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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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A B S T R A C T

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.

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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 (EIA) 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

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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 et al. (2004)described the use of SDS-EDTA pretreated chromatography filter paper strips for collection, transport, and storage of rotavirus samples. 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 et al. (2011) reported the use of rapid test strips (dipsticks) for collection of rotavirus samples 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 30 mg 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 et al., 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 (40 μL) 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 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 × 3 inch polyethylene zipper seal sample bags (Fisher Healthcare, PA USA) with a SORBIT 0.5 G 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 40 μL of Wa lysate product were dried on Sensi-Discs™ and FTA® cards. Virus isolation was attempted from Sensi-Discs™ and FTA® cards after 24 h of incubation at room temperature and after 30 days (Sensi-Discs™ only). Each Sensi-Disc™ and FTA® card was placed into a 1.5 mL Eppendorf tube containing 400 μL of IMDM, vortexed and incubated at room temperature for 4 h. 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 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