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Collection, preservation and transportation of water samples for laboratory analysis and process

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We use this protocol and it's working

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Abstract

Introduction:

Diarrhea is seventh cause of mortality in the world. Children younger than 5 years are mainly affected. Diarrhea cases are related to water consumption in a 84%, Because water is the vehicle to transport microorganisms. Data about water quality in our región are scarce.

Objective: This work aim to do both, molecular identification of viruses and protozoan parasites in water for human consumption, and sanitation conditions to analyze the relationship with acute diarrhea cases in children younger than years in Ciénaga-Magdalena.

Methods: A population-based, un-paired case-control study with explanatory scope was carried out. Water samples were collected in each home of neighborhoods located in south of Ciénaga-Magdalena; where answered a previously validated questionnaire to evaluate hygienic habits. Cases included households where a case of diarrhea occurred in 12 months previously.

A multiplex PCR was performed to identify viruses and protozoa.



Materials

EQUIPMENT

Filtration unit with vacuum pump.

MATERIALS AND REAGENTS

- Commercial paper filters (coffee filters)
- 0.8 μ M nitrocellulose membrane filters
- PBS + Tween 80
- 10 μ L, 100 μ L, and 1000 μ L micropipettes
- Filter tips for 10 μ L, 200 μ L, and 1000 μ L micropipettes
- Distilled water
- MN Bead Tube Type A
- 15 mL conical tubes
- Small metal tweezers
- 0.45 μ M nitrocellulose membrane filters
- Sulfuric acid (H₂SO₄)
- Aluminum chloride (AlCl₃)
- Scissors
- Petri dishes

Manual extraction of viral RNA/DNA using the MagMAX™ Viral/Pathogen II kit

EQUIPMENT

- Shaker
- Heating block
- Electric pipettor
- 2 to 20 μ L micropipettes
- 20 to 200 μ L micropipettes
- 100 to 1000 μ L micropipettes

MATERIALS

- Sterile 1.5 mL tubes
- Sterile 0.2 mL tubes
- Sterile 50 mL conical-bottom tubes
- Sterile 15 mL conical-bottom tubes
- Filter tips for 2 to 20 μ L micropipettes
- Filter tips for 20 to 200 μ L micropipettes
- Filter tips for 100 to 1000 μ L micropipettes

- Racks for 1.5 mL tubes
- Magnetic stand
- Racks for 0.2 mL tubes
- Reagent disposal container with lid
- Tip disposal container
- Absorbent paper
- 70% ethanol
- MN Bead Tube Type A

Real-Time PCR for the detection of parasites (*Giardia*, *Cryptosporidium*, and *Entamoeba*) with the PathID™ qPCR Mastermix kit

EQUIPMENT

- QuantStudio 5 or 7 Real-Time PCR Systems (Thermo Fisher Scientific).

MATERIALS

- Micropipettes 10 µl, 100 µl, 1000 µl.
- Multichannel micropipette 2-10 µl.
- Micropipette filter tips (for 10 µl, 200 µl and 1000 µl)
- MicroAmp™ Optical 96-Well Reaction Plate
- MicroAmp™ Optical 8-Tube Strip 0.2 mL (Thermo Fisher Scientific).
- MicroAmp® Optical 8-Cap Strips (Thermo Fisher Scientific).
- MicroAmp™ Optical Adhesive Film (Thermo Fisher Scientific).
- Ultrapure water.
- Primers and probes.

Real-Time RT-PCR for the detection of viruses (*Rotavirus*, *Norovirus*, and *Astrovirus*) with the PathID™ multiplex One-Step qRT-PCR kit

EQUIPMENT

- QuantStudio 5 or 7 Real-Time PCR Systems (Thermo Fisher Scientific).

MATERIALS

- Micropipettes 10 µl, 100 µl, 1000 µl.
- Multichannel micropipette 2-10 µl.
- Micropipette filter tips (for 10 µl, 200 µl and 1000 µl)
- MicroAmp™ Optical 96-Well Reaction Plate
- MicroAmp™ Optical 8-Tube Strip 0.2 mL (Thermo Fisher Scientific).
- MicroAmp® Optical 8-Cap Strips (Thermo Fisher Scientific).



- MicroAmp™ Optical Adhesive Film (Thermo Fisher Scientific).
- Ultrapure water.
- Primers and probes.

Before start

NOTE: Before the filtration process, the 0.5 mM sulfuric acid (H₂SO₄) and 25 mM aluminum chloride (AlCl₃) solutions must be prepared.

