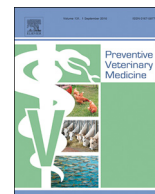




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A systematic review of FTA cards® as a tool for viral RNA preservation in fieldwork: Are they safe and effective?



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ABSTRACT

BackgroundDetection and characterization of viral RNA pathogens from fieldwork are challenging due to the instability of the RNA molecule. FTA cards® have proved useful for sample storage and latter identification of pathogens with importance for agricultural, animal and human health: however, for optimal handling, processing, and biosafety measures are not well-established.

ObjectiveThis systematic review aims to summarize the reported effectiveness of FTA cards® for storage and transport of viral RNA, as well as the conditions for their handling and use in downstream processes. Finally, the biosafety measures required to protect researchers and clinical lab workers are considered.

MethodsWe performed a systematic review following the PRISMA statement. We searched MEDLINE (PubMed), Scopus and Web of Science using the keywords “FTA cards” AND “RNA”. Articles were screened by title and abstract, and after examination of inclusion and exclusion criteria, relevant information was extracted. The quality of the studies was assessed, and the evidence was qualitatively summarized.

ResultsA total of 175 records were retrieved, and 11 additional documents were found by checking references of the eligible articles. A total of 47 articles were included. Samples from animals accounted for 38.3% of the publications, which identified viruses that cause disease in poultry, wild birds, suids, or bovids. Three different methods for RNA extraction were reported. Other factors that vary across reports include the size of RNA amplicon, storage temperature, and duration of storage. Only fourteen articles tested the inactivation of the virus on the FTA card®, and in one case, the virus remained infective.

ConclusionFTA cards® could be a suitable option for RNA virus storage and transport for fieldwork in areas where proper conditions for RNA preservation are difficult to achieve. Three different protocols have been used for RNA detection from this matrix. Biospecimens in the form of dried blood spots should be considered potentially infectious unless specifically treated to inactivate viral pathogens.

1. Introduction

1.1. Rationale

Flinders Technology Associates (FTA) cards® have been widely used for DNA preservation and analysis, particularly in forensic sciences and genetic studies (Hsiao et al., 1999; Smith and Burgoyne, 2004). This

technology consists of filter paper impregnated with a patented chemical mixture that contains chemical denaturants and a free radical scavenger. The chemical mixture lyses cells and organelles, prevents overgrowth of bacteria, and denatures proteins. The DNA is captured in the matrix and remains tightly bound while proteins and inhibitors are washed. This keeps DNA stable during long-term storage at room temperature (GE Healthcare, 2019).

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This method is attractive for several applications, including research of bacterial genetics, as DNA can be maintained at ambient temperature (Reeve et al., 2018). FTA cards® have also been used for storing samples and molecular detection of both DNA and RNA viruses. Recently, they have been proposed as an alternative to liquid media in the screening of cervical cancer for HPV detection (Dong et al., 2017), and improved biosafety has been cited as an advantage in the storage and transport of specimens containing avian influenza virus (Kraus et al., 2011). However, viral RNA is an unstable molecule, susceptible to ubiquitous RNases, complicating its transport and detection.

This challenge is particularly relevant in settings where proper technical support for RNA handling may not be available (hereafter referred to as fieldwork). Although some studies have evaluated the FTA® cards for viral RNA preservation (Ndunguru et al., 2005; Muthukrishnan et al., 2008; Flies et al., 2015), the optimal conditions for their handling have not been extensively studied. Notwithstanding, it is crucial to determine the appropriate temperature and maximum time for storing RNA samples on FTA cards, as well as their biosafety, in order to fieldwork with these pathogens.

1.2. Objectives

In this context, this systematic review aims to assess published studies that used FTA cards® as a mean for viral RNA preservation before molecular detection.

2. Methods

2.1. Literature search

For conducting this systematic review, we followed the recommendations of the PRISMA statement (Moher et al., 2009). In June 2018, MEDLINE (PubMed), Scopus and Science Citation Index (Web of Knowledge) were searched to identify potentially relevant articles using the search strategy (“FTA cards” AND “RNA”). No limit was set for the year, and only articles published in English or Spanish were assessed. The references of the included articles were also reviewed looking for eligible articles.

2.2. Study eligibility and selection

Two different authors conducted the review independently, and it was completed on July 1st, 2018. The resulting articles were initially selected by title and abstract to identify possibly eligible studies according to the selected criteria. Subsequently, duplicates were excluded, and two authors reviewed full-text of the eligible articles. When there was disagreement in the inclusion of a study, a third researcher made the final decision. We assessed studies that used FTA cards® for storage and later isolation of RNA of viral origin with subsequent amplification. Different conditions of storage, RNA extraction, or cDNA synthesis were separately summarized when the information was available. We excluded opinion articles, letters to the editor that did not present original data, reviews, as well as articles for which full-text was not accessible through our institutional subscriptions or their information was incomplete. The potentially eligible articles were entered in a database, screened based on title and abstract and reviewed independently by two research team members. Articles included in the review by either team member were submitted to full-text screening.

2.3. Data extraction

Data extraction was performed by a third author not involved in screening and selection. The data extracted were: author, title, year of the study, institution, source of sample (e.g. blood, serum, plasma, allantoic fluid, other tissues), origin of the sample (i.e. human, poultry, plants), method of extraction of RNA from the FTA card®, time and

temperature of storage of FTA card, method of assessment of RNA integrity and purity, and amplification method of the viral genome fragment.

For studies that addressed different storage times, temperatures and amplicon sizes, the maximum time and maximum temperature at which the viral RNA was detectable as well as the largest amplicon size (bp) reported were recorded. Elution characteristics were compiled if RNA extraction was conducted through elution. If the study assessed the infectivity of the virus isolated from the FTA card®, it was included.

