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Evaluation of BBL™ Sensi-Discs™ and FTA® cards as sampling devices for detection of rotavirus in stool samples

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Abstract

Rotavirus is the most important cause of severe childhood gastroenteritis worldwide. Rotavirus vaccines are available and rotavirus surveillance is carried out to assess vaccination impact. In surveillance studies, stool samples are stored typically at 4°C or frozen to maintain sample quality. Uninterrupted cold storage is a problem in developing countries because of power interruptions. Cold-chain transportation of samples from collection sites to testing laboratories is costly. In this study, we evaluated the use of BBL™ Sensi-Discs™ and FTA® cards for storage and transportation of samples for virus isolation, EIA, and RT-PCR testing. Infectious rotavirus was recovered after 30 days of storage on Sensi-Discs™ at room temperature. We were able to genotype 98–99% of samples stored on Sensi-Discs™ and FTA® cards at temperatures ranging from −80°C to 37°C up to 180 days. A field sampling test using samples prepared and shipped from Cameroon, showed that both matrices yielded 100% genotyping success compared with whole stool and Sensi-Discs™ demonstrated 95% concordance with whole stool in EIA testing. The utilization of BBL™ Sensi-Discs™ and FTA® cards for stool sample storage and shipment has the potential to have great impact on global public health by facilitating surveillance and epidemiological investigations of rotavirus strains worldwide at a reduced cost.

Keywords

rotavirus; stool; storage; shipment; ambient; virus isolation; enzyme immunoassay; genotyping

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Disclosure of Potential Conflicts of Interest

The authors report no potential conflicts of interest.

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1. Introduction

Group A rotavirus is the most common cause of pediatric gastroenteritis and is estimated to kill over 453,000 children annually, mostly in low-income countries (Tate et al., 2012). Two live-attenuated oral vaccines, RotaTeq® (Chandran and Santosham, 2008) and Rotarix® (Ward and Bernstein, 2009), have shown efficacy against severe rotavirus disease in large clinical trials. The World Health Organization (WHO) has recommended routine immunization against rotavirus in all countries. Between 2006 and 2015, 75 countries introduced rotavirus vaccines into national immunization programs, and subsequently, the burden of severe rotavirus disease decreased substantially in those countries (http://sites.path.org/rotavirusvaccine/rotavirus-advocacy-and-communications-toolkit/country-introduction-maps-and-list/; Patel et al., 2012; Steele, 1998).

Rotavirus surveillance is carried out to monitor circulating rotavirus genotypes, detect possible emerging or novel rotavirus strains, and assess the impact of vaccination (Gentsch et al., 2009; Hull et al., 2011; Santos and Hoshino, 2005). In surveillance studies, stool samples from gastroenteritis cases are transported to reference laboratories for confirmatory testing and genetic characterization and are typically stored at 4°C or frozen to maintain sample quality. Uninterrupted cold storage is a problem in many laboratories due to power interruptions, particularly in developing countries. Cold-chain transportation of samples from collection sites to testing laboratories requires dry ice or cold-packs thus making international shipping very expensive and, in some locations, dry ice cannot be obtained easily.

Routine diagnosis of rotavirus is often based on rapid detection of group A rotavirus antigen in stool, generally by enzyme immunoassay or latex agglutination assay (van Doorn et al., 2009). Since these serological assays do not distinguish accurately between different rotavirus serotypes (Gouvea et al., 1990), genotyping is typically performed for analysis of the rotavirus genomic RNA. Reverse transcription PCR (RT-PCR) is used to detect rotavirus RNA and identify VP7 (G) and VP4 (P) genotypes (Matthijnssens et al., 2008). In addition to conventional RT-PCR, quantitative real-time RT-PCR (qRT-PCR) assays offer several advantages for detection such as increased sensitivity, higher-throughput and faster turn-around time, as well as the ability to perform quantification of viral loads (Mijatovic-Rustempasic et al., 2013).

