JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 5876–5880 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.5876–5880.2005

Novel Rotavirus VP7 Typing Assay Using a One-Step Reverse Transcriptase PCR Protocol and Product Sequencing and Utility of the Assay for Epidemiological Studies and Strain Characterization, Including Serotype Subgroup Analysis

Daniel J. DiStefano,[1]* Nikolai Kraiouchkine,[1] Laura Mallette,[2] Marianne Maliga,[2] Gregory Kulnis,[2] Paul M. Keller,[1] H. Fred Clark,[3] and Alan R. Shaw[1]

Vaccine & Biologics Research, Merck Research Laboratories, Merck & Co. Inc., West Point, Pennsylvania[1]*; Vaccine & Biologics Research, Merck Research Laboratories, Merck & Co., Inc., Wayne, Pennsylvania*[2]*; and University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania*[3]

Received 27 April 2005/Returned for modification 21 June 2005/Accepted 9 September 2005

Rotavirus is the most common cause of severe dehydrating gastroenteritis in infants. To date, 10 different serotypes of rotavirus have been identified in human stools. While four or five serotypes dominate, serotype circulation varies with season and geography. Since our laboratory has been involved in the development of a multivalent rotavirus vaccine, it is important to identify the serotypes of rotavirus encountered during our clinical trials. We have developed methodologies for the molecular identification of rotavirus strains based on VP7 gene segment sequence. A 365-bp reverse transcriptase PCR product was generated from the VP7 gene segment using a pair of novel degenerate primers. All serotypes tested (both animal and human) yielded an identically sized product after amplification. Sequencing of these products is performed using truncated versions of the original primers. The sequence generated is compared against a database of rotavirus VP7 sequences, with the G type determined, based on the sequence homology. Using this assay, we have correctly identified human VP7 strains from a panel of available serotypes, as well as numerous animal strains. The assay was qualified using rotavirus positive stool samples, negative stool samples, and rotavirus-spiked stool samples. In addition, samples from cases of acute gastroenteritis collected at Children's Hospital of Philadelphia have been evaluated and indicate that the assay is able to discriminate subtle differences within serotypes. The assay has been utilized in the testing of >3,000 antigen-positive (enzyme immunoassay) samples collected during clinical trials of a rotavirus vaccine (RotaTeq) and identified a serotype in ~92% of samples (3, 17, 19).

Rotavirus is the most common cause of severe gastroenteritis in young children (11). Rotaviruses are ubiquitous in nature. Most vertebrates have their own rotaviruses with serotypes that are relatively, but not absolutely, species specific. Fourteen different serotypes of rotavirus have been identified from human and animal samples (12). Identification of rotavirus serotypes has evolved over the past 3 decades as new techniques became available. The foundation of serotyping was based on polyclonal antiserum directed primarily against the major coat glycoprotein of the virus, VP7, commonly called the G protein. Serotypes were numbered more or less in the order in which they were identified: G1, G2, G3, etc. Currently, both molecular and immunological methods are used for G-type identification of rotavirus strains in human stool samples. These include nested reverse transcriptase PCR (RT-PCR) (7, 8) and immunoassays using monoclonal (18) or polyclonal (2) antibodies. Further examination of samples typed by these methods, such as by sequence analysis, is usually required to further characterize diversity within serotypes. Recently, restriction fragment length polymorphism (15) analyses have been used to compare the genetic diversity of viral isolates within a G serotype.

Our laboratory is engaged in the development of a pentavalent rotavirus vaccine containing human-bovine reassortants with human serotypes G1, G2, G3, and G4 VP7 plus human VP4 (the other rotavirus coat protein) of phenotype P1a (serotype) and genotype [8] (5). The primary endpoint of the clinical studies of this vaccine is protection against rotavirus acute gastroenteritis caused by the serotypes included in the vaccine (G1, G2, G3, and G4). This has raised the need for a robust, accurate, efficient, and documentable typing system. The development of the described assay was pursued because of several potential issues. Typing of samples using monoclonal antibodies (MAbs) has proven problematic, with some samples not detected due to low antigen levels, as well as a potential for cross-reactivity between serotypes (1, 6). Nested RT-PCR was not pursued due to concerns related to gel documentation (inability to account for spurious bands) and the need for laboratory environmental controls to minimize the risk of cross-contamination inherent in nested amplification methods.

