



Establishing conditions for the storage and elution of rabies virus RNA using FTA® cards

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Introduction

Flinders Technology Associates filter paper cards (FTA cards) can be used to store nucleic acids from various samples and are easily portable.



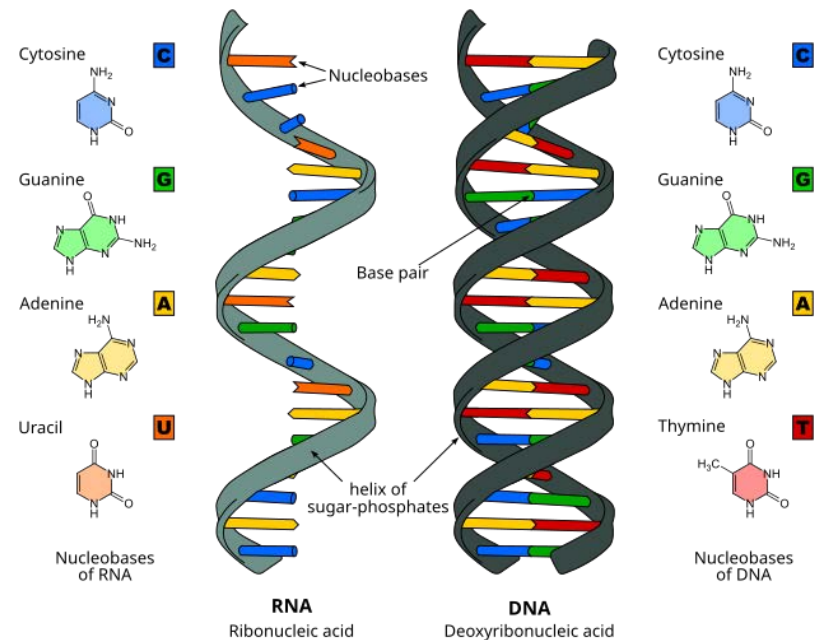
They dissolve the cells and fix the nucleic acid in fibers for storage.

FTA cards protect nucleic acids from nucleases, oxidation and UV damage.

Can be used to store both DNA and RNA samples.

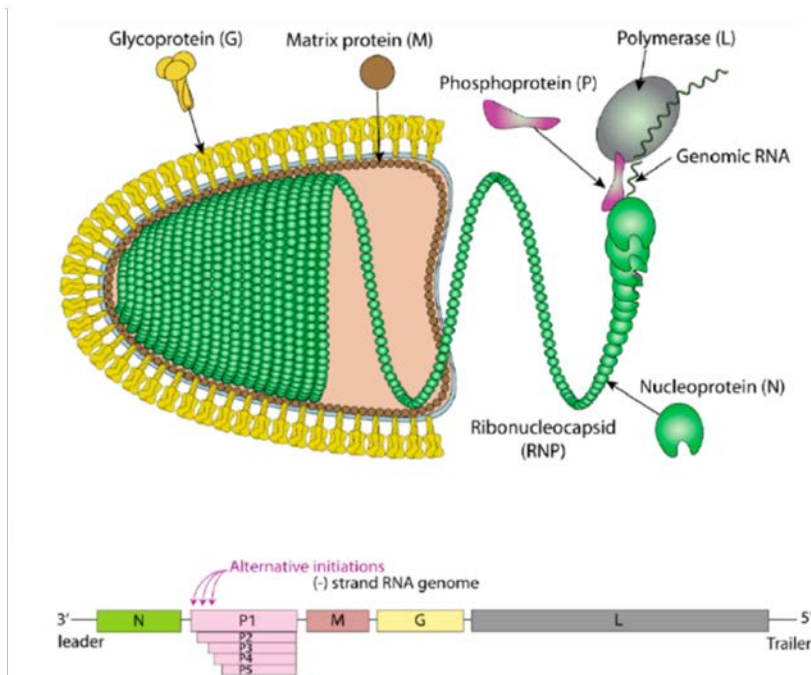
RNA is more unstable than DNA.