In many rotavirus surveillance studies and/or vaccine trials, stool samples need to be transported to reference laboratories for genetic characterization. Currently, this involves shipping stool-filled containers or soiled diapers under cooled and biohazardous conditions. In field situations in some developing countries, where electricity and/or skilled laboratory technicians are not available, the simple task of sample collection, aliquoting, and freezing can present as a challenge. To remedy these issues, Rahman and coworkers described the use of SDS-EDTA pretreated chromatography filter paper strips for collection, transport, and storage of rotavirus samples (Rahman et al., 2004). Although rotavirus RNA was stable for up to 30 days at room temperature and higher temperatures, no live virus or rotavirus antigen were detected in the culture supernatant inoculated with the pretreated strip. Shulman and coworkers reported the use of rapid test strips (dipsticks) for collection of

rotavirus samples (Shulman et al., 2011) and was able to G and P genotype 40–92% of rotavirus samples that had been stored on dried dipsticks at room temperature for up to 5 years. Several bioscience companies also offer commercially available RNA stabilization and storage reagents (e.g., RNA*later*® Life Technologies, Grand Island, NY USA) that protect cellular RNA and inactivate RNase activity to allow later processing. However, these collection and storage methods are purposely designed for RNA isolation and molecular testing (genotyping and/or sequencing), do not permit antigen detection and/or virus isolation from stool samples, and are costly. For example, RNA*later*® costs US$1.87 per sample if purchased in bulk (2015 list price from Life Technologies.)

BBL™ Sensi-Disc™ antimicrobial susceptibility test discs (Sensi-Disc™, Fisher Scientific, MA USA) were intended to be used for semi-quantitative *in vitro* antibiotic susceptibility testing of common, rapidly growing and certain fastidious bacterial pathogens by the agar disc diffusion test procedure (BDDiagnostic, 2011). Sensi-Discs™ are available impregnated with 30mg of Cefepime, a semi-synthetic fourth-generation cephalosporin with broad spectrum of activity against gram-positive and gram-negative bacteria (Yahav et al., 2007) that are commonly found in stool samples. We hypothesize that the presence of Cefepime would inhibit the growth of stool bacteria on these discs thus preserving rotavirus particles, proteins, and RNA for subsequent testing.

The Whatman FTA® card (GE Healthcare, UK) is a commercial product for the collection, storage, preservation, and processing of nucleic acids. The paper contains proprietary chemicals which lyse cellular material and fix and preserve DNA and RNA within a fiber matrix (http://www.whatman.com.cn/upload/starjj_200941413246.pdf). Once immobilized on the cards, the samples are no longer infectious and thus do not pose a biohazard (Picard-Meyer, Barrat, and Cliquet, 2007). DNA bound to FTA® cards can be stored at room temperature for years with high stability (http://www.whatman.com.cn/upload/starjj_2009414132332.pdf). The FTA® cards have been used effectively for a variety of infectious agents, such as human papillomavirus, malaria, avian influenza, rabies, and *Mycobacterium leprae* (Aye et al., 2011; Gonzalez et al., 2012; Keeler et al., 2012; Picard-Meyer et al., 2007; Zhong et al., 2001) but have not previously been evaluated for the detection of rotaviruses.

The purpose of this study is to evaluate two novel methods for collection, storage and shipping of stool specimens for rotavirus testing that will potentially allow us to perform EIA, genotyping, as well as isolation of live virus (Sensi-Disc™). These two novel methods will potentially allow researchers to store and ship specimens at ambient temperature which will greatly reduce shipping costs and avoid problems with storage and/or shipping when the cold chain is interrupted. In this study, different incubation temperatures of a cultured rotavirus strain and rotavirus positive stool samples were investigated for their effect on the stability of rotaviruses for subsequent detection using EIA, qRT-PCR and conventional RT-PCR for both the Sensi-Disc™ and the FTA® card. We believe that the wide range of temperatures tested simulate different conditions that the samples might encounter during storage and shipment internationally.

