ISSN 0120-4157

Biomédica

Revista del Instituto Nacional de Salud

PUBLICACIÓN ANTICIPADA EN LINEA

El Comité Editorial de *Biomédica* ya aprobó para publicación este manuscrito, teniendo en cuenta los conceptos de los pares académicos que lo evaluaron. Se publica anticipadamente en versión pdf en forma provisional con base en la última versión electrónica del manuscrito pero sin que aún haya sido diagramado ni se le haya hecho la corrección de estilo.

Siéntase libre de descargar, usar, distribuir y citar esta versión preliminar tal y como lo indicamos pero, por favor, recuerde que la versión impresa final y en formato pdf pueden ser diferentes.

Citación provisional:

Villamizar RA, Peláez-Carvajal D, Acero LF. Identification of enteric viruses from raw water using fluoro-immuno-magnetic separation coupled to RT-PCR.

Biomédica. 2021;41 (4).

Recibido: 21-02-21

Aceptado: 09-08-21

Publicación en línea: 10-08-21

Identification of enteric viruses from raw water using fluoro-immuno-magnetic separation coupled to RT-PCR

Identificación de virus entéricos en aguas crudas usando separación inmuno-magnética acoplada a RT-PCR

Identificación de virus entéricos usando FIMS-RT-PCR

Raquel A. Villamizar [1], Dioselina Peláez-Carvajal [2], Luis Felipe Acero [2]

[1] Departamento de Microbiología, Facultad de Ciencias Básicas, Universidad de Pamplona, Pamplona, Colombia

[2] Grupo de Virología, Dirección de Redes en Salud Pública, Instituto Nacional de Salud, Bogotá D.C., Colombia

Corresponding author:

Raquel Amanda Villamizar, Laboratorio de Nanotecnología y Gestión Sostenible, Universidad de Pamplona, Km 1 vía Bucaramanga, Ciudadela Universitaria, Edificio Eduardo Cote EC 202, Pamplona, Colombia.

Telefax: 5685303.

raqvillamizar@unipamplona.edu.co

Author contributions:

Raquel A Villamizar: Design, data analysis, test performance and wrote the paper.

Dioselina Peláez-Carvajal: Test performance and wrote the paper.

Luis Felipe Acero: Test performance and data analysis.

Introduction: Enteric viruses have been associated with the production of a variety of diseases transmitted by the fecal-oral route, carried through contaminated food and water. Given their structure and composition, they are highly resistant to environmental conditions and most of the chemical agents used in the purification processes. Therefore, a systematic monitoring of raw water is necessary to ensure its quality, especially, when it is used as feedstock for the production of drinking water for human consumption.

Objective: In the present work the presence of Rotavirus and Hepatitis A Virus was identified by means of the fluoro-immuno-magnetic separation technique (FIMS) in raw water taken from four purification plants in the Norte de Santander department including their water supplies.

Materials and methodos: The viruses were captured and separated from the water samples, using magnetic microparticles functionalized with monoclonal anti-Hepatitis A and anti-Rotavirus antibodies. Confocal microscopy was used to monitor the viral concentration process and transmission electron microscopy for morphological visualization of the separated viruses. The reverse transcriptase-coupled polymerase chain reaction (RT-PCR) was applied to confirm the presence of pathogens.

Results: The two enteric viruses were identified in most of the analyzed water samples, including their water supply sources.

Conclusion: It was possible to determine that the FIMS technique coupled to RT-PCR is highly effective technique in the detection of viral pathogens, in complex matrices such as raw water.

Keywords: rotavirus infections; hepatitis A; antibodies; raw water; water purification; magnetic segregation.

Introducción. Los virus entéricos han sido asociados con la producción de una variedad de enfermedades transmitidas por vía fecal-oral, vehiculizados a través de alimentos y agua contaminada. Dada su estructura y composición son altamente resistentes a condiciones ambientales y a la mayoría de agentes químicos empleados en los procesos de potabilización, por lo que un monitoreo sistemático del agua cruda es necesario para asegurar su calidad, máxime cuando esta se emplea como materia prima para la producción de agua potable para consumo humano.

Objetivo. En el presente trabajo se identificó la presencia de Rotavirus y el Virus de la Hepatitis A, mediante la técnica de separación fluoro-inmuno-magnética (FIMS) en agua cruda procedente de cuatro plantas de potabilización del departamento Norte de Santander incluyendo sus fuentes hídricas de abastecimiento.

Materiales y métodos: Los virus fueron capturados y separados a partir de las muestras de agua, empleando micropartículas magnéticas funcionalizadas con anticuerpos monoclonales anti-Hepatitis A y anti-Rotavirus. Se empleó microscopía confocal para hacer seguimiento al proceso de concentración viral y TEM para la visualización morfológica de los virus separados. La reacción en cadena de la polimerasa acoplada a transcriptasa inversa (RT-PCR) se aplicó para confirmar la presencia de los patógenos.

Resultados: Se logró determinar la presencia de los dos virus entéricos en la mayoría de las muestras de agua analizadas, incluyendo sus fuentes hídricas de abastecimiento.

Conclusión: Se pudo concluir que la técnica FIMS acoplada a RT-PCR es altamente efectiva en la detección de patógenos virales, en matrices complejas como el agua cruda.

Palabras clave: infecciones por rotavirus; hepatitis A; anticuerpos; agua cruda, purificación del agua; separación magnética.