2.4. Quality assessment

Because there is not a previously validated tool for assessment of the quality of studies assessing methods for sample preservation on FTA cards®, we built a checklist consisting of ten criteria that were scored using a discrete scoring. Each item was scored with 2 = if the information provided was complete or sufficient to replicate the methods, 1 = if the information was incomplete or unclear, or 0 = if no information was provided. For data visualization a heatmap was generated using the scores.

The included items were: 1. Source of the sample: Report of the kind of sample obtained for storage in a FTA card®. 2. Time of storage: Report of the time the FTA card® was stored between sample collection and FTA card® processing for RNA isolation and RT-PCR. 3. Temperature of storage: Report of the temperature at which the FTA card® was stored. 4. Amplicon size: Report of the size (bp) of the targeted viral gene for amplification. If the work tried complete genome sequencing the answer to this question was also scored with two. 5. Extraction method: specification with precise detail to guarantee the reproducibility of the methods used for RNA extraction from the FTA card®. 6. Purification method: Specific information to assure the reproducibility of the methods used for RNA purification. 7. cDNA synthesis conditions: enough information about reagents and conditions to ensure the reproducibility of the methods for cDNA synthesis, including primers and cycling conditions. 8. PCR conditions: enough information concerning the methods and reagents used for PCR, including primers and cycling conditions. 9. Quantification of RNA copies: Report of quantification through RT-qPCR of the RNA isolated from the FTA card®. 10. Sequencing: Report about the sequencing of the desired amplicon and confirmed identity of the targeted virus, or confirmation through whole-genome sequencing with next-generation sequencing techniques (NGS).

2.5. Assessment of RNA isolation and definition of viral non-infectiousness

Experiments of viral RNA detection from FTA cards® were considered successful if the RNA fragment of interest was finally amplified from cDNA (Retro-transcription, RT) and PCR irrespective of the fragment size or if the RNA isolation allowed further genome sequencing through NGS. For studies assessing infectivity, we considered FTA card® specimens to be noninfectious when virus isolation was negative through cell culture or bio-assays using material obtained of the cards.

3. Results

We found 175 records using the search strategy, and 11 additional records by checking references of the included articles. After title and abstract screening, 44 articles were excluded because they were duplicates, and 108 papers were selected for screening by title and abstract. After the examination of exclusion criteria, a total of 47 articles were finally included (Fig. 1). All the included articles were published in English, and all of them achieved viral RNA amplification. The assessment of the quality of the information in each article is shown in Fig. 2.

A total of 95.7% of the included studies extracted total RNA before targeted amplification of the viral RNA, while the 4.3% directly isolated

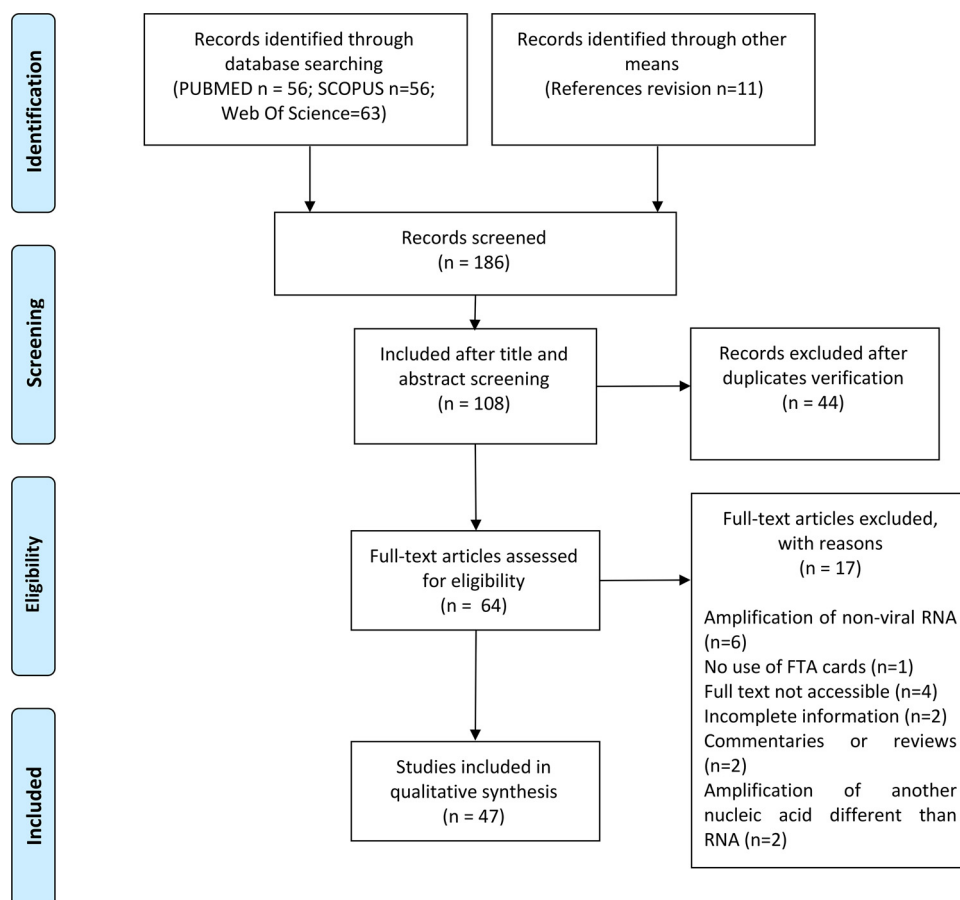


Fig. 1. PRISMA 2009 Flow Diagram of retrieved and selected articles during the systematic review.