The importance of diversity analysis within subgroups is highlighted by the identification of four individual G1 genetic lineages (10), as well as diversity within G2 (16) and G4 (14) serotypes. RT-PCR and sequence analysis have also been recently developed for VP7 typing (13). This method also utilizes degenerate

* Corresponding author. Mailing address: Vaccine & Biologics Research, Merck Research Laboratories, Merck & Co., Inc., West Point, PA 19486. Phone: (215) 652-1747. Fax: (215) 652-2142. E-mail: daniel_distefano@merck.com.

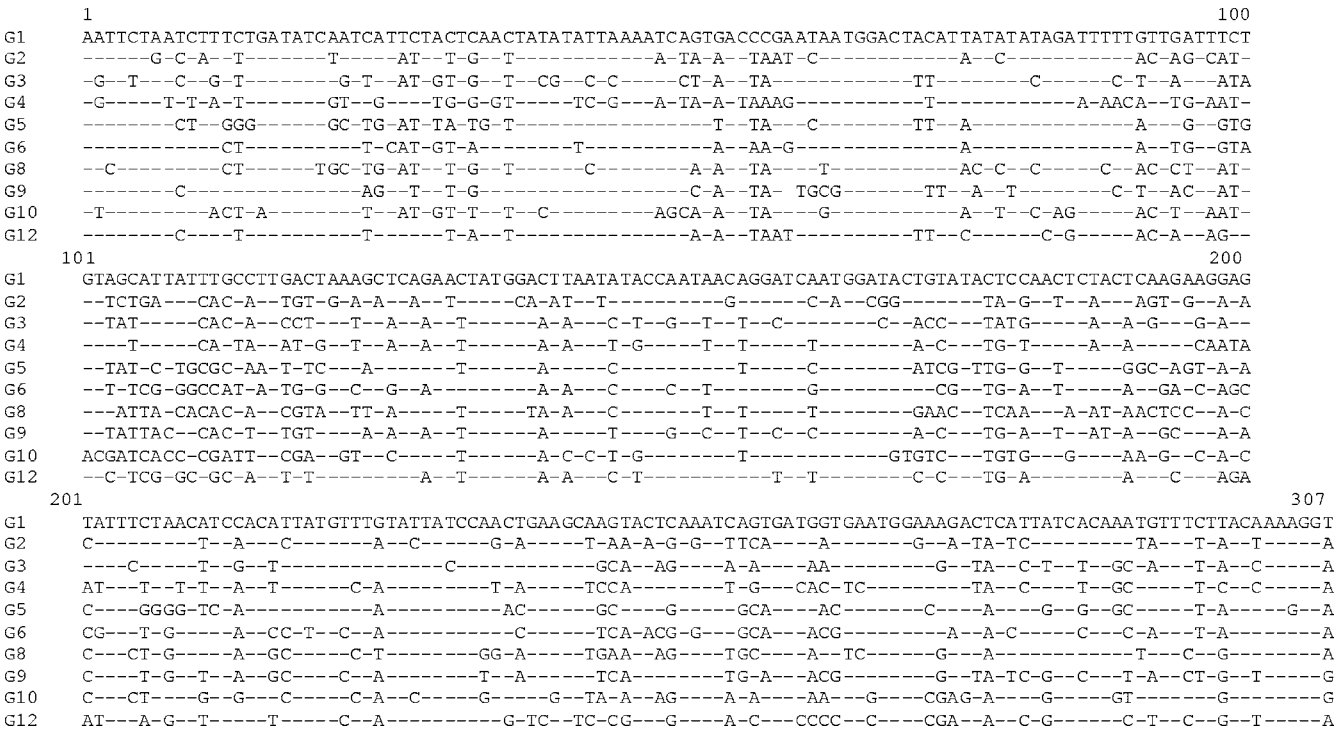

FIG. 1. Comparison of nucleic acid sequences of various VP7 types across the assay target region.

primers but generates a large amplicon for sequencing. The method described here generates a relatively short amplicon spanning a region of known hypervariability (Fig. 1), allowing for one-pass sequencing in both directions with very short run times, yielding information that permits serotype and subtype identification. The assay is amenable to high-volume testing that is necessary for large-scale clinical trials.

