cultivable. In addition, samples of viral concentrates must be highly purified and contaminants free prior to inoculation on cell lines. Cell culture assays are time consuming and expensive (Reynold et al. 2004). These limitations in cell culture methods paved a way for the use of molecular tools for the monitoring of enteric viruses in aquatic environment.

Polymerase chain reaction methods are based on amplification of Nucleic acid from the target organism. The PCR methods are rapid and more sensitive than cell culture methods with detection limits up to single genomic copy of virus per liter of sample. These methods are highly specific and can also be used for detection of non-culturable viruses. There are, however, some disadvantages with PCR techniques. Higher potential for contamination of samples, amplification of non-specific target sequences, and limitations due to the fact that only a small portion of the concentrated sample is analyzed. Furthermore, there is no way to distinguish between infective and non-infective viruse particles. In spite of this, PCR still can provide information about virus existence in aquatic environment(wolf et al. 2009; Girones et al., 2010). The implementation of the combination of molecular methods and cell culture techniques and high-throughput analytical assay like microarray are also used as valuable tool for accurate and better reflection of enteric viruses in aquatic reservoirs ( Connell et al. 2012; Kim et al. 2012)

### CONCLUSION

Enteric viruses are known to cause different kind of infectious diseases in humans. Detection and monitoring of these organisms in aquatic environment is challenging and helpful in control and reduction of water borne illnesses. It is evident that no single approach or method provides comprehensive information. Current methods in use like cell culture assay, Polymerase chain reaction or microarray based tools have some advantages and drawbacks upon each other side by side. Furthermore it is not easy to adopt them as routine laboratory procedures. So development of new approaches and efficient methods with higher sensitivity and specificity are needed.

### References

1. Allwood P.B., Malik Y.S., Hedberg C.W., Goyal S.M. 2003- Survival of F-specific RNA coliphages, feline calicivirus, and Escherichia coli in water: a comparative study- *Appl. Environ. Microbiol*, 69: 5707-5710.
2. Bosch A. 1998- Human enteric viruses in the water environment: A minireview- *Int. Microbiol*, 1: 191-196.
3. Chen C.H., Hsu B.M., Wan M.T. 2007- Molecular detection and prevalence of enterovirus within environmental water in Taiwan- *J. Appl. Microbiol*, 104: 817-823.
4. Ehlers M. M., Grabow W .O .K.2005- Pavlov D N., Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. *Water Research- 39:(11), 2253-2258.* doi: 10.1016/j.watres.2005.04.014
5. Fong T.T., Phanikumar M.S., Xagorarakis I., Rose J.B.2010- Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river- *Appl. Environ. Microbiol*, 76: 715-723.