2. Materials and Methods

2.1 Propagation of virus

Reference rotavirus strain Wa (G1P[8], ATCC VR-2018) was propagated in the monkey kidney cell line MA-104 (ATCC CRL-2378). Briefly, MA-104 cells were grown in monolayer cultures using Iscove's Modified Dulbecco's Media (IMDM) (GIBCO© Laboratories, Grand Island, NY USA). A standard plaque assay was used to determine the titer of Wa stocks (Albert and Bishop, 1984; Wyatt et al., 1983). Aliquots of Wa lysate product at 10^7 PFU/mL were stored at −80°C until further use.

2.2 Sample preparation

Cell culture lysates (40μL) or rotavirus positive stool samples were spotted onto the center of 6 mm diameter Sensi-Disc™ discs (BD, Cat. No 231695) and 10 mm diameter pre-punched FTA® cards (Whatman, Cat. No WB120305). The FTA cards were pre-punched from card stock in the lab with a sterilized 10 mm hole punch and stored in zipper seal bags until used. The samples then were allowed to dry at room temperature (22±2°C) inside a biosafety cabinet overnight. The samples (1–3 per experiment) were then stored in 2 x 3 inch polyethylene zipper seal sample bags (Fisher Healthcare, PA USA) with a SORBIT 0.5G desiccant canister (AGM Container Controls, AZ USA) inside cardboard boxes at 5 different temperatures (37°C, 22±2°C, 4°C, −20°C, and −80°C) for 1–180 days. The wide range of temperatures was chosen to mimic temperatures that one might encounter when samples are stored and then shipped internationally on dry ice, cold packs, or ambient temperature.

2.3 Viability of rotavirus on FTA cards and Sensi-Discs

To study the viability of rotavirus on Sensi-Discs™ and FTA® cards, replicates of 40μL of Wa lysate product were dried on Sensi-Discs™ and FTA® cards. Virus isolation was attempted from Sensi-Discs™ and FTA® cards after 24 hours of incubation at room temperature and after 30 days (Sensi-Discs™ only). Each Sensi-Disc™ and FTA® card was placed into a 1.5mL Eppendorf tube containing 400μL of IMDM, vortexed and incubated at room temperature for 4 hours. Trypsin-activated supernatants were used to infect MA-104 cells as described previously (Albert and Bishop, 1984). A second and third blind passage was carried out in case of negative results on initial virus isolation attempts. The elution and all passages were tested for the presence of rotavirus antigen by performing an enzyme-linked immunosorbent assay (EIA) using the Premier™ Rotaclone® kit (Meridian BioScience, Cincinnati, OH USA). RNA was extracted from the supernatant using the MagMax 96 Viral RNA Isolation kit (Applied Biosystems, Inc., Foster City, CA USA) on the KingFisher Flex Magnetic Particle Processor (Thermo Fisher Scientific, Pittsburgh, PA USA) according to the manufacturer’s instructions. NSP3 gene qRT-PCR was conducted using an ABI 7500 Fast real time PCR instrument (Applied Biosystems, Inc., Foster City, CA USA) and rTth enzyme (Life Technologies, Grand Island, NY USA) as described previously (Mijatovic-Rustempasic et al., 2013). Positive-control RNA template was generated from the Rotavirus A NSP3 gene of laboratory strain Wa RNA as described previously (Mijatovic-Rustempasic et al., 2013); 10-fold dilutions of the transcript from

10^{-4} to 10^{-10} were prepared tested by qRT-PCR to generate a standard curve for calculation of copy numbers (Mijatovic-Rustempasic et al., 2013).

2.4 Stability of rotavirus nucleic acid on Sensi-Discs™ and FTA® cards

To study the stability of rotavirus nucleic acid on Sensi-Disc™ and FTA® cards, replicates (3 per data point) of 40 μL of Wa lysate product were spotted onto Sensi-Discs™ and FTA® cards, dried, and then stored as described above for 28 days. Each Sensi-Disc™ and FTA® card then was eluted in 400μ.L of TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) at room temperature for 4 hours in a 1.5mL Eppendorf tube. RNA extraction was performed on the elutions using a MagMAX™ Viral RNA isolation kit (Applied Biosystems, Inc., Foster City, CA USA) according to the manufacturer's instructions and then tested with the NSP3 gene qRT-PCR as described previously (Mijatovic-Rustempasic et al., 2013).