MATERIALS AND METHODS

Test samples. For rotavirus strains of known VP7 serotypes, cell culture supernatants of the following strains were used: WI79 (G1), Wa (G1), SC2 (G2), DS1 (G2), WI78 (G3), P (G3), BrB (G4), CC4 (G4), HCR3 (G3 subtype), VR5-08 (G5), NG8-038 (G8), WI61 (G9), RRV (Rhesus G3), EDIM (murine G3), OSU (porcine G5), WC3 (bovine G6), and B10-01 (bovine G10).

RotaTeq vaccine components and human-bovine reassortants were VP7 serotypes of G1, G2, G3, G4, and G6 (bovine).

A total of 12 rotavirus samples previously serotyped using MAbs at Children's Hospital Medical Center, Cincinnati, Ohio, were used: eight G1 samples, one G2 sample, one G3 sample, and two G4 samples.

A total of 20 stool samples were obtained at Children's Hospital of Philadelphia. Of these samples, 15 were antigen positive for rotavirus and 5 were antigen negative. Samples were blinded as to antigen test result. Samples were collected from inpatients (6 months to 4 years of age) diagnosed with gastroenteritis.

A total of 104 rotavirus-positive (enzyme immunoassay [EIA]) stool samples collected during the 1999 to 2000 rotavirus season at The Children's Hospital of Philadelphia. Samples were collected from inpatients (15 days to 7 years old) diagnosed with gastroenteritis and were positive for rotavirus by antigen detection.

RNA extraction. RNA was initially extracted from 140 μl of cell culture supernatant fluids or of clarified 10% stool suspensions with the QIAamp Viral RNA Extraction kit (QIAGEN, Inc., Valencia, CA) as per the manufacturer's protocol, with the following exception: the elution used 60 μl of molecular biology-grade water preheated to 80°C. The elution was performed at higher temperatures to maximize RNA recovery. Extractions were carried out using the QIAvac24 vacuum manifold to allow for greater sampling throughput.

A secondary RNA extraction was used for samples initially found to by RT-PCR following QIAamp RNA extraction. Samples were extracted by the Boom silica method as previously described (4).

Primers. Primers used for amplification were as follows (degenerate positions are indicated in parentheses): forward, 5′ GCTC(C/T)TTTT(A/G)ATGTATG GTATTGAATATACCAC 3′; reverse, 5′ CTTTAAAATA(A/C/G/T)A(C/T)(A/G/T)GA(A/G/T)CC(A/T)(A/G)T(C/T)GGCCA 3′. Primers used for the sequencing reaction were as follows: forward, 5′ (C/T)TTTT(A/G)ATGTATGGTATT GAATATACCAC 3′; reverse, 5′ AAATA(A/C/G/T)A(C/T)(A/G/T)GA(A/G/T) CC(A/T)(A/G)T(C/T)GGCCA 3′.

RT-PCR. RT-PCR was performed on purified RNA using the One-Step RT-PCR kit (QIAGEN, Inc., Valencia, CA) as per the manufacturer's recommendations. Forward and reverse amplification primers were used at a final concentration of 600 nM. Amplification was performed on a Tgradient thermocycler (Whatman Biometra, Goettingen, Germany). The following cycling parameters were used; 50°C for 30 min; 95°C for 15 min; 94°C for 45 s, 54°C for 45 s, and 72°C for 1 min, repeated for a total of 40 cycles; 72°C for 10 min; and 4°C hold. Amplification reactions were held on ice (4°C) until the thermocycler was stabilized at 50°C.