About 783 million people around the world lack access to drinking water and at least 2.5 billion people have no proper sanitation (1). In Colombia, most of the rural population faces a critical situation due to the lack of proper quality water. According to Colombian legislation, the physicochemical and microbiological quality of raw water is regulated by decree 1594 of 1984 (2). Nonetheless, this regulation does not establish any type of virological analysis, converting this aspect in an important concern.

Viruses may enter to the food chain through wastewater containing excretions from infected people with contents ranging from 10^5 to 10^{11} viral particles per gram of feces (3). Enteric viruses such as Enterovirus, Astrovirus, Calicivirus, Hepatitis A and Rotavirus have a devastating effect around the world. They have been classified by the WHO as moderate to highly significant, depending on the country and its socio-economic conditions (4). They are usually transported by water generating diseases such as gastroenteritis, hepatitis, meningitis or encephalitis, although it can also remain in latent way in the final consumer (5).

Many methods have been reported for virus detection. However, most of them require pre-concentration steps because viruses are highly diluted in water. Among the most widely used techniques it can be found matrices based on activated carbon (6) or ion exchange resins (7), as well as absorption-elusion tests and others (8,9).

The immunomagnetic separation (IMS) method has been proven to be effective in the detection of several enteric virus including Hepatitis A (10). Recently, some of the authors of the present research work, reported the use of a new concentration technique called Fluoro-Immunomagnetic Separation (FIMS), which was highly

efficient in the capture, concentration and determination of Rotavirus in drinking water in just two hours (11). Therefore, the present scientific research applied FIMS to detect the enteric viruses, this time in a complex matrix such as raw water. Surface water contaminated with sewage waste are generally used to produce drinking water around the world. Several reports at the national and international level have detected the presence of enteric viruses in both, raw and drinking water (12,13). However, in this work, we report for the first time the presence of enteric viruses in raw water from the department of Norte de Santander, Colombia using FIMS coupled to RT-PCR. In this sense, the present study is in line with the provisions of the Environmental Protection Agency of the United States, which recently published a draft with a list of contaminants (CCL4) that should be monitored in waters, including the Hepatitis A Virus (HAV) (14).

Materials and methods

Reagents

Monoclonal anti-Hepatitis A (AMSBIO) and anti-Rotavirus (Millipore) antibodies at a concentration of 100 µg/mL were used. Then, diluted in PBS (Dulbecco, Sigma Aldrich) at a final concentration of 10 µg/mL (pH 7.2) and stored at −20 ◦C until use. The magnetic microparticles (1% w/v; Spherotech Inc), were diluted in distilled water and stored at room temperature until use. Viral RNA extraction was carried out with a QIAamp kit (QIAGEN, Germany). RT-PCR was done with a SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase kit (Invitrogen). Primers sequence for Rotavirus and Hepatitis A amplification are shown in table 1.

Equipment

Virus separation and concentration was carried out with a manual magnetic separator (Spherotech Inc., USA). A FluoViewTM 1000 Olympus Confocal Microscope was used to obtain images of the captured viruses and an Electronic Transmission Microscope (Tecnai F20 Super Twin TMP) was used to confirm their presence. Negative staining and an 80 Kw electric current were applied to visualize their morphology. Viral RNA was quantified in a NANODROP 2000 spectrometer (Thermo Scientific, USA). RT-PCR was performed in a Veriti thermocycler (Applied Biosystems). The electrophoretic run gels were visualized in a Gel Doc XR system (BIO-RAD, USA), using a 100 bp DNA Ladder (INVITROGEN).

Description of the Studied Water Treatment Plants

In the present research, four water treatment plants located in two cities of the department of Norte de Santander were analyzed, including their primary sources (rivers Pamplonita and Zulia). A code was assigned to each of them in order to avoid conflicts of interest with water treatment companies in the region. Each one of these facilities are described below:

Treatment Plant 1 (P1). The treatment applied in this plant is conventional type, involving hydraulic operation and uptake, sedimentation, filtration, disinfection and storage processes. The aqueduct system of the plant has 2 surface catchments, with an average processing capacity of 110 Lt/sec. Its main source of water supply is the Pamplonita River.

Treatment Plant 2 (**P2**)**.** Corresponding to an integral plant model, P2 fits in a small space all the normal unit processes found in a conventional plant. Its aqueduct system is supplied by two different streams, one of them coming from the

sources of the Pamplonita River. The plant has an average processing capacity of 48 Lt/sec.

Treatment Plant 3 (P3). In this facility, the purification process begins at the catchment with a lateral intake on the Pamplonita River, after which sand is extracted in 4 grit chambers. Flow is measured in three Parshall gutters and then conducted to a pre-sedimentation tank. The settled water is divided in two. A first water flow is directed to a Parshall gutter, while the second flow goes to a concrete structure called rectangular weir, where the coagulant and the polymer are applied. The water is flocculated, settled and filtered in both flows, to finally be chlorinated and delivered to the distribution network by pumping and re-pumping.

Treatment Plant 4 (P4). This plant processes water from the Zulia River by means of three large capacity electric pumps. At the plant, coagulant and polymer agents are added to the water which, once flocculated, passes through the sedimentation unit, where the particles formed during flocculation are extracted. Next, the water goes to the filtering bed, to be finally chlorinated and sent by pumping to the peripheral areas of the city it serves.