viral RNA from the FTA card. The studies used a wide variety of sources and samples, which included samples from mammals, birds, insects, plants, cells, and humans (Table 1). The targeted viral genomes belonged to all classes of RNA virus of the Baltimore Classification except Class VI, with 8.5% of the studies reporting amplification of RNA fragments of Class III virus (dsRNA), 68.1% of Class IV virus ((+) ssRNA), 21.3% of Class V virus ((-) ssRNA), and 2.1% of the three classes mentioned above. An animal specimen source accounted for 38.3% of the reports (Moscoso et al., 2005; Maw et al., 2006; Moscoso et al., 2006; Perozo et al., 2006; Purvis et al., 2006; Inoue et al., 2007; Narayanan et al., 2010; Abdelwhab et al., 2011; Kraus et al., 2011; Keeler et al., 2012; Linhares et al., 2012; Madhanmohan et al., 2013; Awad et al., 2014; Biswal et al., 2016; Foss et al., 2016; Jozwiak et al., 2016; Madhanmohan et al., 2016; Manswr et al., 2018). Among them, twelve reports isolated RNA from viruses that can cause disease in poultry or wild birds, like Newcastle virus disease, avian influenza, or infectious bursal disease (Table 1). Six reports isolated RNA of viruses that can cause disease in suids or bovids, like respiratory syndrome virus, and foot-and-mouth disease virus (Table 1).

Plant samples were assessed in 31.9% of the included articles, and samples obtained from insects corresponded to 14.9%. These works aimed to amplify viral RNA of arboviruses that can cause disease in humans, and also viruses capable of causing disease in fishes and plants (Hall-Mendelin et al., 2010; Price et al., 2014; Flies et al., 2015; Yang et al., 2015; Navaneeth Krishnan et al., 2016; Hall-Mendelin et al., 2017; Melanson et al., 2017; Chikh-Ali et al., 2016). Viral specimens were derived from cell culture or from a commercial inactivated vaccine in 10.6% of the reports (Muthukrishnan et al., 2008; Li et al., 2012; Bankamp et al., 2013; Sakai et al., 2015; Montmayeur et al., 2017). Finally, two reports isolated virus using human samples: stool and blood. (Dauner et al., 2015; Tam et al., 2015).

Infectious virus was isolated from one of only fourteen included studies that tested for infectivity of the virus from an FTA card® (Maw et al., 2006). In this study, ADVANTEC chromatography paper No. 526, (Toyo Roshi, Tokyo, Japan), ADVANTEC filter paper No. 2 (Toyo Roshi), and stationery copy paper were compared with FTA cards® for the isolation and inactivation of the dsRNA from the infectious bursal disease virus, a virus member of the *Birnaviridae* family, genus *Avibirnavirus*, with a segmented genome. In this study, every paper-based matrix allowed the isolation of viral RNA and infectious virus. However, virus inactivation was achieved after phenol fixation on the FTA card®. Thirteen other studies demonstrated a lack of infectious virus from the FTA card (Moscoso et al., 2005; Moscoso et al., 2006; Perozo et al., 2006; Muthukrishnan et al., 2008; Narayanan et al., 2010; Abdelwhab et al., 2011; Keeler et al., 2012; Linhares et al., 2012; Bankamp et al., 2013; Awad et al., 2014; Tam et al., 2015; Jozwiak et al., 2016; Madhanmohan et al., 2016) when examining (-) ssRNA viruses of the families *Paramyxoviridae* (Newcastle disease virus, genus *Avulavirus*, *Measles morbillivirus*, subfamily *Orthoparamyxovirinae*, genus *Morbillivirus*), *Orthomyxoviridae* (Avian influenza virus, genus *Alphainfluenzavirus*), and *Pneumoviridae* (*Avian metapneumovirus*, genus *Metapneumovirus*), (+) ssRNA virus members of the family *Picornaviridae* (Foot-and-mouth disease virus, genus *Aphthovirus*), *Arteriviridae* (Porcine reproductive and respiratory syndrome virus), and *Coronaviridae* (Avian coronavirus, genus *Gammacoronavirus*), and dsRNA virus of the family *Birnaviridae* (Infectious bursal disease virus, genus *Avibirnavirus*) and *Reoviridae* (Rotavirus A, genus *Rotavirus*). These studies attempted viral isolation from FTA cards impregnated with infected material using cell culture (Moscoso et al., 2006; Muthukrishnan et al., 2008; Keeler et al., 2012; Linhares et al., 2012; Bankamp et al., 2013; Awad et al., 2014; Tam et al., 2015; Madhanmohan et al., 2016), bioassays infecting specific pathogen-free embryonated chicken eggs (Moscoso et al., 2005;