2.5 VP7 and VP4 genotyping of rotavirus

To evaluate the molecular detection and VP7 and VP4 genotyping of samples inoculated onto Sensi-Discs™ and FTA® cards, replicates of three rotavirus positive stool samples were spotted onto Sensi-Discs™ and FTA® cards, dried, and stored as described above. The rotavirus positive samples selected were liquid, semi-solid, and solid, representing the different consistencies of stool that one would encounter when testing. Each Sensi-Disc™ and FTA® card was eluted in 400μ.L of TE buffer and extractions were performed on elutions as described above. Elutions were tested for rotavirus antigen by performing EIA using the Premier™ Rotaclone® kit. RNA extraction was performed as described and RT-PCR genotyping and sequencing of rotavirus VP7 and VP4 genes were carried out as described previously (Hull et al., 2011) on randomly selected samples to ensure there was no cross contamination from other rotavirus strains during the study.

2.6 Field sampling test

For a field sampling evaluation, 50 stool samples (40 rotavirus positive and 10 rotavirus negative) collected at the rotavirus sentinel surveillance site at the Mother and Child Center, Chantal Biya Foundation Yaoundé, Cameroon and previously screened for rotavirus antigen as well as genotyped for VP7 and VP4, were dried onto Sensi-Discs™ and FTA® cards as described and shipped 10 days later at ambient temperature to the Centers for Disease Control and Prevention, Atlanta GA USA. Aliquots of each whole stool sample were shipped also on dry ice/ice packs. After preparing 10% suspensions of whole stool in PBS, EIA, sample elution, RNA extraction, and RT-PCR genotyping for VP7 and VP4 genes were carried out on all samples as described previously.

2.7 Statistics

Statistical analysis was performed by using EXCEL 2010.

3. Results

3.1 Viability of rotavirus on Sensi-Discs™ and FTA® cards

Virus isolation was attempted from elutions of Wa spotted Sensi-Discs™ and FTA® cards. For Sensi-Discs™, both viral antigens and viral RNA were detected in the eluted material,

as well as in successive passages inoculated with the elution of Sensi-Discs™ after 1 day of incubation. The same result was observed from elution of Sensi-Discs™ after 30 days of incubation, showing that the virus remained intact and infectious after being dried on Sensi-Discs™ with prolonged storage at room temperature (Table 1). For FTA® cards, both viral antigens and viral RNA were detected in the eluted material on day 1 but not in cell culture passages inoculated with the FTA® card eluate, indicating that live virus was not recovered from the matrix. Since live virus could not be recovered from the FTA® cards on day 1, virus isolation was not attempted on day 30. There was no apparent change in appearance of both Sensi-Discs™ and FTA® cards after 30 days of incubation.

3.2 Stability of rotavirus nucleic acid

To test the stability of rotavirus nucleic acid dried on Sensi-Discs™ and FTA® cards, RNA extraction was performed on eluted material and tested by NSP3 gene qRT-PCR for quantification of recovered RNA. Viral RNA was preserved with little loss over 28 days of storage at various incubation temperatures for both the Sensi-Discs™ and FTA® cards (Figure 1). The mean copy number per μL of stool ranged within a 3 fold difference in RNA concentration recovered after 28 days of storage at 37°C, for both the Sensi-Discs™ and FTA® cards. It should be noted that there was an approximate 10-fold decrease in RNA recovered after the sample was dried on the Sensi-Discs™ overnight, but was not observed for RNA dried on FTA® cards.

3.3 VP7 and VP4 genotyping of rotavirus

For molecular characterization of rotavirus eluted from FTA cards and Sensi-Discs™, we attempted genotyping for the VP7 and VP4 genes. Sequence confirmation of both genes was performed on randomly selected samples to ensure there was no cross contamination from other rotavirus strains during the study. Three rotavirus positive stool samples were tested with 3 replicates at each time point for each storage temperature. We were able to genotype 312/315 (99%) and 253/260 (98%) of the samples that were recovered from Sensi-Disc™ and FTA® cards, respectively (Table 2). Weaker PCR product bands were seen by gel electrophoresis for nucleic acid stored on Sensi-Discs™ and FTA® cards that were stored at 37°C (data not shown), and 3 out of the 9 samples were not genotyped when nucleic acid was stored on FTA® cards for 180 days at 37°C (Table 2).