Raw Water Sampling and Pre-treatment

One (1) Lt of raw water was collected from each treatment plant's catchment and its primary source (table 2). Samples were taken by triplicate and placed into sterile portable refrigerators containing dry ice and transported to the laboratory to perform physicochemical and virological analysis. Due to the complex nature of the sample, virological analyses were preceded by a filtration process through cotton and Whatman No.1 filter paper, followed by centrifugation at 3500 rpm for 15 min (Figure 1A).

Physico-Chemical Analyses of Raw and Potable Waters

Raw waters were analyzed under some of the parameters stipulated in Chapter IV-article 39, contained in decree 1594 of 1984 of the Ministry of Agriculture of Colombia (2) (table 2).

Microparticle functionalization

A 10% microparticle solution was incubated with a 10 ppm solution of anti-Hepatitis A and anti-Rotavirus antibodies for 1 h at 37 °C. Subsequently, the microparticles were washed twice with a 0.15 mM PBS solution containing 0.1% BSA. The same particles were also washed just once with distilled water. The functionalized microparticles were stored at 4 °C until use (11).

Detection of Hepatitis A and Rotavirus in raw water samples

A volume of 500 µL of functionalized microparticles with anti-Hepatitis A and anti-Rotavirus antibodies was added to 1 L of pre-treated raw water. The particles were kept in constant agitation for 2 h at room temperature for the immunoreaction to take place. Subsequently, an external magnetic field was applied in order to concentrate and separate the microparticle-antibody-virus complex, as shown in Figure 1B. The magnetized sample was stored in an Eppendorf tube and then microscopically and molecularly characterized. The procedure was carried out by triplicate (11).

Molecular characterization

Viral RNA extraction

The antigen-antibody interaction was broken by heating at 95 °C for 5 minutes and further cooling at 4 °C. Subsequently, the microparticles were centrifuged at 10,000 rpm for 2 min (13). The supernatant was used to extract the viral RNA using

Qiagen Kit, according to manufacturer´s instruction. 140 µL of supernatant were treated to obtain 60 µL of final RNA extract. The amount of nucleic acid was determined by spectrophotometry (11)**.**

RT-PCR

The amplification of the viral genetic material was carried out by RT-PCR one step. To obtain a final reaction volume of 25 µl, the following components were added: 12.5 µl of 2X PCR buffer, 1 µl of each primer's at 10 µM, 1 µl of SuperScript™ III one-step RT-PCR enzymes, 3 µl of RNA template and 6.5 µl of reagent grade water. For reverse transcription, a 30 min cycle at 55 °C was followed by a 2 min cycle at 94 °C. Denaturation was carried out at 94 °C for 3 min. PCR was carried out through 35 cycles programmed as follows: 94 °C for 30 s, 55 °C for 30 s and 68 °C for 1 min. Finally, a 5 min extension cycle at 68 °C was added. Electrophoresis was run at 90 volts for 45 min in a 1.8% agarose gel in 1X TAE. The loading buffer was BlueJuice ™ (Invitrogen) containing 1 µl of the molecular marker. The gel was stained with SYBR® Green for the visualization of the DNA bands. The corresponding images were documented in BioRad Gel Doc XR equipment. The amplified products of interest exhibited sizes of 192 bp for HAV and 211 bp for Rotavirus (Table 1).

Results

Table 3 shows the results obtained from the physico-chemical tests carried out on the samples taken from the catchments of each of the four water purification plants including their primary sources, Pamplonita and Zulia Rivers (table 4).

A comparison of the obtained results was made considering those stipulated in Colombian regulations. Conductivity, pH, alkalinity and total hardness are in the

range according to Decree 1594 (2), as also previously reported García et al (15). Since the temperature was found to vary according to altitude, it was optimal. However, the color significantly exceeded the norm. This is attributed to the dragging of decomposing sand, clay and biological material aggregates, which dissolve mineral compounds in the river waters, thus affecting their color. In the case of turbidity, all treatment plants except P-2 were found to surpass the due limits. This originates small suspended colloidal particles that are dragged along the basins and reach the catchments of the water treatment plants.

To determine the presence of enteric viruses, confocal microscopy characterization was performed. Formation of small clusters in some of the studied samples were detected. Figure 2 shows the results obtained through this characterization. Positive control of these assays was conducted using artificially spiked water with Rotavirus particles.

To confirm that clusters were induced by viruses, they were analyzed by TEM. Previously, antigen-antibody interaction was broken by applying thermal shock to facilitated the release of the viral particles. Figure 3A shows a TEM image of Rotavirus, ranging from 50 to 80 nm diameter, which is due to the presence of a protein coat that can sometimes be double or triple (16). Figure 3B shows morphological features of Hepatitis A. This virus is similar to other enteroviruses. It is rounded, not wrapped, and about 30 nm diameter (17).

The released virions from each analyzed sample were also molecularly characterized by RT-PCR. The electrophoretic run shown in Figures 4A and 4B, allowed to confirm the presence of RT in all raw water samples from all treatment

plants including their primary sources, while HAV was no detected in plant 3 and Pamplonita river.

Discussion

According to the Regional Autonomous Corporation of the Northeast Frontier (*Corporación Autónoma Regional de la Frontera Nororiental - CORPONOR*), the greatest threat posed on the two main water sources of the department of Norte de Santander (i.e., the Pamplonita and Zulia Rivers) is the dumping of wastewater without any treatment. In this way, the Pamplonita River performs two simultaneous functions. On the one hand, it supplies 10 municipalities and at the same time, it collects all their wastewaters, which receive no pretreatment. In the case of the Zulia River, it receives discharges from the capital of the department, which has a population of approximately 800 thousand inhabitants (18). In this city, there are food, textile, tanneries, slaughterhouses, exploitation mines, dry cleaners and laundries, which generate solid waste and putrescible organic matter and other liquid effluents, with highly polluting chemical materials.