Collection, preservation and transportation of water samples for laboratory analysis and process

- 1 Washing and disinfecting the intake points: The water discharge fitting must be cleaned and disinfected with a clean cloth soaked in a sodium hypochlorite solution with a concentration of 5 to 10% active chlorine. Gloves must be worn to avoid skin burns caused by hypochlorite. If the dispensing fitting is metallic, disinfection can be done by temperature by applying the flame (flaming) of an alcohol burner to it for one (1) minute.
- 2 Before taking the sample, any standing water in the sampling facility must be drained, allowing it to flow and run for at least 1 to 2 minutes. This is to ensure that the sample taken next is representative of the quality of the water flowing in the distribution pipeline.
- 3 Sample Collection:
 - Uncap the bottle without taking your hand off the lid to avoid contaminating it with external substances or microorganisms.
 - Collect the sample quickly, filling the container two-thirds full.
 - After sampling, the sample container should be tightly capped, taking care not to contaminate the lid and thus prevent subsequent accidental contamination.
- 4 Perform analyses and record the results obtained for pH, temperature, conductivity, salinity, dissolved solids, and dissolved oxygen.
- 5 Sample Transport:
 - Pack the vials in refrigerated Styrofoam boxes (coolers) with ice packs to prevent breakage or loss during transport.
 - During transport, refrigeration at 4°C and protection from light are recommended. When refrigerating samples, all necessary precautions and measures must be taken to prevent any contamination from melting ice.
- 6 Take additional notes such as site conditions such as appears to be contaminated with organic matter, fish or plants are present, algae is present, etc., watercolor and odor, water clarity, water appears clear, cloudy, or muddy, or any other observations you consider significant.

Vacuum filtration of drinking water samples using nitrocellulose membranes to obtain parasites: cryptosporidium, giardia and entamoeba

- 7 NOTE: Prefiltration should be performed to remove any large particles from the water samples using a commercial paper filter (coffee filter).
- 8 Position the 0.8 µM membrane filter so that it covers the surface of the filter holder. Assemble the filtration system (the funnel, the filter holder, and the attached membrane

- filter) by securing it with an aluminum clamp.
- 9 The above assembly is secured to the flask with a rubber adapter and connected to the vacuum system.
 - 10 Next, slowly pour the water sample over the filter, ensuring that no liquid spills.
 - 11 Turn on the vacuum system until all the water content is filtered.
 - 12 The water sample, drawn through the filter paper by the vacuum, is collected in another container for subsequent procedures.
 - 13 Finally, the membrane filter is stored in MN Bead Tube Type A tubes containing 500 μL of MWA1 solution (provided with the NucleoMag® DNA/RNA Water extraction kit) or in 15 mL conical tubes containing PBS – Tween 80.
 - 14 The tubes (MN Bead Tube Type A or conical) are refrigerated until further processing.

Vacuum filtration of drinking water samples using nitrocellulose membranes to obtain rotavirus, norovirus, and astrovirus

- 15 NOTE: Before the filtration process, the 0.5 mM sulfuric acid (H_2SO_4) and 25 mM aluminum chloride (AlCl_3) solutions must be prepared.
- 16 Pour 5 mL of 25 mM AlCl_3 into a Petri dish, in which the 0.45 μM filter membrane will be immersed for 10 minutes, forming a cation-laden filter (Al^{3+}).
- 17 Next, position the membrane filter so that it covers the surface of the filter holder. The funnel, along with the filter holder and the membrane filter attached to it, is held with an aluminum clamp.
- 18 The above assembly is fitted to the flask with a rubber adapter and connected to the vacuum system.
- 19 Next, slowly pour the water sample over the filter paper to prevent spillage.
- 20 Turn on the vacuum system until all the water content is filtered.

- 21 Optionally, the water sample drawn through the filter paper by vacuum is collected in another container for subsequent procedures.
- 22 The filter paper is washed with 0.5 mM H₂SO₄ to remove aluminum ions.
- 23 Finally, the membrane filter is cut into small pieces and stored in MN Bead Tube Type A tubes or 1.5 mL Eppendorf tubes containing PBS-Tween 80 at a temperature of -80°C.