Study	1. Source	2. Time of storage	3. Temperature of storage	4. Size of amplicon	5. Extraction method	6. Purification method	7. cDNA synthesis conditions	8. PCR conditions	9. Quantification of copies	10. Sequencing
Bankamp B, et al., 2013	2	2	2	2	2	2	2	2	1	1
Muthukrishnan M, et al., 2008	2	2	1	2	1	2	1	0	0	0
Manswr B, et al., 2018	1	2	2	2	2	2	2	2	0	2
Moscoso H, et al., 2006	2	2	2	2	2	2	2	1	0	2
Chang PG, et al., 2011	2	2	1	2	2	0	2	2	0	2
Linhares DC, et al., 2012	2	2	1	0	2	2	1	1	0	0
Maina S, et al., 2017	1	0	0	0	1	2	0	0	0	0
Madhanmohan M, et al., 2013	2	2	2	2	2	2	1	0	0	0
Navaneeth Krishnan A, et al., 2016	2	2	2	2	0	2	2	2	0	2
Melanson VR, et al., 2017	2	2	2	0	1	2	2	2	0	0
Maina S, et al., 2018	2	0	0	0	0	2	1	1	0	2
Flies EJ, et al., 2015	2	2	2	2	1	2	1	2	0	0
Grund E, et al., 2010	2	0	0	2	2	2	2	2	0	0
Yang Y, et al., 2015	2	2	2	2	2	2	1	1	1	2
Abdelwhab EM, et al., 2011	2	2	2	0	2	2	1	1	0	0
Li Y, et al., 2012	2	0	0	1	2	2	2	2	0	0
Keeler SP, et al., 2012	2	2	2	1	2	2	1	1	0	0
Kraus RH, et al., 2011	2	2	2	2	2	2	2	2	0	2
Sakai T, et al., 2015	1	2	2	2	2	2	1	1	1	0
Maw MT, et al., 2006	2	2	2	2	1	2	2	2	0	0
Purvis LB, et al., 2006	2	2	1	2	2	2	2	2	0	2
Biswal JK, et al., 2016	2	2	2	2	2	2	1	1	0	0
Price JA, et al., 2014	2	0	0	2	2	2	2	2	2	1
Moscoso H, et al., 2005	2	2	1	2	2	2	2	2	1	2
Karavina C, et al., 2017	2	0	0	2	2	2	2	2	1	2
Tam KI, et al., 2015	2	2	2	0	2	2	1	1	1	1
Roy Y, et al., 2005	2	2	1	2	2	1	1	1	0	0
Awad F, et al., 2014	2	2	2	2	1	1	1	1	0	0
Narayanan MS, et al., 2010	2	2	2	2	2	0	1	1	0	1
Ndunguru J, et al., 2005	2	0	0	2	2	1	1	2	0	1
Dauner AL, et al., 2015	2	2	2	0	2	2	1	1	2	0
Madhanmohan M, et al., 2016	2	2	2	0	2	1	1	1	0	0
Jozwiak M, et al., 2016	2	2	1	0	0	2	1	1	0	1
Montmayer AM, et al., 2017	2	0	0	2	1	1	2	2	0	1
Foss L, et al., 2016	2	0	0	0	2	0	1	1	0	0
Inoue R, et al., 2007	2	0	0	2	1	2	2	2	0	1
Hall-Mendelin S, et al., 2017	2	2	1	0	2	2	1	1	2	0
Perozo F, et al., 2006	2	2	2	2	1	2	2	2	2	2
Hall-Mendelin S, et al., 2010	2	2	2	0	2	2	1	1	0	0
Maina S, et al., 2017b	2	0	0	0	1	2	0	0	0	1
Maina S, et al., 2016d	1	0	0	0	1	2	0	0	0	1
Maina S, et al., 2016e	1	0	0	0	1	2	0	0	0	1
Maina S, et al., 2016c	2	0	0	0	1	2	0	0	0	1
Maina S, et al., 2016b	2	0	0	0	1	2	0	0	0	1
Maina S, et al., 2016a	1	0	0	0	1	2	0	0	0	1
Maina S, et al., 2017a	2	0	0	0	0	2	1	1	1	2
Chikh-Ali M, et al., 2016	2	0	0	2	2	2	2	2	0	2

Fig. 2. Quality assessment chart. Each included study was assessed in order to determine the information provided for each variable. Each variable was scored with 2 = green, if the information provided was complete or sufficient to replicate the methods, 1 = yellow, if the information was incomplete or unclear, or 0 = red, if no information was provided. For data visualization a heatmap was generated using the scores (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Perozo et al., 2006; Abdelwhab et al., 2011; Jozwiak et al., 2016) or both (Narayanan et al., 2010).

Regarding storage of the FTA cards® before further viral RNA purification, the cards were stored for periods that varied from one to eight months (median time: 30 days, IQR 21–90), at temperatures that ranged between -20 °C to 37 °C (median temperature: 25 °C, IQR 4 °C–25 °C). Three protocols for RNA amplification from the FTA card® were reported: 1. Elution of RNA from the FTA card®, 2. Direct viral RNA purification, and 3. Disk cleaning and soaking.

Elution of RNA from FTA cards® before RNA amplification was the most common method for RNA extraction. It was performed in 55.1% of the assessed studies. The elution conditions and eluents used in those studies are summarized in Table 2. Briefly, viral RNA was obtained by soaking fragments of the FTA card® in an eluent solution, followed by incubation with or without agitation with temperatures that ranged between 4–95 °C. After elution, the RNA underwent a purification step using commercial kits (Moscoso et al., 2005; Ndunguru et al., 2005; Maw et al., 2006; Moscoso et al., 2006; Purvis et al., 2006; Abdelwhab et al., 2011; Keeler et al., 2012; Li et al., 2012; Bankamp et al., 2013; Sakai et al., 2015; Tam et al., 2015; Yang et al., 2015; Biswal et al., 2016; Foss et al., 2016; Madhanmohan et al., 2016; Karavina and Gubba, 2017; Manswr et al., 2018) or standard protocols like sodium acetate and isopropanol precipitation (Grund et al., 2010; Price et al., 2014). In one case, magnetic beads were used for purification and concentration of RNA (Linhares et al., 2012)

Direct virus RNA purification was performed in 30.6% of the studies. It consisted of a protocol in which FTA card® punches were used for RNA purification without a previous step of elution from the FTA

card®. The punches were directly soaked in the lysis and extraction solutions of different commercial kits, TRIzol reagent® or Guanidinium thiocyanate-phenol-chloroform method (Table 1). Most of the studies used methods for total RNA extraction and only one study used a commercial kit for viral RNA isolation (Montmayer et al., 2017). In this particular case, the genome of polioviruses, (*Enterovirus C*, genus *Enterovirus*, family *Picornaviridae*), a virus with a (+) ssRNA non-segmented genome of 6.7–10.1 kb with a poly(A) tail, was isolated from FTA cards®. This method allowed the use of NGS techniques for complete genome sequencing with a total of 206,156 reads (90% of reads mapping to poliovirus, giving 99% genome coverage) (Montmayer et al., 2017).