Appropriate methods have not been established for storage and extraction of RNA from FTA cards.



Objective

The present study investigated the optimum conditions for storage and elution of viral RNA (vRNA) using rabies virus (RABV) applied to FTA cards.



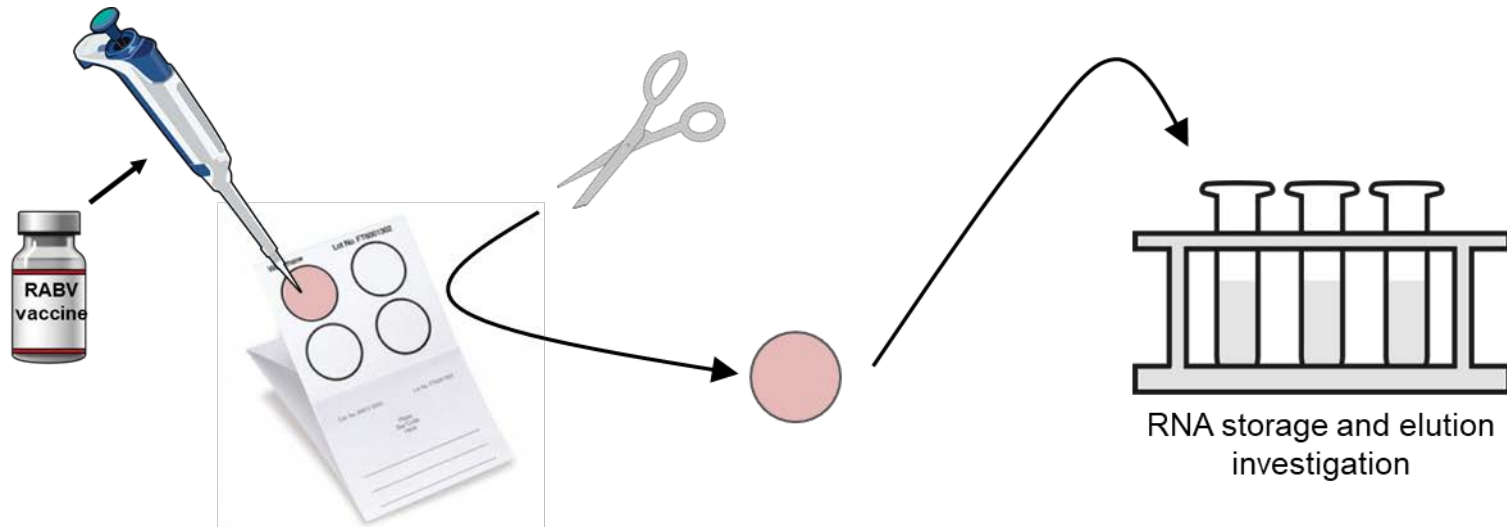
Materials and methods

Using a cutting mat with RNase and DNA removed with RNase AWAY and a Harris Uni-Core punch, we cut 6mm-diameter disks from FTA classic cards.

FTA cards were impregnated with 10 μ L of RC-HL strain (The Chemo-Sero Therapeutic Research Institute, >108.5 TCID₅₀/mL), rabies TC vaccine for animals.

The disks were placed in 1.5 mL micro centrifuge tubes for storage with silica gel after drying at room temperature for 24 hours.

Four disks punch-out from the FTA cards were used for each condition.



Elution of vRNA from FTA® cards

150 µL of eluent was added to soak the disks, and the disks tubes were left at room temperature.

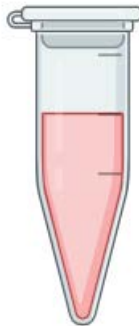
The disk was removed from the microtube, and the remaining solution was considered as RNA eluate.

In addition, RNA was extracted directly from the vaccine for use as a positive control.

QIAmp Viral RNA Mini Kit (QIAGEN, Japan, Tokyo) was used to extract the vRNA from the eluates.