By comparing the current physico-chemical analyses, to the parameters stipulated in RAS (2000) (19), which classifies water quality according to its level of pollution, the sources under study would be classified according to their color as very deficient. Regarding turbidity, water from P1 and P2 would be of intermediate quality, while water from P3 and P4 would be classified as very poor. This matches with previous reports on different rivers along the country. The physico-chemical dynamics of several segments of the Opia River in the department of Tolima reported low-to-medium water quality (20). Similarly, research conducted on surface waters supplying the municipality of Bahía Solano (department of

Chocó) found that the physicochemical parameters of the basin corresponded to low-intermediate quality as a result of sewage and anthropogenic solid waste disposition (21).

The physico-chemical features of the studied water samples are adequate for viral particles to survive. These pathogens are able to survive in a 3-10 pH range for periods of up to 120 days in superficial and waste waters, at temperatures ranging from 20 to 30 °C (22). These facts undoubtedly represent a significant public health issue since these hydric sources are used for recreation (watering places and natural pools), irrigation and even potabilization. Since the physicochemical properties of the studied waters allow classifying them as “very deficient”, they require specific treatments to eliminate the presence of both chemical and biological contaminants. However, the treatment plants studied in this research used conventional technologies for purification and thus, would turn these water resources into a transmission carrier of enteric viruses such as Rotavirus and HAV.

Virological analysis by using FIMS technique coupled to RT-PCR facilitated the concentration and detection of the two viral pathogens under study. The use of magnetic microparticles with a fluorescent core represent an advantage allowing simultaneously to observe the viral concentration process. Formation of aggregates suggested presumptively the presence of enteric viruses in the samples (Figure 2A-BCF). Virus-mediated clustering has been previously reported by Koh I and Josephon L, who indicate that a mono-dispersed solution of magnetic particles conjugated with antibodies are capable of producing aggregates in the presence of viral particles with binding specificity for conjugated

antibodies. This results in supramolecular structures with improved magnetic properties (23) (Figure 5).

The concentration of the enteric viruses was possible due to the complex formation between monoclonal antibodies and fluoro-magnetic microparticles. Antibodies are Y-shaped protein molecules which selectively detect structurally intact epitopes present only in infectious particles. The recognition event is mediated by supramolecular interactions based on hydrogen bonds, ionic bonds and van der Waals forces (24). The genus Rotavirus is divided into seven serological groups ranging from A to G, which, contain several subgroups. Groups A and C include most of the human pathogens of the genus, while the other groups exhibit more affinity for animals. Group A is the most common human pathogen (17). In this research, specific monoclonal antibodies were used to detect epitope VP6 in the viral capsid of group A, which allowed confirming its presence in the catchments of the 4 studied treatment plants and their primary sources (the Pamplonita and Zulia rivers) (Figure 4A). According to the CDC Rotavirus has a long persistence in water supplies and a high infectivity (25), which is in agreement with our findings. In contrast, HAV was detected only in 3 of the 4 plants under analysis (P1, P2 and P4) and in the waters from the Zulia River, which supplies P4 (Figure 4B). This was found to be in agreement with the formation of aggregates observed through confocal microscopy (Figure 2). This is a highly contagious virus, with a low infective dose exhibiting tropism for the epithelial cells of the digestive tract, from where it travels through the bloodstream to the liver, which is the affected organ in the well-known "infectious Hepatitis" (16).

The finding of these two viruses coincides with a previous report of the highly prevalent presence of Rotavirus and HAV in samples of raw and drinking water from different municipalities of Colombia (12). Additionally, the current results generate a particular concern since an earlier study in drinking water from the distribution phase of the same four treatment plants determined the presence of Rotavirus in P1 (11)**.** This clearly indicates that water disinfection treatments are not efficient enough when it comes to removing this type of enteric virus. All these findings suggest that the water under analysis is transporting viral pathogens which could cause public health problems. This same fact was reported by Lodder JW and collaborators, who analysed surface waters in 10 different locations in Netherlands. They found that this type of water sources, which are used to produce drinking water, are likely to contain a high amount of human viral pathogens, among which they highlighted the presence of Rotavirus and Norovirus (22).

It was possible to identify the presence of Rotavirus and HAV in raw water samples from four treatment plants of the department of Norte de Santander (Colombia), including their primary water sources. The fluoro-immunomagnetic separation (FIMS) technique which combined the use of antibodies together with magnetic microparticles, allowed to efficiently capture, concentrate and separate the enteric viruses from low water volumes, in few time and without energy consumption. In addition, coupling FIMS to RT-PCR it was possible to confirm with high sensitivity and specificity the presence of the pathogens. In this sense, this research is a proof of concept about enteric viruses determination by using an easy handle method that could be introduced for monitoring virological water quality at the regional and national laboratories.

Acknowledgements

Authors would like to thank Colciencias and Universidad de Pamplona for financial support through project 112156934429 of, which was assigned through national open call 569.

Conflict of interest

Authors declare not having any conflict of interest around the work reported in the present paper.