On the other hand, 8.2% of the included studies used disk cleaning and soaking for RNA purification, which consisted of punching a disk from the card and placing in a solution to remove contaminants before proceeding one or two-step RT-PCR by placing the cleaned disk into a second solution (the reaction mix). Note that this method did not include an RNA purification step (Roy and Nassuth, 2005; Inoue et al., 2007; Narayanan et al., 2010; Chang et al., 2011).

The third strategy was to perform one or two-step RT-PCR after RNA isolation or preparation from the FTA cards®. In 57.1% of these studies, the targeted viral genes were amplified by one-step RT-PCR. Most of them (96.4%) used specific primers for the RT step and the PCR step. Only one study used random primers for RT and then specific primers for PCR (Yang et al., 2015). The reaction mix composition, as well as the cycling conditions in each work, varied depending on the PCR kit used and the desired amplicon. Eleven studies quantified the product of the RT-PCR, 26 used the product of these reactions for sequencing, and

Table 1
General characteristics of the included works that performed viral RNA amplification/sequencing from samples immobilized in FTA cards. N/A = Not Apply, (-) = Information not provided.

Study	Year	Institution	RNA sample	Source	RNA isolated	Virus	Viral genome	Infectivity	Maximum time of storage	Temperature of storage	Extraction method	RT-PCR method	Primer strategy (RT)
Bankamp B, et al.	2013	Centers for Disease Control and Prevention	Vero/hSLAM cells	Cells	Total	Measles morbillivirus	ssRNA(-)	-	6 months	4 °C	Elution	one step RT-qPCR	Specific
Muthukrishnan M, et al.	2008	Indian Immunologicals Limited	BHK21 cell	Cells	Total	Foot-and-mouth disease virus	ssRNA(+)	No	120 days	Room temperature	Disc cleaning	one step RT-qPCR	Specific
Mansvr B, et al.	2018	University of Liverpool	Allantoic fluid	Animal	Total	Infectious bronchitis virus	ssRNA(+)	-	21 days	Room temperature	Elution	one step RT-qPCR	Specific
Moscoso H, et al.	2006	The University of Georgia	Allantoic fluid	Animal	Total	Infectious bronchitis virus	ssRNA(+)	-	21 days	Room temperature	Elution	two step RT-PCR	Specific
Chang PG, et al.	2011	Virginia Polytechnic Institute and State University	Bursal tissue	Animal	Total	Infectious bursal disease virus	dsRNA	-	8 months	-20 °C	Elution	one step RT-PCR	Specific
Linhares DC, et al	2012	College of Veterinary Medicine	Leaf	Plant	Total	Tobacco etch virus, Soybean mosaic virus, Turnip mosaic virus, Cucumber mosaic virus, and Peanut stunt virus	ssRNA(+)	-	-	-	Disc cleaning	two-step RT-PCR	Specific
Maina S, et al.	2017	University of Western Australia	Serum and lung	Animal	Total	Porcine reproductive and respiratory syndrome virus	ssRNA(+)	-	14 days	Room temperature	Elution	one step RT-PCR	Specific
Madhanmohan M, et al.	2013	Indian Immunologicals Limited	Leaf	Plant	Total	Cucurbit aphid-borne yellows virus	ssRNA(+)	-	-	N/S	Purification	RNA-seq	-
Navaneeh Krishnan A, et al.	2016	Central Institute of Brackishwater Aquaculture	Tongue epithelium	Animal	Total	Foot-and-mouth disease virus	ssRNA(+)	-	1 month	25 °C	Purification	RT-LAMP	Specific
Melanson VR, et al.	2017	United States Army Research Institute of Infectious Diseases	Larval homogenate, cell culture extract, milt extract, seawater spiked with larval homogenate	Insect	Total	Dengue virus	ssRNA(+)	-	-	-	-	one step RT-PCR	Specific
Maina S, et al.	2018	The University of Western Australia	Leaf	Plant	Total	Sweet potato feathery mottle virus and Sweet potato virus C	ssRNA(+)	-	-	-	Purification	one step RT-PCR	Specific
Flies EJ, et al.	2015	University of Tasmania	Mosquito saliva	Insect	Total	Arbovirus (Ross River virus, Barmah Forest virus, and Stratford virus)	ssRNA(+)	-	Seven days	25 °C	Elution	two-step RT-PCR	Specific
Grund E, et al.	2010	University of Hamburg	Leaf	Plant	Total	Watermelon chlorotic stunt virus, Mycovirus China 9, Tomato spotted wilt virus, Tobacco mosaic virus, Cucumber mosaic virus, Little cherry virus 1, Little cherry virus 2, Potato spindle tuber viroid	dsRNA, ssRNA(+), ssRNA(-)	-	-	-	Elution	two-step RT-PCR	Specific
Yang Y, et al.	2015	Walter Reed Army Institute of Research	Blood	Insect	Total	Dengue virus type 2	ssRNA(+)	-	1 month	Room temperature	Elution	one step qRT-PCR, MiSeq	Specific and random
Abdelwhab EM, et al	2011	Free Berlin University	Amnio-allantoic fluid	Animal	Total	Avian influenza virus	ssRNA(-)	No	5 months	Room temperature	Elution	one step RT-qPCR	Specific

(continued on next page)

Table 1 (continued)