The extracted RNA was kept at -80°C until use.

RNA eluate



RNA eluate
(positive
control)



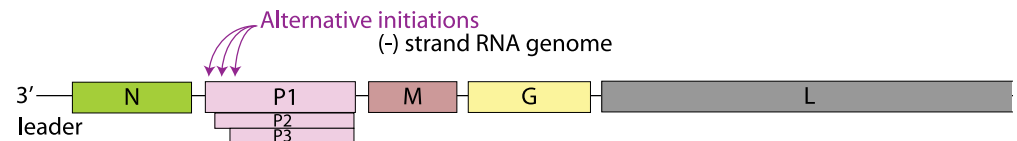
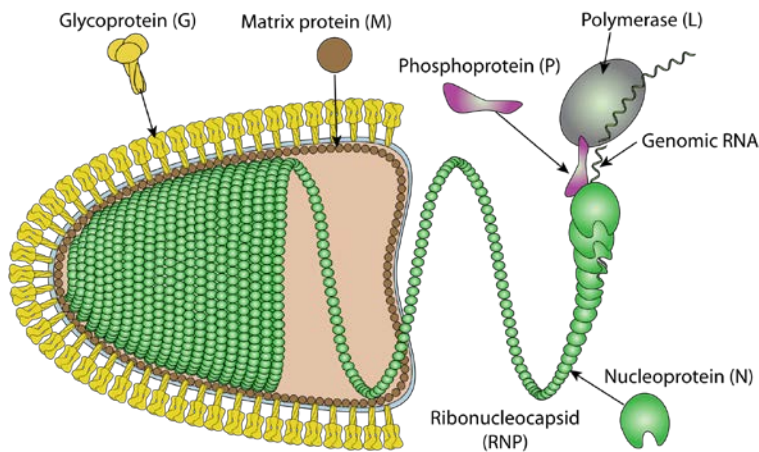
vRNA copy assessment through RT-PCR

The RABV full length N gene region (base position: 71–1,423) was amplified using RT-PCR with the primer pairs of RCHL-Nfull-F and RCHL-Nfull-R.

Recombinant plasmid DNA was prepared by incorporating the amplified product into pGEM-T Easy Vector and transformed into JM109.

After extraction of the plasmids, the plasmid containing target sequence inserts were linearized at 37°C for 1 hr and purified.

In vitro transcription was performed, and the concentration of synthesized RNA was measured using a NanoDrop.



vRNA damage assessment

RT-PCR was conducted with the primers pairs of RCHL-Nfull-F and RCHL-Nfull-R for amplification of full length N gene (1,353 bp).

Amplified products were subjected to electrophoresis in 1.5% agarose gel and checked under UV light after staining with ethidium bromide.

The intensity of target bands of amplified products was compared with that of the control and evaluated semiquantitatively as integrated optical density.

Table 1. Primers used in this study

Primer	Sequence (5'–3')	Size of amplicon	Use
RCHL-Nfull-F	ATGGATGCCGACAGGATTG	1,353 bp	RT-PCR
RCHL-Nfull-R	TTAAGAGTCGCTCGAATACGTCTTG		
P1	CTACAATGGATGCCGACAAGA	964 bp	RT-PCR
P2	CCCATATAACATCCAACAAAGTG		
Nes-S	ATGGATGCCGACAAGATTGT	290 bp	RT-PCR
Nes-C	GCWATCAGGATTCCATAGCT		
realNgeneF1	CGGCTGTTCTCACTCTTATTTC	133 bp	Real time-PCR
realNgeneR1	CTGATTTGACCCATATAGCATCC		



Elution rate comparisons

150 μ L of eluent was added to the disks in the microtubes and were left soaking at room temperature.

Five different RNA eluents:

- Nuclease free water
- TE-buffer
- Rapid extraction solution (Ambion)
- Trizol
- Buffer AVL

Elution times 5, 15, 30, 60 min and 24 hours.