References

1. **Organización Mundial de la Salud y el Fondo de las Naciones Unidas para la Infancia (UNICEF)**. Progresos en materia de agua potable, saneamiento e higiene: informe de actualización de 2017 y línea de base de los ODS. Fecha de consulta: 15 de marzo de 2019. Disponible en: https://apps.who.int/iris/bitstream/handle/10665/260291/9789243512891-spa.pdf?sequence=1

2. **Ministerio de Agricultura**. Decreto 1594 de 1984. “En cuanto a usos del agua y residuos líquidos”. Fecha de consulta: 8 de abril de 2019. Disponible en: www.ideam.gov.co › documents › Dec_1594_1984.pdf

3. **Purparia G, Macalusoa G, Di Bellaa S, Gucciardia F, Miraa F, Di Marco P**, **et al.** Molecular characterization of human enteric viruses in food, water samples, and surface swabs in Sicily. Int J Infect Dis. 2019;80:66–72. https://doi.org/10.1016/j.ijid.2018.12.011

4. **World Health Organization**. Guidelines for Drinking-water Quality, Fourth ed., Geneva, 2011. Fecha de consulta: 20 de abril de 2019. Disponible en*:* whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf

5. **Falman JC, Fagnant-Sperati CS, Kossik AL, Boyle DS, Meschke JS**. Evaluation of Secondary Concentration Methods for Poliovirus Detection in Wastewater. Food Environ Virol. 2019;11:20-31. https://doi.org/10.1007/s12560-018-09364-y

6. **Cormier J, Gutierrez M, Goodridge L, Janes M**. Concentration of enteric virus indicator from seawater using granular activated carbon. J Virol Methods. 2014;196:212-8. https://doi.org/10.1016/j.jviromet.2013.11.008

7. **Chandler JC, Schaeffer JW, Davidson M, Magzamen SL, Pérez-Méndez A, Reynolds SJ,** *et al*. A method for the improved detection of aerosolized influenza viruses and the male-specific (F+) RNA coliphage MS2. J Virol Methods. 2017;246:38-41. https://doi.org/10.1016/j.jviromet.2017.04.004

8. **Coughlin BA, Kniel KE**. Protocol Recovery and Detection of Enteric Viruses from Non-Traditional Irrigation Water Sources. Methods Protoc. 2019;2:55-63. https://doi.org/10.3390/mps2030055

9. **Haramoto E, Kitajima M, Hata A, Torrey JR**, **Masago Y, Sano D**, et al. A review on recent progress in the detection methods and prevalence of human enteric viruses in water. Water Res. 2018;135:168-86. https://doi.org/10.1016/j.watres.2018.02.004

10. **Ha JH, Choi, C, Ha, SD.** Evaluation of immunomagnetic separation method for the recovery of hepatitis A virus and GI.1 and GII.4 Norovirus strains seeded on oyster and mussel. Food Environ Virol. 2014;6:290-6. https://doi.org/10.1007/s12560-014-9156-2.

11. **Villamizar R, Osma JF, Ortíz OO**. New technique for direct fluoroinmunomagnetic detection of rotavirus in water samples. J Water Health. 2017;15:932-41. https://doi.org/10.2166/wh.2017.028

12. **Peláez D, Guzmán B, Rodríguez J, Acero F, Nava G.** Presencia de virus entéricos en muestras de agua para el consumo humano en Colombia: desafíos de los sistemas de abastecimiento. Biomédica. 2016;36:169-78. https://doi.org/10.7705/biomedica.v36i0.2987

13. **Elmahdy EM, Fongaro G, Schissi CD, Petrucio MM, Barardi CRM.** Enteric viruses in surface water and sediment samples from the catchment area of Peri Lagoon, Santa Catarina State, Brazil. J Water Health. 2016;14:142-54. https://doi.org/10.2166/wh.2015.295

14. **Environmental Protection Agency**. Drinking Water Contaminant Candidate List 4– Draft. EPA-HQ-OW-2012-0217. Fecha de consulta: 20 de agosto 2017. Disponible en: http://federalregister.gov/a/2015-02210.

15. **García Alzate C, Valencia CR, Taphorn R, Gonzalez I**. Physicochemical and biological characterization of the Roble river, Upper Cauca, western Colombia. Rev Mus Argent Cienc Nat. 2010;12:5-16. https://doi.org/10.22179/REVMACN.12.247

16. **Rowlands J.** Human hepatitis A virus is united with a host of relations. Proc Natl Acad Sci U S A. 2015;112:15010-1. https://doi.org/10.1073/pnas.1520121112

17. **Mathew D, Gautum R.** Rotavirus. Clin Lab Med. 2015;35:363-91. https://doi.org/10.1016/j.cll.2015.02.012

18. **Departamento Administrativo Nacional de Estadística (DANE).** Censo general 2005. Fecha de consulta: 24 de junio de 2019. Disponible en:

https://www.dane.gov.co/index.php/estadisticas-por-tema/demografia-y-poblacion/censo-general-2005-1

19. **Ministerio de Desarrollo Económico.** Dirección de Agua Potable y Saneamiento Básico Reglamento Técnico del Sector de Agua Potable y Saneamiento Básico. RAS 2000. Título B. Fecha de consulta: 8 de abril de 2019. Disponible en: https://www.minvivienda.gov.co/sites/default/files/documentos/titulob-030714.pdf

20. **Forero-Céspedes A, Reinoso-Flórez G**. Water quality assessment of the Opia River (Tolima-Colombia), using macroinvertebrates and physicochemical parameters. Caldasia. 2013;35:371-87.