Electrophoresis. RT-PCRs were electrophoresed on 1% agarose gels for ~45 min at 100 V. Samples were identified as positive based on the presence of the expected 365-bp amplicon with ethidium bromide staining.

Product purification. Samples positive for amplification were purified by QIAquik spin column (QIAGEN, Inc., Valencia, CA) as per the manufacturer's protocol, prior to sequencing reactions.

Sequencing. Sequencing reactions were performed in duplicate using 5 μl of purified RT-PCR products as template, using DYEnamic ET Terminators (Amersham Biosciences, Piscataway, NJ) with both the forward and reverse sequencing primers. Cycle sequencing was performed on a Tgradient thermocycler (Whatman Biometra, Goettingen, Germany) with the following amplification parameters: 25 cycles, each consisting of 94°C for 20 s, 50°C for 15 s, and 60°C for 1 min. The temperature ramp parameter was set to 1°C/s rather than the typical 3 to 4°C/s for the thermocycler used. Sequence reactions were gel purified using 96-well plates (Edge Bioscience, Princeton, NJ) and were eluted in molecular biology-grade water. Sequence reactions products were electrophoresed on a MegaBACE 1000 DNA Sequencing system (Amersham Biosciences, Piscataway, NJ). Optimized parameters for the sequencing runs were injection at

TABLE 1. Results obtained from 17 rotavirus strains of known serotype

Strain	Serotype		Top database hit (strain, accession no.)	Avg % homology to top database hit
	Designated	Determined		
WI79	G1	G1	Wa, M21843	99.0
Wa	G1	G1	Wa, M21843	99.8
SC2	G2	G2	95B, U73956	98.9
DS1	G2	G2	No name, L20813	99.6
WI78	G3	G3	YO, D86284	97.0
P	G3	G3	Au-17, D86272	98.4
BrB	G4	G4	NB111/86, AF161819	98.7
CC4	G4	G4	GR828/86, AF170834	97.8
HCR3	G3 subtype	G3 subtype	HCR3, L21666	100
VR5-08	G5	G5	IAL-28, L79916	98.1
NG8-038	G8	G8	B37, J04344	98.9
WI61	G9	G9	US1212, AJ250272	95.2
RRV	Rhesus G3	Rhesus G3	RRV, M21650	99.2
EDIM	Murine G3	Murine G3	EDIM, AF039220	99.8
OSU	Porcine G5	Porcine G5	OSU, X04613	99.5
WC3	Bovine G6	Bovine G6	RF, X65940	98.0
B10-01	Bovine G10	Bovine G10	B223, X52650	98.5

2 kV for 10 s and electrophoresis at 6 kV for 150 min. Automated base-calling was performed using Cimmaron 1.5.3 Slim Phredify software, as supplied. Sequence data files were saved with the extension ".abd" for sequence analysis.

Sequence analysis. Generated sequences were imported into Sequencher (Gene Codes Corp., Ann Arbor, MI), and contiguous sequences of the forward and reverse reactions were assembled. Sequence disagreements were resolved, and a consensus sequence was exported for serotype determination by sequence homology analysis.

Serotype determination by sequence homology analysis. Generated sequences were compared to a database of rotavirus VP7 sequences using the "Align to Folder" function in MacVector (Accelrys, San Diego, CA). The results returned from this analysis (a list of serotypes and/or strains in order of sequence homology) determined the called serotype of the sample. The database consists of 309 VP7 sequences of rotavirus strains of known serotype (as designated in the submitted sequence). The database includes 273 strains from human hosts, with the distribution of serotypes as follows: serotype G1 (104 strains), G2 (48 strains), G3 (23 strains), G4 (28 strains), G5 (3 strains), G6 (4 strains, G8 (13 strains), G9 (33 strains), G10 (2 strains), G12 (6 strains), as well as 36 animal strains (simian, murine, bovine, equine, canine, and rabbit).