3.4 Field sampling test

For the field sampling test, we were able to genotype all 40 rotavirus positive samples regardless of which condition the samples were shipped (Table 3). However, 2 (5%) and 7 (17.5%) out of 40 samples that were dried on Sensi-Discs™ and FTA® cards, respectively, were negative by EIA upon retest; all 40 whole stools were positive when retested (Table 3). The 10 rotavirus negative samples, included to ensure that there were no false positives or that cross contamination didn't occur during sampling, all tested negative for EIA and genotyping in all three storage formats (Table 3). Virus isolation was not attempted because of the inefficiency and complexity of isolating rotavirus from stool. The total cost for the Sensi-Disc™ and FTA® cards shipment (US$138.47) at ambient temperature from Yaounde, Cameroon to Atlanta, USA was 5.4 fold less than the cost (US$ 747.79) of the dry ice shipment.

4. Discussion

In rotavirus surveillance studies, stool samples from gastroenteritis cases typically are transported from sentinel sites to reference laboratories for EIA testing and genetic characterization. In this study, we dried stool samples onto Sensi-Discs™ and FTA® cards in an attempt to replace high-cost cold-chain storage and transportation, which is often difficult to maintain developing countries. We decided to store dried samples over a large range of temperatures over an extended period of time in order to mimic temperature(s) that samples might encounter during shipment internationally. Also, we decided to elute at room temperature for a longer period of time, versus at higher temperature for a shorter period of time like most standard protocols recommended by manufacturers, in consideration of some laboratories in developing countries which may not have the luxury of a heating apparatus. The two matrices evaluated in this study will allow researchers to store and ship specimens at ambient temperature with greatly reduced shipping costs and avoid problems with storage and/or shipping where the cold chain might be interrupted. Although other sampling devices (Rahman et al., 2004; Shulman et al., 2011) and commercially available RNA stabilization storage reagents such as RNA*later*® are available, we have shown that RNA can be stably stored on FTA® card and Sensi-Disc™ at temperatures as high as 37°C for up to 180 days. FTA® cards allow transportation of samples without special handling (i.e., biohazardous material shipping). Sensi-Discs™ allows antigen detection and potentially virus isolation from stool samples which could be important in some studies.

This study revealed stability of rotavirus RNA on FTA® cards and Sensi-Discs™ stored at different temperatures over prolonged incubation periods. RNA extracted from elutions of FTA® cards were detectable by qRT-PCR, as well as by conventional genotyping and sequencing. For FTA® cards, complete inactivation of cultured strain Wa was demonstrated along with negative results for rotavirus antigen detection. . This demonstrated that samples spotted on FTA® cards are non-infectious and can be sent internationally without biohazard shipping at ambient temperature. Although Sensi-Disc™ requires biohazard shipment packaging, it serves as a possible alternative when virus re-isolation is desired as we were able to propagate cultured strain Wa from Sensi-Disc™ elutions even after 30 days of storage.

The loss of sensitivity of RNA detection compared to conventional methods of sample transport and loss of some EIA positive samples after a field sampling test are limitations. However, initial EIA testing was routinely done on site, and (EIA) positive samples were usually sent for further molecular analysis. For EIA positive samples, virus concentration has been estimated to be minimum of 10^6 virus particles/mL, therefore a 10 fold loss should not affect the quality and accuracy of molecular results which can detect rotavirus RNA at much lower concentrations.