21. **Valverde-Solis A, Moreno-Tamayo E, Ortiz-Palacios NY***.* Análisis de la calidad de varios cuerpos de aguas superficiales en Bahía Solano utilizando índices de contaminación. Rev Cient Inst Biod Des. 2015;34:14-21. https://doi.org/10.18636/riutch.v34i1.553

22. **Lodder WJ, van den Ber L, Rutjes SA, de Roda Husman AM**. Presence of Enteric Viruses in Source Waters for Drinking Water Production in the Netherlands. Appl Environ Microbiol. 2010;76:72:17. https://doi.org/10.1128/AEM.00245-10

23. **Koh I, Josephson L**. Magnetic Nanoparticle Sensors. Sensors. 2009;9:8130-45. https://doi.org/10.3390/s91008130

24. **Zhang H, Williams S, Zborowski M, Chalmers J.** Binding Affinities/Avidities of Antibody–Antigen Interactions: Quantification and Scale-Up Implications. Biotechnol Bioeng. 2006;95:5. https://doi.org/10.1002/bit.21024

25. **Center for Diseases Control and Prevention (CDC).** Effect of Chlorination on Inactivating Selected Pathogen. Fecha de consulta: 28 de junio de 2021. Disponible en https://www.cdc.gov/safewater/effectiveness-on-pathogens.html

Table 1. Primers sequence used in RT-PCR one step for Rotavirus and Hepatitis A amplification

NAME	SEQUENCE	PRODUCT SIZE (pb)
RTV	5`TTGCCACCAATTCAGAATAC 3` 3`ATTTCGGACCATTTATAACC 5`	211
HAV-1	5`CAGCACATCAGAAAGGTGAG 3` 3`CTCCAGAATCATCTCCAAC 5`	192

WATER SOURCE	DESCRIPTION
Pamplonita River	It is born at Altogrande hill in the Fontibón Páramo, which belong to the municipaly of Pamplona. It flows into the Zulia river, in the department Norte de Santander, Colombia. This 160 km length river is located at 8°19¨47¨N 72° 26¨33¨W. It has an average flow of 15 m^3/s and drains a basin of 1.345 Km^2
Zulia River	Born in the Cachirí Páramo at 4222 m asl, in the department of Norte de Santander (Colombia). The Zulia river flows into Lake Maracaibo, in Venezuela, the length of its Colombian section being 154 km. The surface of its basin is 3.484 km^2, with an average flow of 50 m^3/s. Its geographical coordinates are 9°03¨23¨N 72° 17¨44¨W

Table 2. Geographical description of two of the most important water sources that supply water to the department of Norte de Santander, Colombia.

PARAMETER	ANALYSIS METHOD
Real color	Spectrophotometric
pH	Electrometric
Temperature	Electrometric
Turbidity	Nephelometric
Conductivity	Electrometric
Alkalinity	Volumetric
Total hardness	Volumetric

Table 3. Physicochemical parameters analyzed in raw waters

ANALYSIS PARAMETER	PRIMARY SOURCE		CATCHMENT				ADMISSIBLE MAXIMUM/ DECREE 1594/84
	P/NITA RIVER	ZULIA RIVER	P1	P2	P3	P4	
Color	1990	483	77	24	76	45	20 UPC
pH	7.31	7.29	7.47	7.73	7.46	7.28	6.5-8.5 Units
Temperature	22	24	10	8	25	23	20º C (Optimal)
Turbidity	976	373	10.5	7.17	389	267	10 NTU
Conductivity	276	97.5	68.6	34.9	201	96.5	< 1.000 microsiemens/cm
Alkalinity	139	47	24	21	62	68	200 mg/L $CaCO_3$
Total hardness	28	17	14	12	22	23	300 mg/L $CaCO_3$

Table 4. Physicochemical analysis of raw water samples from the department of Norte de Santander (Colombia)

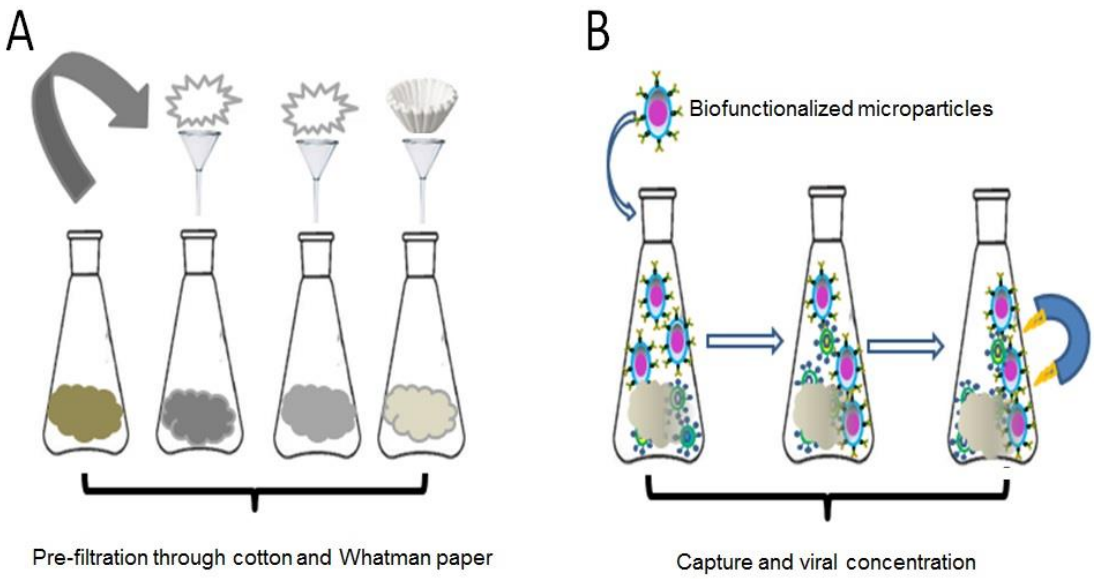

Figure 1. (A) Raw water pre-treatment (B) Schematic representation of the magnetic concentration and separation process of Rotavirus and HAV particles.