QIAamp Viral RNA Mini Kit was used to extract the vRNA from the eluates.

In addition, RNA was extracted directly from the vaccine liquid using this kit for use as a positive control. The extracted RNA extract was kept at -80°C until use.



Effect of storage temperature & duration

The FTA® cards were stored at temperatures of -80°C , -20°C , 4°C and room temperature for periods of 1–4 weeks and 2–3 months.

The degree of RNA damage was evaluated by comparing the amount of RT-PCR-amplified products between the RNA from FTA cards stored in the different conditions and vRNA directly extracted from the vaccine.

Influence of brain emulsion on vRNA storage

The vaccine liquid mixed with brain emulsion was applied to the FTA cards and stored at -80°C .

The mouse brain emulsion was prepared by adding 1 g of frozen mouse brain to 10 mL of PBS, and 10 μL of vaccine liquid was added to 90 μL of this emulsion.

10 μL of the centrifuged supernatant was applied to FTA cards.

Control was prepared by diluting the vaccine liquid with sterile distilled water.

FTA cards with the applied samples were dried for 24 hours at room temperature and kept at -80°C until use.

The extraction of the RNA from FTA cards was conducted using TE-buffer.



Detection of vRNA in field samples

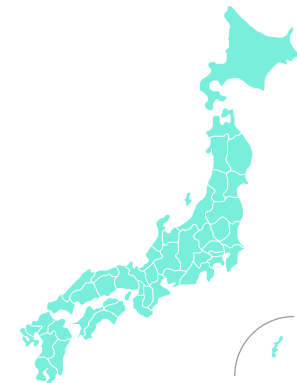
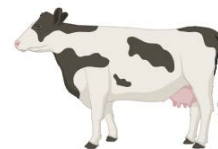
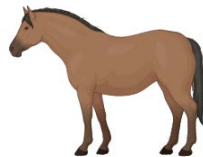
Nine brain samples from cows and horses diagnosed as rabies positive in Brazil were applied to FTA cards.

Cards were transported Brazil to Japan at room temperature.

The cards were stored for 6 months at -80°C after arrival in Japan.

RNA was extracted from the nine samples, RT-PCR was conducted.

RNA was detected by electrophoresis and visual observation, with positive and negative represented as + and –.