Limit of detection. The limit of detection for the assay was determined using rotavirus-negative stool samples spiked with known amounts of vaccine viruses. Samples were then carried through the assay, and the limit of detection was determined as the lowest level of rotavirus that yielded an amplification product and a correct serotype determination. Limit of detection experiments were carried out by both the QIAGEN and Boom silica methods.

RESULTS

Rotavirus strains of known serotype culture-adapted strains. Target VP7 sequences were generated from 17 stock culture supernatants representing both human and animal rotavirus strains (see Materials and Methods). Each sample was extracted in triplicate, and each of the extracted RNAs was amplified in triplicate. A total of nine sequences were generated for each strain. Designation of VP7 type based on sequence homology was performed and compared to the expected serotype for all sequences from all samples. Sequence-based VP7 typing correctly identified the VP7 type for all nine sequences from each of the 17 samples tested (Table 1). Results report the serotype identified and the accession number of the most closely related GenBank sequence. Additionally, the percent homology to the published sequence is reported as an average of the percent homology of all nine sequences. Comparisons of the sequences obtained from each sample showed >99% homology between each obtained sequence.

TABLE 2. Results obtained from five rotavirus vaccine strains (RotaTeq)

Strain	Serotype		Top database hit (strain, accession no.)	Avg % homology to top database hit
	Designated	Determined		
G1.vaccine	G1	G1	Wa, M21843	99.0
G2.vaccine	G2	G2	95B, U73956	99.4
G3.vaccine	G3	G3	YO, D86284	96.9
G4.vaccine	G4	G4	NB111/86, AF161819	98.6
P1.vaccine	Bovine G6	Bovine G6	RF, X65940	97.8

Samples were also evaluated by the Boom silica extraction method. All G types obtained from all samples were identical both to the expected type and to the type obtained using the initial QIAamp extraction.

Vaccine strains. Target VP7 sequences were obtained from each of the five bovine-human reassortant vaccine strains (the individual components of RotaTeq). A total of nine sequences from each of the vaccine strains were generated. All vaccine strains were identified with the correct VP7 type. A comparison of these sequences to known rotavirus sequences was performed with the results listed in Table 2.

Rotavirus-positive stool samples previously typed by monoclonal antibody. A total of 12 rotavirus samples that were previously serotyped with monoclonal antibodies were VP7 typed by this sequence-based method. The sequence-based VP7 typing method showed 92% agreement with expected serotypes based on the monoclonal antibody-based serotyping (Table 3). The lone sample with discordant results was a sample identified as a G4 serotype. Sequence analysis designated this as a G9 type. The high nucleic acid homology of this sequence to known G9 nucleic acid sequences indicated that the G4 monoclonal antibody may cross-react with G9 strains. These findings were consistent with previous reports of G9 strains reacting with G4 monoclonal antibodies (1, 6). A comparison of sequences from G4 and G9 strains showed 72% nucleic acid and 80% amino acid homology.

Rotavirus antigen-positive and antigen-negative stool samples. VP7 target sequences were generated from 20 stool samples collected at Children's Hospital of Philadelphia. Any viral RNA present was extracted from all samples in triplicate. Each

TABLE 3. Comparison of results from RT-PCR sequence-based typing with monoclonal antibody typing

Monoclonal antibody result	RT-PCR sequence result	Top database hit (strain, accession no.)	% Homology to top database hit
G1	G1	K18, D16319	99.0
G1	G1	TF14, AF183860	99.5
G1	G1	K18, D16319	99.0
G1	G1	TF14, AF183860	99.2
G1	G1	K18, D16319	98.5
G1	G1	K18, D16319	98.5
G1	G1	TF14, AF183860	99.5
G1	G1	K18, D16319	98.5
G2	G2	TD64, AF106292	99.5
G3	G3	TK08, D86281	97.6
G4	G4	GR828/86, AF170834	97.6
G4	G9	1527, AJ279082	99.5

```
G1.Type I     ILIFLISIILLNYILKSVTQIMDYIIYRFLLISVALFALTKAQNYGLNIPI
G1.Type II    -------------------R--------------------------------
G1.Type III   -------------------R--------S---F-------R-----I-L--

G1.Type I     TGSMDTVYSNSTQEGIFLTSLCLYYPTEASTQISDGEWKDSLSQMFLIKG
G1.Type II    ---------------V-------------------------------T--
G1.Type III   ------A-A-----------------------N-------------T--

G2.Type I     ILTILISIILLNYILKTITNTMDYIIYRFLLVIVLISPFVRTQNYGIYLPI
G2.Type II    ------F-------------------F----L-A-M---------------

G2.Type I     TGSLDAVYTNSTSETFLTSTLCLYYPAEAKNEISDDEWENTLSQLFLTKG
G2.Type II    --------------S----------T--------N--------------
```