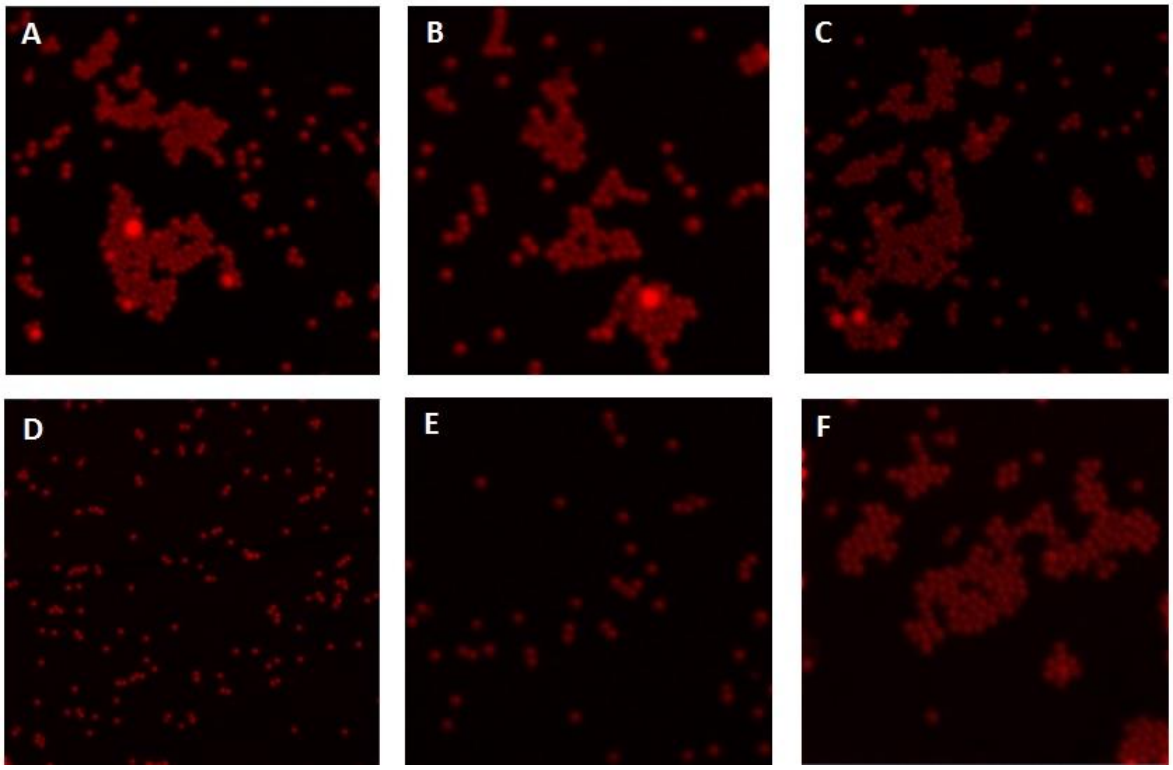

Figure 2. Confocal Microscopy of viral complexes obtained from water samples in four potabilization plants of the department of Norte de Santander (Colombia) and their primary water sources (A: P1, B: P2, C: P3, D: P4; E and F: Pamplonita river and Zulia river) with potential HAV content. The formation of aggregates can be observed in A, B, C and F, while it is absent in D and E. Similar behavior was obtained with Rotavirus.