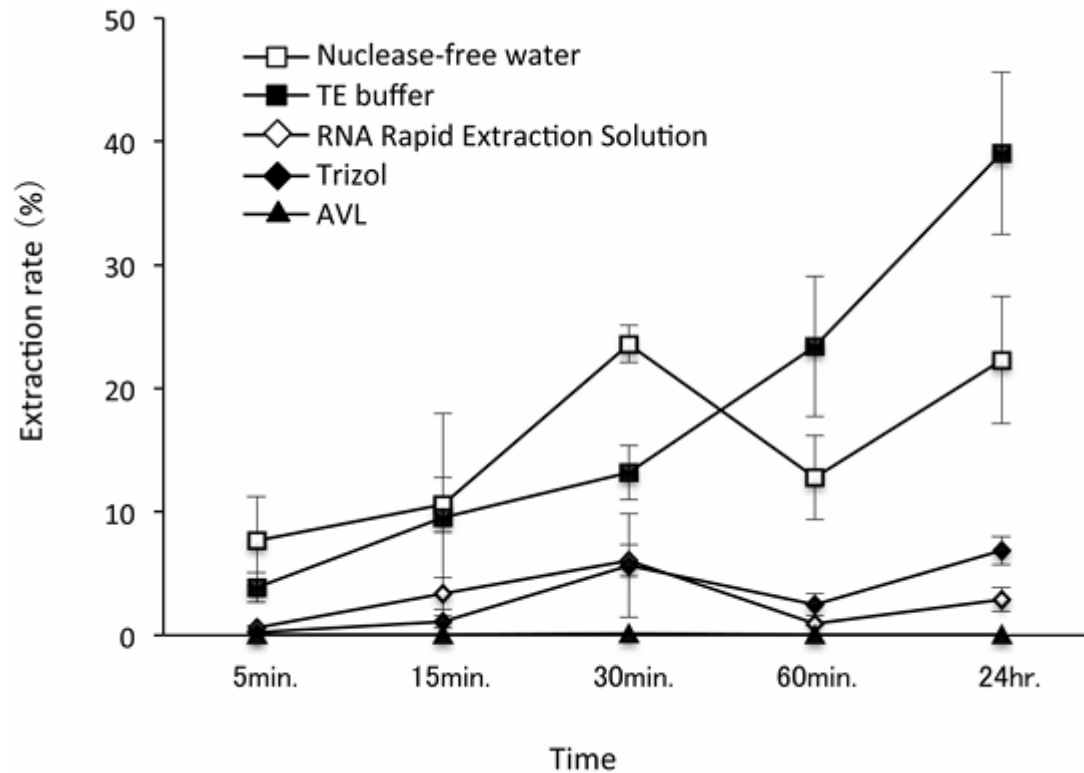


Results

The elution rate of vRNA was highest after 30min in nuclease-free water, similar pattern observed with all eluents except TE buffer.

The RNA elution rate in TE-buffer increased with longer elution times.

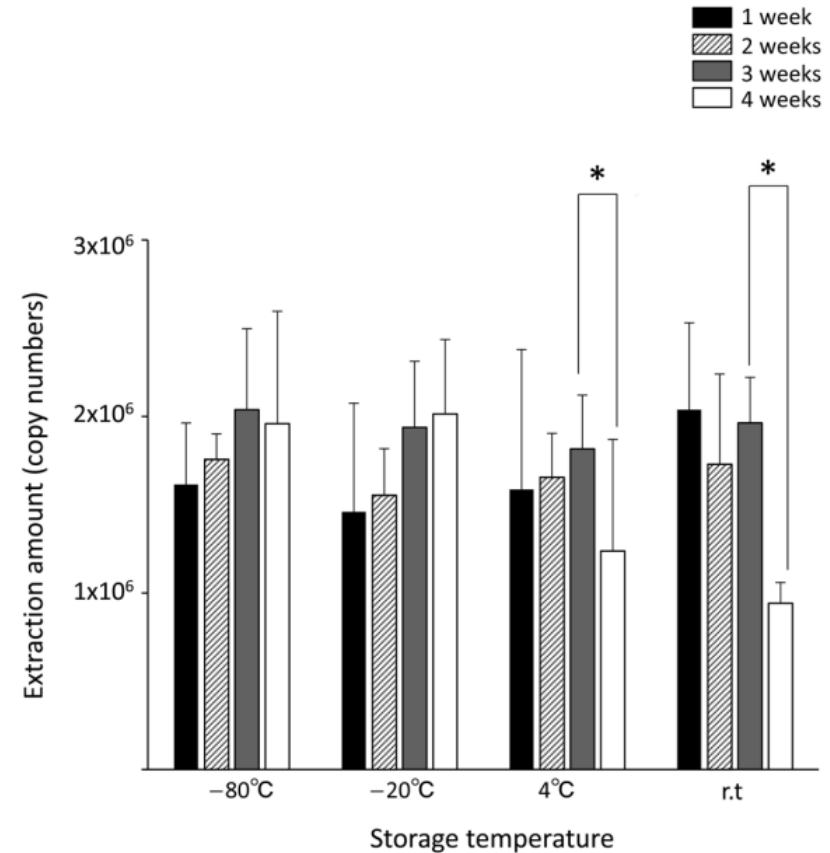
Amount of amplified products were low compared with TE buffer.