6. Fong T., Lipp E.K. 2005- Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools- Microbiology Molecular Biology Reviews, 69:(2), 357-371. doi: 10.1128/mmbr.69.2.357-371.2005
7. Frydenberg A. 2003- Starr M., Hand, foot and mouth disease. Aust. Fam. Physician, 32: 594-595.
8. Gerba C.P., Gramos D. M., Nwachuku N. 2002- Comparative Inactivation of Enteroviruses and Adenovirus 2 by UV Light- Applied and Environmental Microbiology, 68:(10), 5167-5169.
9. Girones R., Ferrús M.A., Alonso J.L., Rodriguez-Manzano J., Calgua B. 2de Abreu Corrêa A., Hundesa A., Carratala A., Bofill-Mas S. 2010- Molecular detection of pathogens in water - The pros and cons of molecular techniques. Water Research, 44: (15), 4325-4339. doi: 10.1016/j.watres.2010.06.030.
10. Griffin D.W., Donaldson K.A., Paul J.H., Rose J.B. 2003- Pathogenic Human Viruses in Coastal Waters- Clinical Microbiology Reviews, 16:(1), 129-143. doi: 10.1128/cmr.16.1.129-143.2003
11. Gutiérrez-Aguirre I., Steyer A., Boben J., Gruden K., Poljšak-Prijatelj., Ravnikar M. 2008- Sensitive detection of multiple rotavirus genotypes with a single reverse transcription real-time quantitative PCR assay- J. Clin. Microbiol, 46: 2547-2554.
12. Hamza I.A., Jurzik L., Uberla K., Wilhelm M. 2011- Methods to detect infectious human entericviruses in environmental water samples- Int j Hygn Environ Health, Nov;214(6):424-36
13. Hunt D.R., Saab S. 2009- Viral hepatitis in incarcerated adults: a medical and public health concern- Am. J. Gastroenterol, 104: 1024-1031.
14. Katayama H., Shimasaki A., Ohgaki, S. 2002- Development of a Virus Concentration Method and Its Application to Detection of Enterovirus and Norwalk Virus from Coastal Seawater- Applied and Environmental Microbiology, 68:(3), 1033-1039. doi: 10.1128/aem.68.3.1033-1039.2002
15. Kim J.M., Kim S.Y., Park Y.B., Kim H.J., Min B.S., Cho J.C., Yang J.M., Cho Y.H., Ko G. 2012- Simultaneous detection of major enteric viruses using a Combimatrix microarray- J Microbiol, Oct 27 [Epub ahead of print]
16. Kittigul L., Raengsakulrach B., Siritantikorn S., et al. 2003- Detection of poliovirus, Hepatitis a Virus, and Rotavirus from Sewage and Water Samples- Southeast Asian J. Trop. Med Public Health, 31: 41-46.
17. Lamont Y., Rze?utka A., Anderson J.G., MacGregor S. J., Given M. J., Deppe C. 2007- Pulsed UV-light inactivation of poliovirus and adenovirus- Letters in Applied Microbiology, 45:(5), 564-567.
18. Lawrence J.E., Steward G.F.2010 - Purification of viruses by centrifugation. In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], Manual of Aquatic Viral Ecology. ASLO, 166-181.
19. Lee S.H., Lee C., Lee K.W., Cho H.B., Kim S.J.2005 - The simultaneous detection of both enteroviruses and adenoviruses in environmental water samples including tap water with an integrated cell culture-multiplex-nested PCR procedure- J. Appl. Microbiol, 98: 1020-1029.
20. Li D., Gu A.Z., Yang W., He M., Hu X.H., Shi H.C.2010 - An integrated cell culture and reverse transcription quantitative PCR assay for detection of infectious rotaviruses in environmental waters- J. Microbiol. Methods, 82: 59-63.
21. Lipp E., Kurz R., Vincent R., Rodriguez-Palacios C., Farrah S., Rose J.2001- The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary- Estuaries and Coasts, 24:(2), 266-276. doi: 10.2307/1352950
22. Ogorzaly L., Tissier A., Bertrand I., Maul A., Gantzer C. 2009 -Relationship between F-specific RNA phage genogroups, faecal pollution indicators and human adenoviruses in river water- Water Research, 43:(5), 1257-1264. doi: 10.1016/j.watres.2008.12.011
23. Okoh A.I., Sibanda T., Gusha S.S.2010 - Inadequately treated wastewater as a source of human enteric viruses in the environment- Int. J. Environ. Res. Public Health, 7: 2620-2637.
24. Parkin R.T., Soller J. A., Olivieri A.W.2003 - Incorporating susceptible subpopulations in microbial risk assessment: pediatric exposures to enteroviruses in river water-Journal of Exposure Analysis and Environmental Epidemiology, 13:(2), 161-168.
25. Pavlov D.N., Van Zyl W.B., Van Heerden J., Grabow W.O.K., Ehlers M.M., -Prevalence of vaccine-derived polioviruses in sewage and river water in South Africa- Water Res, 39: 3309-3319.
26. Rajtar B., Majek M., Polanski L., Polz-Dacewicz M.2008 - Enteroviruses in water environment – a potential threat to public health- Ann Agric Environ Med , 15: 199-203.
27. Reynolds K.A.2004 - Integrated cell culture/PCR for detection of enteric viruses in environmental samples- Methods Mol. Biol, 268: 69-78.
28. Rzezutka A., Cook N.2004 - Survival of human enteric viruses in the environment and food- FEMS Microbiology Reviews, 28:(4), 441-453.
29. Steward G.F., Culley A.I.2010 - Extraction and purification of nucleic acids from viruses In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], Manual of Aquatic Viral Ecology. ASLO, pp: 154-165.
30. Vivier J.C., Ehlers M.M., Grabow W.O.K.2004 - Detection of enteroviruses in treated drinking water- Water Res, 38: 2699-2705.
31. Wetz J.J., Lipp E. K., Griffin D.W., Lukasik J., Wait D., Sobsey M. D., Scott T.M., Rose J.B.2004 - Presence, infectivity, and stability of enteric viruses in seawater: relationship to marine water quality in the Florida Keys. Mar-Poll. Bull, 48:698-704.
32. Wilhelmi I., Roman E., Sanchez-Fauquier A. 2003 - Viruses causing gastroenteritis. Clin. Microbiol- Infect, 9:247-262.
33. Wolf S., Rivera-Aban M., Greening G.E. 2009 -Long-range reverse transcription as a useful tool to assess the genomic integrity of norovirus- Food Environ. Virol, 1: 129-136.
34. Wommack K.E., Sime-Ngando T., Winget D.M., Jamindar S., Helton R.R.2010 - Filtration-based methods for the collection of viral concentrates from large water samples In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], Manual of Aquatic Viral Ecology. ASLO, pp: 110-117.
35. Wyn-Jones A., Carducci A., Cook N., D'Agostino M., Divizia M., Fleischer J., Gantzer C., Gawler A., Girones R., Holler C., de Roda AM., Kay D., Kozyra I., Lopez J., Muscillo M., Nascimento J., Maria S., Papageorgiou G., Rutjes S., Sellwood J., Szewzyk R., Wyer M.2011 - Surveillance of adenoviruses and noroviruses in European recreational waters- Water Research, 45:(3), 1025-1038. doi: 10.1016/j.watres.2010.10.015.
1. Allwood P.B., Malik Y.S., Hedberg C.W., Goyal S.M. 2003- Survival of F-specific RNA coliphages, feline calicivirus, and Escherichia coli in water: a comparative study- Appl. Environ. Microbiol, 69: 5707-5710.
2. Bosch A. 1998- Human enteric viruses in the water environment: A minireview- Int. Microbiol, 1: 191-196.