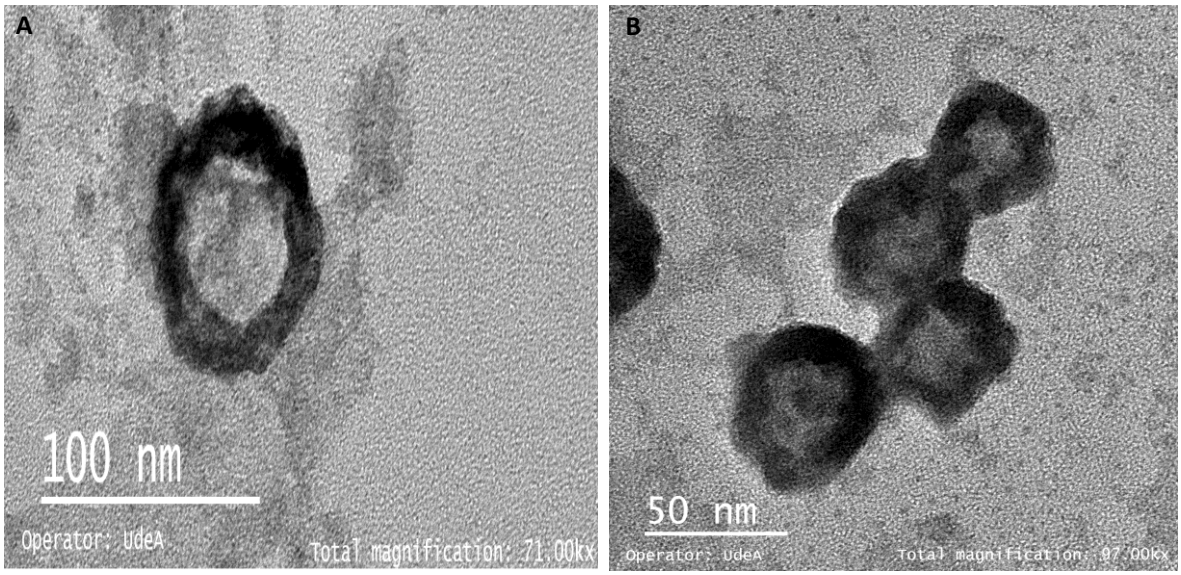

Figure 3. Captured and concentrated virions of A) Rotavirus and B) HAV observed by Electron Transmission Microscopy

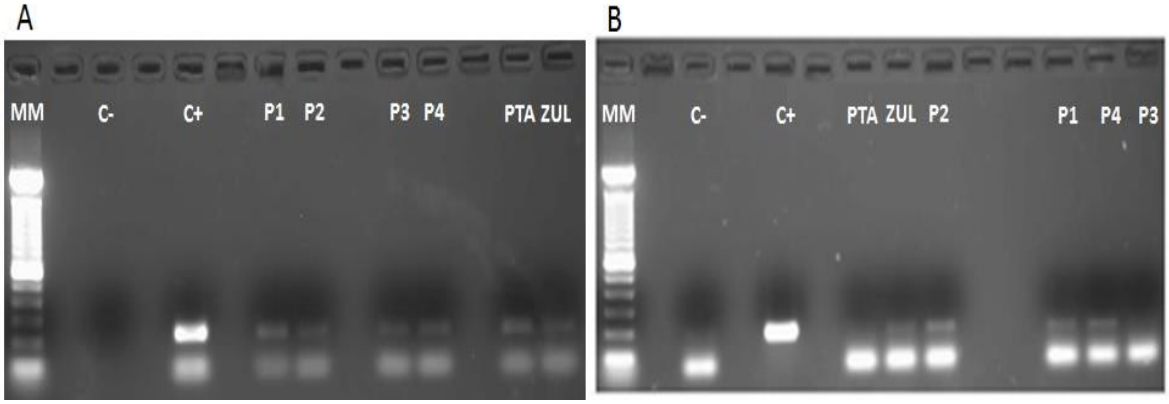

Figure 4. Agarose gel electrophoresis (1.8%) where C+ in A) shows the+211 bp positive control, corresponding to the VP6 gene that encodes the VP6 capsid protein while in B) C+ shows the 192 bp positive control, corresponding to a fragment of the capsid protein precursor region which generates a unique intermediate (VP1-2A) of the HAV. The subsequent columns in both images show the analyzed water samples in the different treatment plants (P1 to P4) including their primary water source (PTA: Pamplonita ZUL: Zulia rivers)

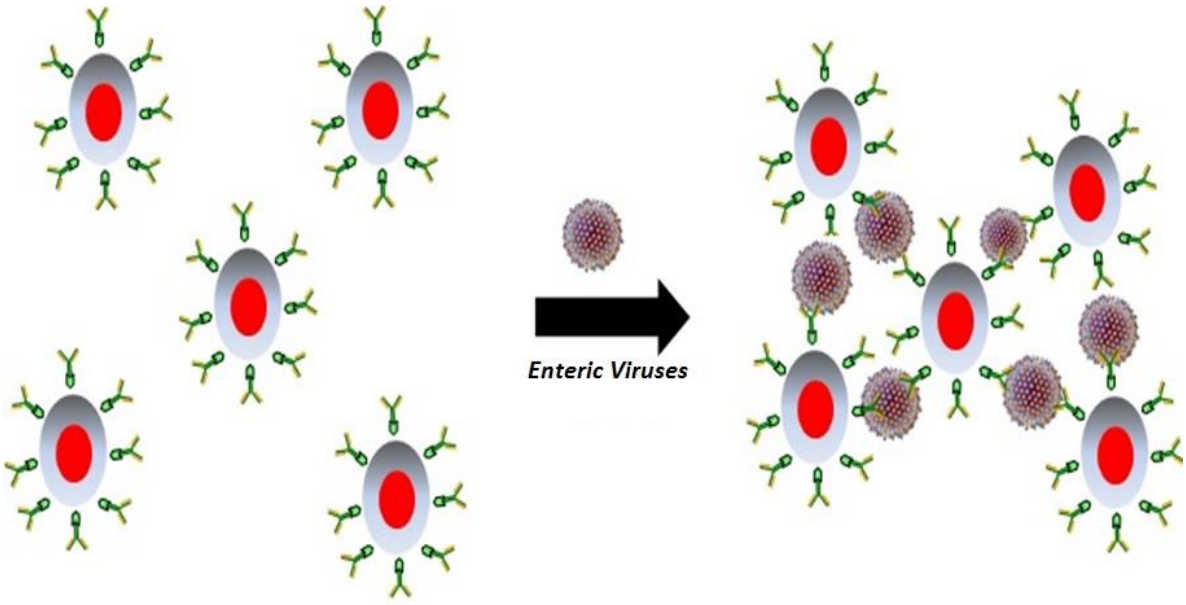

Figure 5. Schematic representation of enteric virus-mediated aggregate formation with fluoro-inmuno-magnetic microparticles.