FIG. 2. Comparison of amino acid sequences of G1 and G2 subtypes identified in the greater Philadelphia area, 1999 to 2000.

of the extracted RNA was amplified in triplicate. For five samples, no RT-PCR amplification was found in any of the nine replicate amplifications, all of which were previously identified as rotavirus negative by EIA. These five samples were also amplification negative by the Boom silica extraction method. The remaining 15 samples were positive for rotavirus based on RT-PCR amplification. Each of these samples yielded a product and the VP7 target sequence for each of the nine replicates per sample. For a given sample, VP7 types obtained were identical for all replicates (data not shown). Sequence homology comparisons within a sample sequence set were >99%.

Stool samples from 1999 to 2000 season, Philadelphia, PA. A total of 104 samples were analyzed by RT-PCR and sequence analysis (the samples were not analyzed by immunological methods). The following results were obtained: G1 (47%), G2 (10%), G4 (1%), and G9 (41%). Sample sequences were additionally analyzed for differences within serotypes. In analyzing VP7 types determined for a set of clinically obtained stool samples, we noticed that some samples that typed the same often had the highest percent homology to different database sequences. For example, samples identified as G1 were of at least two different subsequence groups. Of 47 samples, 40 had >95% homology to sequence TF14 (GenBank AF183860) (arbitrarily referred to as groups I and II, as the sequences fell into two distinct groups based on homology scores), while 4 of 47 had ~94% homology to sequence DC-6 (GenBank U26379) (referred to as group III). The average percent nucleic acid homology between these two subsequences was 92% (I or II versus III). Groups I and II appeared to be slightly different sequences with 96% homology. Amino acid sequence comparisons of the three groups appear in Fig. 2.

Samples typed as G2 also appeared to have more than one subtype. Of 11 samples typed as G2, 8 were arbitrarily designated type I, and 3 of 11 were designated as type II, with 93% homology between types. Amino acid sequence comparisons also appear in Fig. 2. Conversely, samples typed as G9 were of very low diversity. Forty-three of 43 samples showed homology to strain 1527 (GenBank AJ279082). Samples identified as G9 have 98 to 100% homology at the amino acid level with the strain 1527 database entry.

Limit of detection. For the limit of detection, rotavirus samples of known serotype and known titer were spiked into antigen- and RT-PCR amplification-negative stool samples. The limit of detection was defined as the level at which all amplification replicates were positive and were of the correct (expected) serotype after sequence analysis. Both RNA extraction methods described above were evaluated. The limit of detection for QIAamp-extracted samples in (particles per milliliter) was 10^5 for G1, G3, and G4 strains, and 10^6 for G2 and G6 (bovine) strains. The limit of detection for the Boom silica method-extracted samples (PFU per milliliter) was 10^4 for G1, G2, G3, and G4 strains, and 10^5 for the G6 (bovine) strain. The determined limit of detection indicates that the RT-PCR is able to reproducibly amplify RNA from approximately 100 to 10,000 particles of rotavirus per reaction. The limit of detection is equivalent or better than rotavirus detection using VP6 antibodies (9). For clinical samples evaluated, >95% of all antigen-positive samples were also RT-PCR positive by this method (data not shown).