3. Chen C.H., Hsu B.M., Wan M.T. 2007- Molecular detection and prevalence of enterovirus within environmental water in Taiwan- *J. Appl. Microbiol*, 104: 817-823.
4. Ehlers M. M., Grabow W. O. K. 2005- Pavlov D N., Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. *Water Research*- 39:(11), 2253-2258. doi: 10.1016/j.watres.2005.04.014
5. Fong T.T., Phanikumar M.S., Xagorarakis I., Rose J.B. 2010- Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river- *Appl. Environ. Microbiol*, 76: 715-723.
6. Fong T., Lipp E.K. 2005- Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools- *Microbiology Molecular Biology Reviews*, 69:(2), 357-371. doi: 10.1128/mmbr.69.2.357-371.2005
7. Frydenberg A. 2003- Starr M., Hand, foot and mouth disease. *Aust. Fam. Physician*, 32: 594-595.
8. Gerba C.P., Gramos D. M., Nwachuku N. 2002- Comparative Inactivation of Enteroviruses and Adenovirus 2 by UV Light- *Applied and Environmental Microbiology*, 68:(10), 5167-5169.
9. Girones R., Ferrús M.A., Alonso J.L., Rodriguez-Manzano J., Calgua B. 2de Abreu Corrêa A., Hundesa A., Carratala A., Bofill-Mas S. 2010- Molecular detection of pathogens in water - The pros and cons of molecular techniques. *Water Research*, 44: (15), 4325-4339. doi: 10.1016/j.watres.2010.06.030.
10. Griffin D.W., Donaldson K.A., Paul J.H., Rose J.B. 2003- Pathogenic Human Viruses in Coastal Waters- *Clinical Microbiology Reviews*, 16:(1), 129-143. doi: 10.1128/cmr.16.1.129-143.2003
11. Gutiérrez-Aguirre I., Steyer A., Boben J., Gruden K., Poljšak-Prijatelj, Ravnikar M. 2008- Sensitive detection of multiple rotavirus genotypes with a single reverse transcription real-time quantitative PCR assay- *J. Clin. Microbiol*, 46: 2547-2554.
12. Hamza I.A., Jurzik L., Uberla K., Wilhelm M. 2011- Methods to detect infectious human entericviruses in environmental water samples- *Int j Hygn Environ Health*, Nov;214(6):424-36
13. Hunt D.R., Saab S. 2009- Viral hepatitis in incarcerated adults: a medical and public health concern- *Am. J. Gastroenterol*, 104: 1024-1031.
14. Katayama H., Shimasaki A., Ohgaki, S. 2002- Development of a Virus Concentration Method and Its Application to Detection of Enterovirus and Norwalk Virus from Coastal Seawater- *Applied and Environmental Microbiology*, 68:(3), 1033-1039. doi: 10.1128/aem.68.3.1033-1039.2002
15. Kim J.M., Kim S.Y., Park Y.B., Kim H.J., Min B.S., Cho J.C., Yang J.M., Cho Y.H., Ko G. 2012- Simultaneous detection of major enteric viruses using a Combimatrix microarray- *J Microbiol*, Oct 27 [Epub ahead of print]
16. Kittigul L., Raengsakulrach B., Siritantikorn S., et al. 2003- Detection of poliovirus, Hepatitis a Virus, and Rotavirus from Sewage and Water Samples- *Southeast Asian J. Trop. Med Public Health*, 31: 41-46.
17. Lamont Y., Rze?utka A., Anderson J.G., MacGregor S. J., Given M. J., Deppe C. 2007- Pulsed UV-light inactivation of poliovirus and adenovirus- *Letters in Applied Microbiology*, 45:(5), 564-567.
18. Lawrence J.E., Steward G.F. 2010 - Purification of viruses by centrifugation. In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], *Manual of Aquatic Viral Ecology*. ASLO, 166-181.
19. Lee S.H., Lee C., Lee K.W., Cho H.B., Kim S.J. 2005 - The simultaneous detection of both enteroviruses and adenoviruses in environmental water samples including tap water with an integrated cell culture-multiplex-nested PCR procedure- *J. Appl. Microbiol*, 98: 1020-1029.
