Out of two hundred and twenty (220) samples that were tested positive by the predicate kit, two hundred and fourteen (214) were positive on Pulse Adeno-Rota Combo Test.
Out of eight hundred and thirty (830) samples that were tested negative by the predicate kit, eight hundred and twenty four (824) were negative on Pulse Adeno-Rota Combo Test. Twelve (12) samples that had a disparity in results were verified by a third product. Six (6) samples had results in agreement with Pulse Adeno-Rota Combo Test while six (6) samples agreed with the predicate kit. The agreement with the predicate kit is summarized below.
Agreement of positive = 214/220 = 97.27%, Agreement of Negative = 824/830 = 99.28%, Total Agreement = 1038/1050 = 98.86%

Table 2. Accuracy results of Adenovirus

		Predicate Kit		Total
		Pos.	Neg.	
Pulse Adeno-Rota Combo Test	Pos.	197	6	203
	Neg.	7	840	847
Total		204	846	1050

Out of two hundred and four (204) samples that were tested positive by the predicate kit, one hundred and ninety seven (197) were positive on Pulse Adeno-Rota Combo Test. Out of eight hundred and forty six (846) samples that were tested negative by the predicate kit, eight hundred and forty (840) were negative on Pulse Adeno-Rota Combo Test. Thirteen (13) samples that had a disparity in results were verified by a third product. Seven (7) samples had results in agreement with Pulse Adeno-Rota Combo Test while six (6) samples agreed with the predicate kit. The agreement with the predicate kit is summarized below.
Agreement of positive = 197/204 = 96.57%
Agreement of Negative = 840/846= 99.29%
Total Agreement = 1037/1050 = 98.76%

Assay Specificity

The following bacterial and viral strains were used to test the specificity of Pulse Adeno-Rota Combo Test. Positive and negative controls spiked with the bacteria or virus at the indicated concentration showed no interference on the test results.
Adenovirus type 40 $1x10^6$ $TCID_{50}$ (no interference to Rotavirus)
Adenovirus type 41 $1x10^6$ $TCID_{50}$ (no interference to Rotavirus)
Rotavirus Wa $1x10^6$ $TCID_{50}$ (no interference to Adenovirus)
Campylobacter jejuni 7.63×10^7CFU/ml, Candida albicans $1x10^8$CFU/ml
Clostridium perfringens A $1x10^8$CFU/ml , Citrobacter freundii $1x10^8$CFU/ml
Enterococcus faecalis $1x10^8$CFU/ml , Escherichia coli $1x10^8$CFU/ml
Klebsiella pneumonia $1x10^8$CFU/ml, Listeria monocytogenes $1x10^8$CFU/m
Moraxella catarrhalis $9.9x10^6$CFU/ml, Neisseria gonorrhoeae $1x10^8$CFU/ml
Pseudomonas aeruginosa $1x10^8$CFU/ml, Stapylococcus epidermidis $1x10^8$CFU/ml
Stapylococcus aureus $1x10^8$CFU/ml, Shigella flexneri $1x10^8$CFU/ml
Shigella sonnel $1x10^8$CFU/ml, Streptococcus dysgalactiae $1x10^8$CFU/ml
Streptococcus agalactiae $1x10^8$CFU/ml, Streptococcus pyogenes $1x10^8$CFU/ml

Cross Reactivity

Pulse Adeno-Rota Combo Test may cross-react with the rotavirus antigen from monkey and porcine.

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Adeno-Rota Virus Antigen Combo Test

INTENDED USE

The PULSE Adeno-Rota Combo Test is an *in vitro* qualitative immunochromatographic assay for the rapid detection of *adenovirus* and *rotavirus* antigens in human stool specimen. The test results are intended to aid in the diagnosis of *adenovirus* and *rotavirus* infection and to monitor the effectiveness of therapeutic treatment.
This product is intended for Laboratory use only.

SUMMARY AND PRINCIPLES

Rotavirus is the primary causative agent of pediatric gastroenteritis and diarrhea worldwide. Almost every child on the planet may get infected by age 5. Scientists say that 900,000 young children around the world die each year from rotaviruses. In tropical climates, rotavirus infection can occur all year round. The age groups most susceptible to the disease are that of infants and children. The infection usually begins with a fever. Soon the little one begins to vomit and has a nasty stomach ache. The vomiting goes away, followed by watery diarrhea that lasts from 3 to 9 days. Most of the time, kids recover easily. Sometimes, severe dehydration will result. The infection starts suddenly and lasts for an average of four to six days. Rotaviruses are extremely contagious. Only a few particles are needed to transmit infection. They originate in the stool, but are found throughout the environment wherever young children spend much time. They are resistant to disinfectants used to clean surfaces and to anti-bacterial hand-washing agents. Rotavirus particles remain active on human hands for at least 4 hours, on hard dry surfaces for 10 days, and on wet areas for weeks. Rotavirus induced dehydration is a major cause of infant morbidity in both developed and underdeveloped countries, and a major cause of infant mortality in the developing countries.
Adenovirus is the second most common cause of viral gastro-enteritis in Children (10-15%). This virus may also cause respiratory diseases and, depending on the serotype, also diarrhea, conjunctivitis, cystitis, etc. At lease 47 serotypes of adenovirus have been described, all sharing a common hexon antigen. Serotypes 40 and 41 are the ones associated with gastro-enteritis. The main syndrome is diarrhea that may last between 9 and 12 days associated with fever and vomiting.
Pulse Adeno-Rota Combo Test is a sandwich solid phase immunochromatographic assay. To perform the test, an aliquot of diluted stool sample is added to the sample well of the test cassette. The sample flows through a pad containing antibodies against adenovirus and rotavirus coupled to red-colored colloidal gold. If the sample contains adenovirus or rotavirus antigens, the antigen will bind to the antibody coated on the colloidal gold particles to form antigen-antibody-gold complexes. These complexes move on the nitrocellulose membrane by capillary action toward the test line region on which adenovirus and rotavirus specific antibodies are immobilized separately. As the