To the best of our knowledge, this is the first study using FTA® cards for rotavirus sampling and is the first use of Sensi-Discs™ as a sampling device. The use of Sensi-Disc™ and FTA® cards will be of potential great value for transport of samples at a greatly reduced shipping cost without the necessity of a cold chain. Sometimes delivery delays of dry ice shipments due to customs clearance delays or other issues can result in thawing of rotavirus

positive stool samples and degradation of sample quality. The temperature stability of samples collected on these discs also will allow laboratories to store specimens without using refrigerators or freezers. In developing countries laboratories, where uninterrupted cold storage is often a problem due to electrical interruptions, the use of these discs can greatly improve sample quality since the stool samples would not be subjected to repeated temperature changes. In addition, the reduced costs of shipping samples to reference laboratories for analysis will enhance the ability to maintain long term surveillance in vaccination monitoring programs, as we demonstrated a greater than 80% reduction in shipping costs in our field test. The cost per sample is also very low for the FTA card punched discs and Sensi-Discs™, US$0.29 and US$0.26 per disc, respectively. This approach will support and strengthen rotavirus surveillance and epidemiology programs as well as improve our laboratory services. The use of FTA® cards would be advantageous for the transportation of material from site to site and during outbreak investigations, would be fast and safe, while the use of Sensi-Discs™ can replace the routine collection of stool samples in vials or larger containers that would eventually be tested by virus isolation, EIA, and genotyping. We believe that it would be highly advantageous to use FTA® cards as sampling device in genotyping studies and Sensi-Disc™ in studies that require EIA testing, genotyping, and possibly virus isolation.

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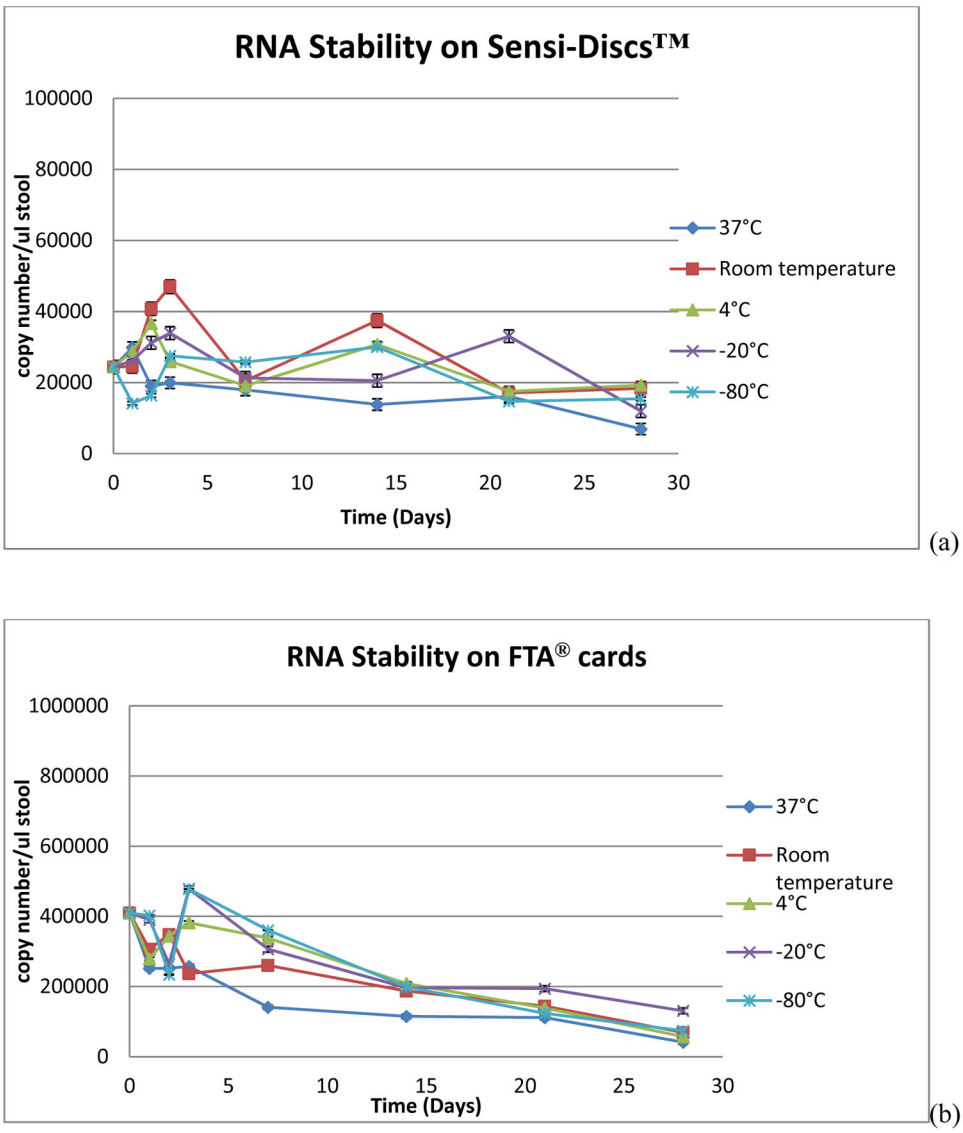

