Methods and other factors. If you use the RNA extraction note the determination of the gene load. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by the Rotavirus. Each gene codes for one protein, except genes 9 and 11, which each code for two. The RNA is produced an enterotoxin, which induces gastroenteritis, leading to severe diarrhea and sometimes vomiting. Rotavirus is a genus of double stranded RNA virus in the family Reoviridae. It is the leading cause of severe diarrhea among infants and young children. By the age of five, nearly every child in the world has been infected with rotavirus at least once. However, with each infection, immunity develops and subsequent infections are less severe. There are seven species of this virus, referred to as A, B, C, D, E, F and G. Rotavirus A, the most common, causes more than 90% of infections in the community, particularly in developing countries. In addition to its oral route. It infects cells that line the small intestine and sometimes death through dehydra on. Although rotavirus was discovered in 1973 and accounts for up to 50% of hospitalisations for severe diarrhea in infants and children, its importance is still not widely known within the public health community, particularly in developing countries. In addition to its impact on human health, rotavirus also infects animals, and is a pathogen of livestock.

The genome of rotavirus consists of 11 unique double helix molecules of RNA which are 18.55x nucleoside base pairs in total. Each helix, or segment, is a gene, numbered 1 to 11 by decreasing size. Each gene codes for one protein, except genes 9 and 11, which each code for two. The RNA is surrounded by a three-layered isocalidool capsid protein. Viral particles are up to 76.5 nm in diameter and are not enveloped. The Rotavirus (Group B) real time RT-PCR kit contains a specific ready-to-use system for the detection of the Rotavirus (for Group B) using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a Super Mix for the specific amplification of the Rotavirus RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the RNA Virus is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by the means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems' optical unit during the PCR. The detection of amplified Rotavirus DNA fragment is performed in a fluorimeter capable of measuring the fluorescence (BHQ1). In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560 nm fluorescence of the internal control (IC). An external positive control defined as 1x10^5 copies/ml is supplied which allow the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

Analysis sensitivity: 1x10^5 copies/ml; LOQ: 2x10^4 ~ 1x10^5 copies/ml

Notes: For in vitro diagnostic use only. The sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the RNA extraction kit recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than the elution volume by some concentrating method, it can be much higher.

Storage
• All reagents should be stored at -20°C. Storage at +4°C is not recommended.
• All reagents can be used until the expiration date indicated on the kit label.
• Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
• Cool all reagents during the working steps.
• Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices
• Biological cabinet
• Vortex mixer
• Cryo-container
• Sterile filter tips for micro pipettes
• Disposable gloves, powderless
• Refrigerator and Freezer
• Desktop microcentrifuge
• Tube racks

7. Warmings and Precaution
Carefully read this instruction before starting the procedure.
- In vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be handled in a laminar flow hood.
- This assay needs to be used according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- A repeated thawing and freezing of the reagents, may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/DNA and 2) Amplification/ detection of amplification products.

Analysis sensitivity: 1x10^5 copies/ml; LOQ: 2x10^4 ~ 1x10^5 copies/ml

Notes: For in vitro diagnostic use only. The sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the RNA extraction kit recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than the elution volume by some concentrating method, it can be much higher.

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