DISCUSSION

The ability to determine the VP7 type of rotavirus strains in clinical trials and postvaccine licensure surveillance is important. Determining vaccine efficacy against serotypes included in the vaccines, as well as those not included, depends on accurate and sensitive typing of rotavirus strains. The method we have developed is an accurate and sensitive tool for the VP7 typing of rotavirus strain. We have shown that the assay can consistently detect and type a variety of VP7 types in both culture supernatants and stool samples. For samples of known rotavirus strains, 198 out of 198 observed results (153 laboratory strain amplifications and 45 vaccine strain amplifications) were identical to expected results. For the 32 stool samples analyzed, 5 samples were negative 100% of the time (45 amplifications), and 27 were positive 100% of the time (243 amplifications). Each of the nine generated serotypes (based on sequence homology) for any given sample was identical. Of the 12 stool samples previously serotyped by monoclonal antibody, only 1 was typed as a different serotype by this assay. In this case, the sample was typed by MAb EIA as VP7 type 4 but by our method as a G9, with 99% homology to GenBank sequence (strain 1527; GenBank AJ279082), indicating that the monoclonal antibody may recognize epitopes on both G4 and G9 strains.

The described VP7 typing assay has been demonstrated to consistently and correctly identify rotavirus strains. It has also been shown to be able to discriminate between two distinct subsequences within a given G type. This information may prove of some utility in the analysis of vaccine efficacy of different subtypes within serotypes. The ability to discriminate

between subtypes of a given serotype may also be useful for detailed epidemiological studies, allowing investigators to trace the transmission of single strains of rotavirus through the community or nosocomial or other single-source outbreaks, even in situations where only a single VP7 serotype is prevalent in a given season.

The assay is amenable to high-throughput methodologies, allowing its use in large-scale clinical trials and postvaccine licensure surveillance. With the use of 96-well formats for RNA extraction, RT-PCR, product purification, and sequence generation would allow nearly 100 samples to be analyzed in approximately 3 to 4 days. This method has been used to sequence thousands of samples from clinical trials, with an average throughput of 100 to 120 samples per week. This method has successfully G typed >95% of all clinical samples tested, with results obtained within 4 days of initial extraction. As results obtained are nucleic acid sequences, these results are unambiguous and require no human judgment as to the final result. Unlike other RT-PCR-based methods, this assay does not rely on gel patterns and migrations to determine VP7 types and therefore offers an advantage both in ease of use and from a regulatory standpoint. In addition, the data collected allow for complex molecular and phylogenetic analyses to be conducted with no additional data generation required.