Figure 1.

Results of qRT-PCR targeting the NSP3 gene (copy number/μL stool) using RNA extracted from (a) Sensi-Discs™ (b) FTA® cards spotted with Wa lysate product and incubated at various temperatures for a duration of 28 days. Each data point represents 3 replicates; error bars are equal to one standard deviation.

Table 1

Rotavirus antigen and RNA detection from elutions and successive passages inoculated with elutions from Sensi-Discs™ and FTA® cards.

			EIA (Rotaclone®[a])				NSP3 gene qRT-PCR[c]			
Sample Device	**Incubation Period**	**Virus Isolation**	**Elution**	**P1[b]**	**P2**	**P3**	**Elution**	**P1**	**P2**	**P3**
Sensi-Disc™	1	Positive	+	+	+	+	$1.05\times10^{4}\pm249$	$2.37\times10^{4}\pm891$	$3.05\times10^{6}\pm763$	$2.90\times10^{6}\pm872$
Sensi-Disc™	30	Positive	+	+	+	+	$9.06\times10^{3}\pm302$	$1.75\times10^{5}\pm328$	$1.86\times10^{6}\pm1458$	$2.16\times10^{6}\pm1874$
FTA®card	1	Negative	+	−	−	−	$2.5\times10^{4}\pm468$	−	−	−

[a] Rotaclone® OD value of ≤ 0.15 is considered negative according to manufacturer protocol, results were consistent for 5 replicates.

[b] P1 = cell culture passage #1; P2 = cell culture passage #2; P3 = cell culture passage #3.

[c] Mean copy number/μL of stool ± standard deviation (SD) of 5 replicates.

Table 2

Number of stool samples spotted on Sensi-Discs™ and FTA® cards that were detected by RT-PCR genotyping of VP7 and VP4 genes after incubation at different temperatures.

Incubation Period (days)	Sensi-Discs™					FTA®Cards				
	Incubation Temperature (°C)					Incubation Temperature (°C)				
	37	22±2	4	−20	−80	37	22±2	4	−20	−80
0	9/9[a]	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
1	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9
7	9/9	9/9	9/9	9/9	9/9	8/9	9/9	8/9	9/9	9/9
14	9/9	9/9	9/9	9/9	9/9	9/9	8/9	9/9	9/9	9/9
30	8/9	9/9	8/9	9/9	9/9	9/9	9/9	9/9	9/9	8/9
60	9/9	9/9	9/9	9/9	9/9	ND[b]	ND	ND	ND	ND
180	8/9	9/9	9/9	9/9	9/9	6/9	9/9	9/9	9/9	9/9

[a] Three rotavirus positive stool samples were used with 3 replicates each, totaling 9 samples.

[b] ND: not done.

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Table 3

Results of RT-PCR genotyping for VP7 and VP4 genes of field samples transported as whole stool and on Sensi-Discs™ and FTA® cards.