complexes reach the test line, they will bind to the antibody corresponding to the virus on the membrane to form of a line. A red control line will always appear in the result window to indicate that the test has been correctly performed and the test device functions properly. If virus is not present or lower than the detection limit of the test, only the control line will be visible. If the control line does not develop, the test is invalid.

MATERIALS SUPPLIED

1. Pulse Adeno-Rota Combo test card : Each cassette contains a test strip with adenovirus and rotavirus specific antibody on the test region of the membrane and colored adenovirus and rotavirus antibody-gold conjugate pad.
2. Sample bottle: Each sample bottle contains 1.5 ml of stool specimen collection buffer. Store at 4-30ºC

Additional Items Required:

1. Specimen collection container.
2. Timer.

STORAGE & STABILITY

The expiration date is indicated on the package label. Store Sample Collection Tubes at 4-30 º C. Store test device at 4-30 º C.

PRECAUTIONS

1. For *In Vitro* diagnostic use only.
2. This product is intended for Laboratory use only.
3. Wear protective gloves while handling kit components and test specimens.
4. Patient specimens and inactivated Positive Control may contain infectious agents and should be handled and disposed of as potential biohazards.
5. Do not use kit components beyond expiration date.
6. Dispose of all used materials in appropriate container. Treat as potential biohazard.

SPECIMEN COLLECTION

Stool samples must be taken as soon as the symptoms appear. Viral particles decrease in number after one week. Stool specimens should be collected in containers that do not contain media, preservatives, animal serum or detergents as any of these additives may interfere with the Rapid Rotavirus Antigen Test.

REAGENT PREPARATION

Bring all reagents, including test device, to room temperature before use.

SPECIMEN PREPARATION

1. Unscrew the sample bottle, use the attached applicator stick attached on the cap to transfer small piece of stool (4-6 mm in diameter; approximately 50 mg – 200 mg) into the sample bottle containing specimen preparation buffer.
2. For liquid or semi-solid stools, add 100 microliters of stool to the vial with an appropriate pipette.
3. Replace the stick in the bottle and tighten securely. Mix stool sample with the buffer thoroughly by shaking the bottle for a few seconds.

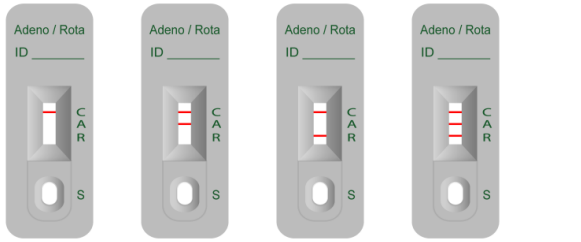
PROCEDURE

1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch.
3. Hold the sample bottle upright with the tip pointed away from the test performer, snap off the tip.
4. Hold the bottle in a vertical position over the sample well of the test card, deliver 3 drops (120 -150 µL) of diluted stool sample to the sample well.
5. Read the result at 10 minutes. A strong positive sample may show result earlier.

Note: Results after 10 minutes may not be accurate.

RESULTS

Positive result: A distinct pink colored band appears on test line regions, in addition to a pink line on the control line region.
Negative result: No line appears in the test line regions. A distinct pink line shows on the control line region.
Invalid: The control line does not become visible within 10 minutes after the addition of the sample.



Negative | Adenovirus Positive | Rotavirus Positive | Adeno/Rota Positive

QUALITY CONTROL PROCEDURE

The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.
Good Laboratory Practice recommends the daily use of control materials to validate the reliability of the device. Control materials which is not provided with this test kit may be commercially available.

LIMITATIONS OF THE PROCEDURE

1. The test is for qualitative detection of rotavirus antigen in stool sample and does not indicate the quantity of the antigens.
2. The test is for in vitro diagnostic use only.
3. The test result should be used only to evaluate a patient with signs and symptoms of the disease. A definitive clinical diagnosis should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES

Pulse Adeno-Rota Combo Test detects the presence of adenovirus and *rotavirus* antigens in stool specimens. Expected values for any given population should be determined for each laboratory. The positivity rate of any given laboratory may vary depending on geographic location, season, and living environment.

PERFORMANCE CHARACTERISTICS

Accuracy

Pulse Adeno-Rota Combo Test was evaluated on 1050 stool samples. The test results were compared with an approved predicate kit.

Table 1. Accuracy results of Rotavirus

		Predicate Kit		Total
		Pos.	Neg.	
Pulse Adeno-Rota Combo Test	Pos.	214	6	220
	Neg.	6	824	830
Total		220	830	1050