Study	Year	Institution	RNA sample	Source	RNA isolated	Virus	Viral genome	Infectivity	Maximum time of storage	Temperature of storage	Extraction method	RT-PCR method	Primer strategy (RT)
Li Y, et al.	2012	Shandong Center for Disease Control and Prevention	HEp-2 Cells	Cells	Total	Enterovirus spp.	ssRNA(+)	-	-	-	Elution	one step qRT-PCR	Oligo dT and Random primers
Keeler SP, et al	2012	The University of Georgia	Cloacal swabs	Animal	Total	Avian influenza virus	ssRNA(-)	-	90 days	Room temperature	Elution	one step RT-PCR	Specific
Kraus RH, et al	2011	Wageningen University	Cloacal swabs	Animal	Total	Avian influenza virus	ssRNA(-)	-	N/S	-	Elution	one step RT-PCR	Specific
Sakai T, et al	2015	Nihon Veterinary Research Center	Vaccine	Vaccine	Viral	Rabies virus	ssRNA(-)	-	4 months	-20 °C	Elution	one step RT-PCR	Specific
Maw MT, et al	2006	United Graduate School of Veterinary Sciences	Mucose	Animal	Total	Infectious bursal disease virus	dsRNA	Yes	30 days	37 °C	Elution	two-step RT-PCR	Specific
Purvis LB, et al	2006	University of Georgia	Bursal tissue	Animal	Total	Infectious bursal disease virus	dsRNA	-	24 hours	Room temperature	Elution	one step RT-PCR	Specific
Biswal JK, et al	2016	ICAR- Directorate on Foot-and-mouth Disease	Clinical samples (cell culture isolates, tongue epithelial suspension, and impression smears)	Animal	Total	Foot-and-mouth disease virus	ssRNA(+)	-	6 weeks	4-37 °C	Elution	two-step RT-PCR	Oligo dT
Price JA, et al.	2014	Texas A&M AgriLife Research and Extension	Wheat curl mite	Animal	Total	Wheat streak mosaic virus	ssRNA(+)	-	1 hour	Room temperature	Elution	one step RT-PCR	Specific
Moscoso H, et al.	2005	The University of Georgia	Allantoic fluid	Animal	Total	Infectious bronchitis virus	ssRNA(+)	-	36 days	Room temperature	Elution	one step RT-PCR	Specific
Karavina C, et al	2017	University of KwaZulu-Natal	Leaf	Plant	Total	Tomato spotted wilt virus	ssRNA(-)	-	-	-	Elution	two step RT-mPCR	Specific
Tam KI, et al	2015	Centers for Disease Control and Prevention	Stool	Human	Total	Rotavirus A	dsRNA	-	28 days	37 °C	Elution	one step RT-PCR	Specific
Roy Y, et al.	2005	University of Guelph	Leaf and fruit	Plant	Total	Pepino mosaic virus and Rupestris stem pitting associated virus	ssRNA(+)	-	8 months	Room temperature	Disc cleaning	one step RT-PCR	Specific
Awad F, et al.	2014	University of Liverpool	Tracheal organ cultures	Animal	Total	Avian metapneumovirus	ssRNA(-)	No	2 months	4 °C	Purification	RT-PCR	Specific
Narayanan MS, et al.	2010	Madras veterinary college	Allantoic fluid	Animal	Total	Avian avulavirus 1	ssRNA(-)	No	20 days	4 °C	Disc cleaning	two-step RT-PCR	Specific
Ndunguru J, et al.	2005	International Laboratory of Tropical Agriculture	Leaf	Plant	Total	Geminivirus	ssRNA(+)	-	-	N/S	Elution	two step RT-PCR	Specific
Dauner AL, et al.	2015	Naval Medical Research Center	Blood	Human	Total	Dengue virus	ssRNA(+)	-	21 days	37 °C	Elution	one step RT-PCR	Specific
Madhanmohan M, et al.	2016	Indian Immunologicals	Smear	Animal	Total	Foot-and-mouth disease virus	ssRNA(+)	No	56 days	4 °C	Elution	one step RT-PCR	Specific
Jozwiak M, et al.	2016	National Veterinary Research Institute	Allantoic fluid	Animal	Total	Avian influenza virus	ssRNA(-)	No	90 days	Room temperature	-	one step RT-PCR	Specific
Montmayeur AM, et al.	2017	Centers for Disease Control and Prevention	Poliovirus culture	Virus	Viral	Poliovirus	ssRNA(+)	-	-	-	Purification	NGS sequencing	Random

(continued on next page)

Table 1 (continued)

Study	Year	Institution	RNA sample	Source	RNA isolated	Virus	Viral genome	Infectivity	Maximum time of storage	Temperature of storage	Extraction method	RT-PCR method	Primer strategy (RT)
Foss L, et al.	2016	California Department of Public Health	Oral swabs	Animal	Total	West Nile virus	ssRNA(+)	-	-	-	Elution	one step RT-PCR	Specific
Inoue R, et al.	2007	Kyoto Prefectural University	Blood	Animal	Total	Porcine reproductive and respiratory syndrome virus	ssRNA(+)	-	-	-	Disc cleaning	one step RT-PCR	Specific
Hall-Mendelin S, et al.	2017	Public Health Virology	Mosquito saliva	Animal	Total	Arbovirus (Zika virus, Chikungunya virus, Dengue virus, and Barmah Forest virus)	ssRNA(+)	-	28 days	Room temperature	Elution	one step RT-PCR	Specific
Perozo F, et al	2006	University of Georgia	Allantoic fluid	Animal	Total	Newcastle disease virus	ssRNA(-)	No	15 days	Room temperature	Purification	one step RT-PCR	Specific
Hall-Mendelin S, et al.	2010	University of Queensland	Mosquito saliva	Animal	Total	Arbovirus (West Nile river, Ross River virus, and chikungunya virus)	ssRNA(+)	-	28 days	23 °C	Elution	one step RT-PCR	Specific
Maina S, et al.	2017	University of Western Australia	Leaf	Plant	Total	Aphid lethal paralysis virus	ssRNA(+)	-	-	-	Purification	Sequencing (metagenomic)	N/A
Maina S, et al.	2016	University of Western Australia	-	Plant	Total	Sweet potato chlorotic fleck virus	ssRNA(+)	-	-	-	Purification	Genome sequencing	N/A
Maina S, et al.	2016	University of Western Australia	-	Plant	Total	Sweet potato virus 2	ssRNA(+)	-	-	-	Purification	Genome sequencing	N/A
Maina S, et al.	2016	University of Western Australia	Leaf	Plant	Total	Suakwa aphid-borne yellows virus	ssRNA(+)	-	-	-	Purification	Genome sequencing	N/A
Maina S, et al.	2016	University of Western Australia	Leaf	Plant	Total	Bean common mosaic necrosis virus	ssRNA(+)	-	-	-	Purification	Genome sequencing	N/A
Maina S, et al.	2016	University of Western Australia	-	Plant	Total	Sweet potato virus G	ssRNA(+)	-	-	-	Purification	Genome sequencing	N/A
Maina S, et al.	2017	University of Western Australia	Leaf	Plant	Total	Papaya ringspot virus biotype W	ssRNA(+)	-	-	-	Purification	two step RT-PCR, RNA-seq	Specific
Chikh-Ali M, et al	2016	University of Idaho	Leaf	Plant	Total	Potato virus Y	ssRNA(+)	-	-	-	Elution	two step RT-PCR	Specific