20. Li D., Gu A.Z., Yang W., He M., Hu X.H., Shi H.C. 2010 - An integrated cell culture and reverse transcription quantitative PCR assay for detection of infectious rotaviruses in environmental waters- *J. Microbiol. Methods*, 82: 59-63.
21. Lipp E., Kurz R., Vincent R., Rodriguez-Palacios C., Farrah S., Rose J. 2001- The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary- *Estuaries and Coasts*, 24:(2), 266-276. doi: 10.2307/1352950
22. Ogorzaly L., Tissier A., Bertrand I., Maul A., Gantzer C. 2009 -Relationship between F-specific RNA phage genogroups, faecal pollution indicators and human adenoviruses in river water- *Water Research*, 43:(5), 1257-1264. doi: 10.1016/j.watres.2008.12.011
23. Okoh A.I., Sibanda T., Gusha S.S. 2010 - Inadequately treated wastewater as a source of human enteric viruses in the environment- *Int. J. Environ. Res. Public Health*, 7: 2620-2637.
24. Parkin R.T., Soller J. A., Olivieri A.W. 2003 - Incorporating susceptible subpopulations in microbial risk assessment: pediatric exposures to enteroviruses in river water- *Journal of Exposure Analysis and Environmental Epidemiology*, 13:(2), 161-168.
25. Pavlov D.N., Van Zyl W.B., Van Heerden J., Grabow W.O.K., Ehlers M.M., -Prevalence of vaccine-derived polioviruses in sewage and river water in South Africa- *Water Res*, 39: 3309-3319.
26. Rajtar B., Majek M., Polanski L., Polz-Dacewicz M. 2008 - Enteroviruses in water environment – a potential threat to public health- *Ann Agric Environ Med*, 15: 199-203.
27. Reynolds K.A. 2004 - Integrated cell culture/PCR for detection of enteric viruses in environmental samples- *Methods Mol. Biol*, 268: 69-78.
28. Rzezutka A., Cook N. 2004 - Survival of human enteric viruses in the environment and food- *FEMS Microbiology Reviews*, 28:(4), 441-453.
29. Steward G.F., Culley A.I. 2010 - Extraction and purification of nucleic acids from viruses In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], *Manual of Aquatic Viral Ecology*. ASLO, pp: 154-165.
30. Vivier J.C., Ehlers M.M., Grabow W.O.K. 2004 - Detection of enteroviruses in treated drinking water- *Water Res*, 38: 2699-2705.
31. Wetz J.J., Lipp E. K., Griffin D.W., Lukasik J., Wait D., Sobsey M. D., Scott T.M., Rose J.B. 2004 - Presence, infectivity, and stability of enteric viruses in seawater: relationship to marine water quality in the Florida Keys. *Mar-Poll. Bull*, 48:698-704.
32. Wilhelmi I., Roman E., Sanchez-Fauquier A. 2003 - Viruses causing gastroenteritis. *Clin. Microbiol- Infect*, 9:247-262.
33. Wolf S., Rivera-Aban M., Greening G.E. 2009 -Long-range reverse transcription as a useful tool to assess the genomic integrity of norovirus- *Food Environ. Virol*, 1: 129-136.
34. Wommack K.E., Sime-Ngando T., Winget D.M., Jamindar S., Helton R.R. 2010 - Filtration-based methods for the collection of viral concentrates from large water samples In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], *Manual of Aquatic Viral Ecology*. ASLO, pp: 110-117.
35. Wyn-Jones A., Carducci A., Cook N., D'Agostino M., Divizia M., Fleischer J., Gantzer C., Gawler A., Girones R.,

Holler C., de Roda AM., Kay D., Kozyra I., Lopez J., Muscillo M., Nascimento J., Maria S., Papageorgiou G., Rutjes S., Sellwood J., Szewzyk R., Wyer M.2011 -

Surveillance of adenoviruses and noroviruses in European recreational waters- Water Research, 45:(3), 1025-1038. doi: 10.1016/j.watres.2010.10.015.

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