Sample ID	Stool Type	EIA (before shipment)	Whole Stool		Sensi-Disc™		FTA® cards	
			EIA	G and P Genotypes	EIA	G and P Genotypes	EIA	G and P Genotypes
1	Solid	+	+	G2P[4]	+	G2P[4]	+	G2P[4]
2	Semi-solid	+	+	G2P[4]	+	G2P[4]	+	G2P[4]
3	Semi-solid	+	+	G6P[6]	+	G6P[6]	+	G6P[6]
4	Liquid	+	+	G6P[6]	+	G6P[6]	−	G6P[6]
5	Solid	+	+	G10P[6]	+	G10P[6]	+	G10P[6]
6	Semi-solid	+	+	G6P[6]	+	G6P[6]	+	G6P[6]
7	Semi-solid	+	+	G12P[8]	+	G12P[8]	+	G12P[8]
8	Liquid	+	+	G12P[8]	−	G12P[8]	−	G12P[8]
9	Semi-solid	+	+	G2P[4]	+	G2P[4]	+	G2P[4]
10	Liquid	+	+	G9P[8]	+	G9P[8]	+	G9P[8]
11	Liquid	+	+	G4P[8]	+	G4P[8]	+	G4P[8]
12	Liquid	+	+	G2P[6]	+	G2P[6]	+	G2P[6]
13	Semi-solid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
14	Liquid	+	+	G4P[6]	+	G4P[6]	+	G4P[6]
15	Solid	+	+	G2P[6]	+	G2P[6]	+	G2P[6]
16	Liquid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
17	Solid	+	+	G4P[6]	+	G4P[6]	+	G4P[6]
18	Liquid	+	+	G1P[8]	+	G1P[8]	+	G1P[8]
19	Liquid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
20	Liquid	+	+	G2P[6]	+	G2P[6]	+	G2P[6]
21	Liquid	+	+	G1P[8]	+	G1P[8]	+	G1P[8]
22	Liquid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
23	Solid	+	+	G4P[6]	+	G4P[6]	+	G4P[6]
24	Solid	+	+	G4P[6]	+	G4P[6]	+	G4P[6]
25	Semi-solid	+	+	G12P[8]	+	G12P[8]	+	G12P[8]
26	Liquid	+	+	G1P[6]	+	G1P[6]	+	G1P[6]
27	Semi-solid	+	+	G1P[8]	+	G1P[8]	+	G1P[8]

Sample ID	Stool Type	EIA (before shipment)	Whole Stool		Sensi-Disc™		FTA® cards	
			EIA	G and P Genotypes	EIA	G and P Genotypes	EIA	G and P Genotypes
28	Liquid	+	+	G1P[8]	+	G1P[8]	−	G1P[8]
29	Solid	+	+	G2P[6]	+	G2P[6]	+	G2P[6]
30	Semi-solid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
31	Liquid	+	+	G1P[8]	+	G1P[8]	−	G1P[8]
32	Semi-solid	+	+	G4P[6]	+	G4P[6]	+	G4P[6]
33	Liquid	+	+	G1P[8]	−	G1P[8]	−	G1P[8]
34	Semi-solid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
35	Liquid	+	+	G1P[8]	+	G1P[8]	+	G1P[8]
36	Liquid	+	+	G3P[6]	+	G3P[6]	−	G3P[6]
37	Liquid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
38	Liquid	+	+	G1P[4]	+	G1P[4]	+	G1P[4]
39	Liquid	+	+	G1P[6]	+	G1P[6]	−	G1P[6]
40	Semi-solid	+	+	G3P[6]	+	G3P[6]	+	G3P[6]
41	Neg	−	−	Neg	−	Neg	+	Neg
42	Neg	−	−	Neg	−	Neg	+	Neg
43	Neg	−	−	Neg	−	Neg	+	Neg
44	Neg	−	−	Neg	−	Neg	+	Neg
45	Neg	−	−	Neg	−	Neg	+	Neg
46	Neg	−	−	Neg	−	Neg	+	Neg
47	Neg	−	−	Neg	−	Neg	+	Neg
48	Neg	−	−	Neg	−	Neg	+	Neg
49	Neg	−	−	Neg	−	Neg	+	Neg
50	Neg	−	−	Neg	−	Neg	+	Neg