LAMP = Loop-mediated Isothermal Amplification.

Table 2
 Characteristics of studies that performed RNA elution from FTA cards® before RT-PCR reaction. (-) Not performed.

Study	Eluent	Elution volume (µL)	Elution time (min)	Agitation	Elution temperature	Purification method
Bankamp B, et al.	PBS + AVL buffer	750	10	-	Room temperature	QIAamp Viral RNA Mini Kit
Mansur B, et al.	TE buffer	1000	15	-	Room temperature	QIAamp viral RNA Mini Kit
Moscoso H, et al.	TE buffer	1000	15	-	Room temperature	QIAamp viral RNA Mini Kit
Linhares DC, et al.	Tris-HCl + EDTA	200	10	-	Room temperature	High Pure Viral RNA Kit
	RNA rapid extraction solution	100	5	-	Room temperature	Magnetic bead-based technology
Flies EJ, et al.	GM + 3%FBS	1000	20	Vortex every 5min	On ice	EZ1 Virus Mini 109 Kit v2.0 (Qiagen, Victoria, AU)
Grund E, et al.	Elution buffer	400	15	Agitation every 5min	4 °C	Sodium acetate and isopropanol precipitation
Yang Y, et al.	AVL buffer + RNA carrier	566	5	Continuous vortex +5 min centrifugation	Room temperature	Qiagen QIAamp Viral RNA kit
Abdelwhab EM, et al	TE buffer	200	10	-	Room temperature	Qiagen Viral RNA Mini Kit
Li Y, et al.	Deionized sterile water + TE-1 + Tris-HCl + Buffer EB (Tris-Cl, elution buffer from the QIAamp Viral RNA Mini Kit)	30	40	Vortex at 100x during 30 min, 10 min vortex at 200xg in intervals of 1 min	65 °C or 95 °C	Roche Pure Viral RNA kit
Keeler SP, et al.	RNA rapid extraction solution	75	60	-	Room temperature	MagMAX™-96 AI/ND Viral RNA Isolation Kit
	RNA rapid extraction solution	75	60	-	Room temperature	MagMAX™-96 AI/ND Viral RNA Isolation Kit
Kraus RH, et al.	RNA rapid extraction solution	70	5	Agitation	Room temperature	MagMAX-96 viral RNA isolation kit
Sakai T, et al.	Nuclease-free water	150	1440	-	Room temperature	QIAamp® Viral RNA Mini Kit
	TE buffer	150	1440	-	Room temperature	QIAamp® Viral RNA Mini Kit
	RNA rapid extraction solution	150	30	-	Room temperature	QIAamp® Viral RNA Mini Kit
	TRIZOL Reagent	150	30	-	Room temperature	QIAamp® Viral RNA Mini Kit
	AVL buffer	150	1440	-	Room temperature	QIAamp® Viral RNA Mini Kit
Maw MT, et al.	Nuclease-free water	75	5	Agitation	Room temperature	TRIZOL Reagent
Purvis LB, et al.	Whatman purification reagent	200	20	Vortex every 5min	Room temperature	High Pure RNA isolation kit
Biswal JK, et al.	AVL buffer	560	10	-	Room temperature	QIAamp Viral RNA mini kit
Price JA, et al.	TE buffer	400	15	Vortex every 5min	Ice incubation	Sodium acetate and isopropanol precipitation
Moscoso H, et al.	Tris-HCl and EDTA	200	10	-	Room temperature	High Pure Viral RNA Kit
Karavina C, et al.	Nuclease-free water	50	30	Vortex 15 seg	95 °C	-
Tam K, et al.	TE buffer	400	240	-	Room temperature	MagMAX™ Viral RNA isolation kit
Ndunguru J, et al.	RNA processing buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 800 U/mL RNase Out™ 200–250 µg/mL glycogen and 2 mM DTT)	500	30	Vortex every 5min	Room temperature	Sodium acetate and isopropanol precipitation
Dauner AL, et al.	AVL buffer	300	60	Shaking	Room temperature	QIAamp viral RNA Mini Kit
Madhanmohan M, et al.	VMM	460	900	-	4 °C	RNeasy® Mini Kit
Foss L, et al.	ABI Magmax lysis binding solution	1000	900	-	Room temperature	-
Hall-Mendelin S, et al.	GM + 3%FBS	1000	20	Vortex every 5min	On ice	QIAamp® Virus BioRobot® MDx Kit
Hall-Mendelin S, et al.	GM + 3%FBS	1000	20	Vortex every 5min	On ice	QIAamp® Virus BioRobot® MDx Kit
Chikh-Ali M, et al	RNA processing buffer	500	30	Vortex every 5min	Room temperature	Sodium acetate and isopropanol precipitation

in one study, RNA-seq was implemented for viral identification. (Maina et al., 2016a,c,b,d,e; Maina et al., 2017a, b; Maina et al., 2017c; Montmayeur et al., 2017).