Manual extraction of viral RNA/DNA using the MagMAX™ Viral/Pathogen II kit

- 24 Preparation of the lysis and binding buffer: The lysis buffer is prepared using the quantities indicated by the manufacturer, which states that 530 µL of Viral/Pathogen Binding Solution and 20 µL of magnetic bead solution should be added for each sample; then gently mix by inversion until homogenized.
- 25 Proteinase K Digestion:
 1. Add 10 µL of proteinase K directly to 400 µL of sample, resuspend with a micropipette, and then add 550 µL of previously prepared lysis-binding buffer to the tube.
 2. Mix vigorously at 1050 rpm using a shaker for 2 minutes, then incubate at 65°C for 5 minutes.
 3. After the incubation time, mix again with a shaker at 1050 rpm for 5 minutes.
 4. Then, place the tubes on the magnetic stand for approximately 5 minutes or until all the beads have been collected.
- 26 Bead Washing:
 5. Discard the supernatant using a micropipette and 1000 µL filter tips while it is in the magnetic rack.
 6. Remove the tubes from the rack and add 1 mL of wash buffer.
 7. Shake at 1050 rpm for 2 minutes.
 8. Place the tubes on the magnetic stand to separate the beads for 2 minutes or until the mixture is clear.
 9. Discard the supernatant using a micropipette and add 1 mL of 80% ethanol (previously prepared as ultrapure water and absolute ethanol).
 10. Shake the mixture with a shaker at 1050 rpm for 2 minutes. Then, place the samples on the magnetic stand until they are clear. Discard the supernatant with a micropipette.
 11. Add 500 µL of 80% ethanol and shake with a shaker at 1050 rpm for 2 minutes. Then, place the samples on the magnetic stand until the mixture is clear. Discard the supernatant with 1000 µL tips. Finally, remove the residue from the ethanol wash with a 10 µL filter tip.
 12. Dry the beads by shaking the open tubes in the shaker at 1050 rpm for 2 minutes.

- 27 Elution:
13. Add 50 μL of eluent solution to each sample.
 14. Shake at 1050 rpm for 5 minutes.
 15. After the shaking time is over, the tubes are incubated in a dry block at 65°C for 10 minutes.
 16. Shake the tubes at 1050 rpm for 5 minutes. After this shaking time, they are placed on the magnetic stand until rinsing.
 17. Finally, after rinsing, the RNA/DNA is removed from the initial tube and transferred to two new tubes (aliquoted); the first volume contains approximately 25 μL for storage, and the remainder is placed in a new tube for RT-qPCR and qPCR.

Real-Time PCR for the detection of parasites (*Giardia*, *Cryptosporidium*, and *Entamoeba*) with the PathID™ qPCR Mastermix kit

- 28 Real-Time Polymerase Chain Reaction (qPCR): qPCR reactions for the detection of *Giardia*, *Cryptosporidium*, and *Entamoeba* are performed using the PathID™ qPCR kit.
- 29 For the detection of *Giardia*, *Cryptosporidium* and *Entamoeba* parasites, primers and probes are used that are directed to a region of the rRNA genes (SSU) and a specific sequence of *C. parvum*.
- 30 Dispense 20 or 25 μL (for singleplex or multiplex, respectively) of the mixture into each well of the plate or tube strip, and select two wells in each zone for the positive and negative controls. Add 5 μL of ultrapure water to the wells designated for the negative controls.
- 31 Next, go to a designated area for dispensing DNA: Add 5 μL of the sample DNA to the reaction mixture in each of the wells. It is recommended that the samples be arranged in parallel on the plate.
- 32 Add 5 μL of the positive control to the designated wells. Then, if using a plate, seal it with adhesive film; or, failing that, with lids if using tube strips.
- 33 Place the plate in the thermal cycler so that it matches the sample placement previously designed in the equipment software.
For the amplification of the genes of interest, incubate the reaction in two stages, the first with a cycle of polymerase activation and DNA denaturation at 95°C for 10 minutes and a second amplification stage with 40 cycles of denaturation at 95°C for 15 seconds and a hybridization/extension and plate reading at 60°C for 60 seconds.

Real-Time RT-PCR for the detection of viruses (*Rotavirus*, *Norovirus*, and *Astrovirus*) with the PathID™ multiplex One-Step qRT-PCR kit

- 34 Real-Time Polymerase Chain Reaction (qRT-PCR): qRT-PCR reactions for the detection of Rotavirus, Norovirus, and Astrovirus are performed using the PathID™ Multiplex One-Step qRT-PCR Kit.
- 35 For the detection of Rotavirus, Norovirus and Astrovirus, primers and probes are used that target a region of the NSP3, RdRp/Capsid junction and ORF1a genes.
- 36 Dispense 20 or 25 µL (for singleplex or multiplex, respectively) of the mixture into each well of the plate or tube strip, and select two wells in each zone for the positive and negative controls. Add 5 µL of ultrapure water to the wells designated for the negative controls.
- 37 Next, go to a designated area for dispensing the RNA: Add 5 µL of RNA from the sample to the reaction mixture in each of the wells. It is recommended that the samples be arranged in parallel on the plate.
- 38 Add 5 µL of the positive control to the designated wells. Then, if using a plate, seal it with adhesive film; or, failing that, with lids if using tube strips.
- 39 Place the plate in the thermal cycler so that it matches the sample placement previously designed in the equipment software.
For amplification of the genes of interest, incubate the reaction in three steps: the first with a cycle for the reverse transcription reaction at 48°C for 10 min; the second step with a cycle of polymerase activation and DNA denaturation at 95°C for 10 minutes; and a final amplification step with 45 cycles of denaturation at 95°C for 15 seconds and a hybridization/extension and plate reading at 60°C for 45 seconds.