Results

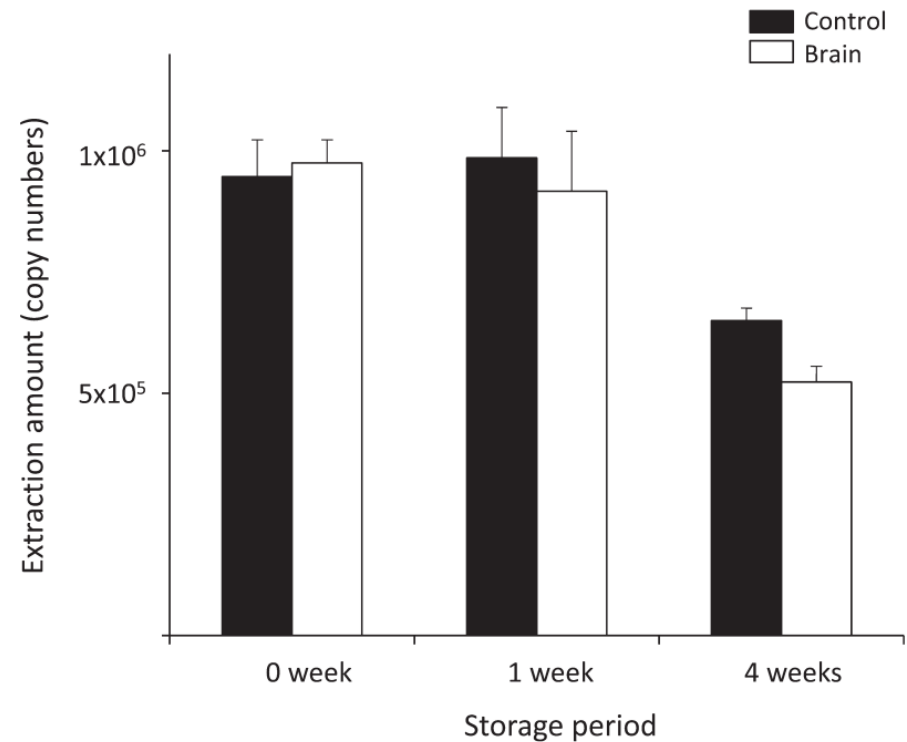
The FTA cards stored at temperatures of -80°C and -20°C there was no significant damage to RNA.

The FTA cards stored at 4°C and room temperature, show significantly decline between 1-2 weeks at room temperature and 3-4 weeks at 4°C .



Results

Samples with or without brain emulsion show no significant difference in the amount of RNA eluted from the FTA cards stored at -80°C .



Results

Table 5. Detection of RABV RNA from brain samples applied to FTA® cards after 6 months storage

Sample	Host	Elution for 30 min		Elution for 24 hr	
		964 bp ^{a)}	290 bp ^{b)}	964 bp ^{a)}	290 bp ^{b)}
BRhr1502	Equine	—	—	+	+
BRbv1503	Bovine	—	+	—	+
BRbv1504	Bovine	—	+	+	+
BRhr1505	Equine	—	+	—	+
BRbv1506	Bovine	—	+	—	+
BRbv1507	Bovine	—	+	+	+
BRhr1508	Equine	—	—	—	—
BRbv1509	Bovine	—	+	—	+
BRbv1510	Bovine	—	+	—	+
No. of detection/No. of samples		0/9	7/9	3/9	8/9

a) The primer pairs of P1 and P2 were used for the amplification of 964 bp of RABV N gene. b) The primer pairs of Nes-S and Nes-C were used for the amplification of 290 bp of RABV N gene.

These results suggest that the elution rate of vRNA increased with elution time of RNA from FTA® cards from 30 min to 24 hr, although short-chain RNA was detected following a 30-min elution in TE-buffer.



Conclusions

FTA cards containing RNA samples should be stored as cool as possible.

Low concentration of the RNA on the cards can affect the RNA stability even when a freezer is used for card storage.

TE-buffer was found to be the most suitable eluent for RABV RNA from FTA cards of those compared in this study, and elution time >30 min from FTA cards.

It was difficult to obtain long-chain amplified products from field samples stored for a long time on FTA cards, we were able to detect short-chain RNA.

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