REFERENCES

1. **Arista, S., G. M. Giammanco, S. De Grazia, C. Colomba, and V. Martella.** 2005. Genetic variability among G4 Italian human rotaviruses. J. Clin. Microbiol. **43:**1420–1425.
2. **Beards, G. M., A. D. Campbell, N. R. Cottrell, J. S. Peiris, N. Rees, R. C. Sanders, J. A. Shirley, H. C. Wood, and T. H. Flewett.** 1984. Enzyme-linked immunosorbent assays based on polyclonal and monoclonal antibodies for rotavirus detection. J. Clin. Microbiol. **19:**248–254.
3. **Block, S. L., T. Vesikari, M. G. Goveia, S. Rivers, B. Adeyi, M. J. Dallas, J. Boslego, and P. Heaton.** 2005. Efficacy, immunogenicity and safety of a polyvalent rotavirus vaccine (RotaTeq) at expiry potency in healthy infants. 39th National Immunization Conference, Washington, D.C., 21–24 March, 2005.
4. **Boom, R., C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
5. **Clark, H. F., P. A. Offit, R. W. Ellis, J. J. Eiden, D. Krah, A. R. Shaw, M. Pichichero, J. J. Treanor, F. E. Borian, L. M. Bell, and S. A. Plotkin.** 1996. The development of multivalent bovine rotavirus (strain WC3) reassortant vaccine for infants. J. Infect. Dis. **174**(Suppl. 1)**:**S73–S80.
6. **Coulson, B. S., J. R. Gentsch, B. K. Das, M. K. Bhan, and R. I. Glass.** 1999. Comparison of enzyme immunoassay and reverse transcriptase PCR for identification of serotype G9 rotaviruses. J. Clin. Microbiol. **37:**3187–3193.
7. **Das, B. K., J. R. Gentsch, H. G. Cicirello, P. A. Woods, A. Gupta, M. Ramachandran, R. Kumar, M. K. Bhan, and R. I. Glass.** 1984. Characterization of rotavirus strains from newborns in New Delhi, India. J. Clin. Microbiol. **32:**1820–1822.
8. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z. Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
9. **Herrmann, J. E., N. R. Blacklow, D. M. Perron, G. Cukor, P. J. Krause, J. S. Hyams, H. J. Barrett, and P. L. Ogra.** 1985. Enzyme immunoassay with monoclonal antibodies for the detection of rotavirus in stool specimens. J. Infect. Dis. **152:**830–832.
10. **Jin, Q., R. L. Ward, D. R. Knowlton, Y. B. Gabbay, A. C. Linhares, R. Rappaport, P. A. Woods, R. I. Glass, and J. R. Gentsch.** 1996. Divergence of VP7 genes of G1 rotaviruses isolated from infants vaccinated with reassortant rhesus rotaviruses. Arch. Virol. **141:**2057–2076.
11. **Kapikian, A. Z., J. Flores, Y. Hoshino, R. I. Glass, K. Midthun, M. Gorziglia, and R. M. Chanock.** 1986. Rotavirus: the major etiological agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. J. Infect. Dis. **153:**815–822.
12. **Kapikian, A. Z., and R. M. Chanock.** 1996. Rotaviruses, p. 1657–1708. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
13. **Kostouros, E., K. Siu, E. L. Ford-Jones, M. Petric, R. Tellier.** 2003. Molecular characterization of rotavirus strains from children in Toronto, Canada. J. Clin. Virol. **28:**77–84.
14. **Maunula, L., and C. H. von Bonsdorff.** 1998. Short sequences define genetic lineages: phylogenetic analysis of group A rotaviruses based on partial sequence of genome segments 4 and 9. J. Gen. Virol. **79:**321–332.
15. **O'Halloran, F., M. Lynch, B. Cryan, and S. Fanning.** 2002. Application of restriction fragment length polymorphism analysis of VP7-encoding genes: fine comparison of Irish and global rotavirus isolates. J. Clin. Microbiol. **40:**524–531.
16. **Piec, T. L., and E. A. Palombo.** 1996. Sequence comparison of the VP7 of serotype G2 rotaviruses from diverse geographical locations. DNA Seq. **9:**369–373.
17. **Vesikari, T., H. F. Clark, P. Offit, F. Schodel, M. J. Dallas, P. Heaton, G. Golm, D. Krah, A. Shaw, A. Garbarg-Chenon, K. M. Kaplan, and J. Sadoff.** 2003. The effect of dose and composition of a pentavalent rotavirus reassortant vaccine (RotaTeq) upon efficacy and immunogenicity in healthy infants. 37th National Immunization Conference, Chicago, IL, 17–20 March, 2003.
18. **Ward, R. L., M. M. McNeal, J. D. Clemens, D. A. Sack, M. Rao, N. Huda, K. Y. Green, A. Z. Kapikian, B. S. Coulson, R. F. Bishop, H. B. Greenberg, G. Gerna, and G. M. Schiff.** 1991. Reactivities of serotyping monoclonal antibodies with culture-adapted human rotaviruses. J. Clin. Microbiol. **29:**449–456.
19. **Ward, R. L., D. I. Bernstein, V. E. Smith, D. S. Sander, A. Shaw, J. J. Eiden, P. Heaton, P. A. Offit, and H. F. Clark.** 2004. Rotavirus immunoglobulin A responses stimulated by each of 3 doses of a quadrivalent human/bovine reassortant rotavirus vaccine. J. Infect. Dis. **189:**2290–2293.