4. Discussion

The findings of this systematic review support that viral RNA from different types of RNA viruses spotted in a FTA card® can be purified and then amplified for viral identification. The RNA can be isolated from a large number of sources and samples, and stored over a wide range of periods of time and temperatures. This highlights the fact that FTA cards® could be used as means for transport and storage of RNA pathogens. However, storage time and temperature are critical variables affecting RNA preservation. Studies reviewed here suggest that viral RNA could be stable in a wide range of temperatures for several months. Cards stored at 4 °C allowed the amplification of fragments up to ~1200 bp, and cards stored for six months allowed amplification of fragments up to ~800 bp.

Use of FTA cards to work with biospecimens appears to be relatively safe since isolation of infectious virus was rarely reported. Nevertheless, the possibility remains that some infectious virus could be present in this type of sample, as shown by the study of bursal disease virus. In this case, additional chemical fixation was used to render the sample completely noninfectious (Maw et al., 2006).

More data is required to explain why the bursal disease virus remained infectious when such a wide variety of viruses were inactivated after spotting on FTA cards® (Perozo et al., 2006; Narayanan et al., 2010; Abdelwhab et al., 2011; Awad et al., 2014; Jozwiak et al., 2016; Madhanmohan et al., 2016). Possible explanations include that infectious bursal disease virus is a non-enveloped virus. While enveloped viruses can persist viable in dried surfaces for five days, non-enveloped viruses can persist for weeks (Firquet et al., 2015). However, the inability to isolate viable foot-and-mouth disease virus from FTA cards®, another non-enveloped virus, is inconsistent with this argument (Madhanmohan et al., 2016). Storage time and temperature and card manipulation and processing during viral isolation are other possible factors that could affect viral infectiousness. It is remarkable that Maw et al., 2006 isolated infective virus after seven days of storage at room temperature, but Jozwiak et al., 2016 were unable of performing viral isolation even as earlier as 1 h after sample spotting on FTA card, and Linhares et al., 2012 after storage at 4 °C for 24 h. In this context, viral viability testing after immobilization on FTA cards® seems like an advisable practice before considering FTA cards® isolates innocuous. Then, FTA cards® should be managed and stored following the required biosafety precautions.

Different methods for extraction and purification were represented in the literature. The elution and subsequent purification of the RNA are the most widely used, and different elution solutions in a wide range of volumes, time, agitation and temperatures have been tested. Based on these protocols, we advise to keep the elution volume below 500 µL and the elution time below 30 min, with vortex or agitation every 5 min at room temperature. Then perform RNA purification using commercial kits or standard protocols before subsequent RT-PCR. This method was effective for amplification of fragments up to ~1700 bp.

For disc cleaning protocols it is advisable to add 200 µL of FTA purification reagent in a tube containing the discs, mix thoroughly for 3 min at room temperature, and repeat this step twice. Then, rinse the discs with 200 µL of TE Buffer and, finally, add the disc to the proper PCR reaction mix. When the source material likely contains PCR inhibitors, an additional step of cleaning with 200 µL of Ethanol 70% can be performed (Roy and Nassuth, 2005; Inoue et al., 2007; Narayanan et al., 2010; Chang et al., 2011). These protocols and direct purification protocols allowed amplification of fragments of ~900 bp, even when the storage times of the FTA card® were up to eight months at room temperature. This suggests that probably the three reported methods for RNA extraction from the FTA card® have similar effectiveness and that

their selection could be related to reagent availability or personal preferences.

4.1. Limitations

Since the stability and integrity of viral RNA for molecular detection is a significant concern during fieldwork, this systematic review focused on the evaluation of FTA cards® as a strategy for detection of these pathogens. However, successful applications of these cards have been also reported for DNA viruses and bacteria (Dong et al., 2017; Reeve et al., 2018). For instance, the use of FTA cards® have been proved effective as a means of sample storage and transport for genotyping isolates of *Campylobacter jejuni* obtained from poultry and stored for three months at room temperature (Sierra-Arguello et al., 2016).

It is possible that publication bias, the lack of publishing reports with negative results, could lead to failure in identifying studies that were unable to amplify RNA extracted from the FTA cards®. The search strategy (“FTA cards” AND “RNA”) was used instead of (“FTA card” AND “RNA”) since the first retrieved more records in two of the assessed databases, and the same number of records in Scopus. Additionally, though we conducted an extensive search in different databases, and the screened works were searched through different institutional subscriptions, four papers were not accessible for full-text assessment. Nevertheless, the search identified more than forty reports evaluating FTA cards® as RNA immobilization matrix with sufficient information to summarize their protocols for potential replication.

Although the goal of this review was to consider fieldwork conditions, some of the identified studies used the FTA card® for RNA spotting in controlled laboratory conditions. Therefore, the effectiveness of this matrix could vary when used in fieldwork and associated handling and shipping conditions. In these particular cases, fieldwork testing should be performed. However, some of the studies identified tested their effectiveness submitting these matrices to transport and shipping, similar to fieldwork conditions (Madhanmohan et al., 2016).

5. Conclusions

The effectiveness of FTA cards® for sample collection and recovery of viral RNA for downstream analysis was demonstrated by several studies. Use of FTA cards® may be an attractive approach for fieldwork involving molecular detection and/or characterization of viral pathogens, particularly when simple and cheap sample collection and transport systems are desirable. The stability of RNA genomic material of many different viruses under a wide range of conditions further supports the advantage of FTA cards® use, even for metagenomic analysis (Yang et al., 2015; Maina et al., 2017b). Though many viruses will become inactivated in the collection and storage process, we suggest that all specimens should be considered potentially infectious unless proven otherwise. Thus, the available evidence suggests that FTA cards® are a good option for virus storage and transport for molecular detection and identification of RNA viral pathogens, although additional field testing would be advisable to further validate and standardize these methods for use in the fieldwork.

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