Letter to the Editor

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Stability of SARS-CoV-2 RNA in FTA card spot-prep samples derived from nasopharyngeal swabs

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To the Editor,

The diagnostic and therapeutic approaches towards the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic are a global challenge, and the reverse transcription polymerase chain reaction (RT-PCR) of virus RNA isolated from nasopharyngeal swabs (NPS) has become a common tool to confirm the clinical diagnosis of SARS-CoV-2 [1].

Despite the Centers for Disease Control and Prevention's (CDC) recommendation to transport diagnostic specimens in viral transport medium (VTM) that preserves SARS-CoV-2 viability and infectivity [2], there is a growing need for the implementation of inactivated virus transportation in order to allow safe handling processes at diagnostics laboratories [3]. Moreover, laboratories must be aware of preanalytical inconsistencies that may adversely affect SARS-CoV-2 RNA stability [1].

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At present, various studies have addressed the topic of virus RNA stability in different VTM [4–7], however, reports on VTM for SARS-CoV-2 inactivation are scarce [8].

Available evidence suggests, that Whatman™ Flinders Technology Associates™ (FTA) cards are a reliable option for safe transport and storage of viral RNA pathogens [9]. Minimal storage space, easy transportation, long-term storage at room temperature (RT), and simple extraction protocols are the main advantages of this non-infectious medium. Especially in endemic and developing countries, sample transportation at ambient temperature without the need of cold chain may be of advantage.

Because data about the utility of FTA cards for the detection and preservation of SARS-CoV-2 RNA are lacking, this work was conducted to evaluate the influence of different storage temperatures and periods on the stability of virus RNA in FTA card samples derived from SARS-CoV-2 positive NPS.

Nineteen SARS-CoV-2 positive anonymized remnant NPS collected in 2 mL 0.9% saline solution using a wooden applicator with small cotton tip (Ø2.2 × 150 mm; nerbe plus GmbH & Co.KG, Winsen/Luthe, Germany) were available. FTA cards (Whatman™ FTA™ Classic Card; GE Healthcare, Little Chalfont, United Kingdom) were prepared by pipetting 150 µL of swab sample onto the center of each printed circle area. Figure 1 shows an FTA card before and after sample application. The FTA cards were then dried at RT for 2 h and stored at four different temperatures (–20, 4–8 °C, RT, 37 °C) for 1–3 weeks with each printed circle area representing a single storage condition. A reference FTA (rFTA) card sample was obtained immediately after the filter paper had dried out. For RNA elution, four punches of diameter 6 mm were obtained from the FTA cards and overnight-incubated in 400 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at RT. Using the chemagic Viral DNA/RNA 300 Kit on the chemagic 360 instrument (both PerkinElmer chemagen Technologie GmbH, Beasweiler, Germany), we extracted 300 µL NPS or FTA card sample according to the manufacturer's instructions. RT-PCR was performed on a cobas z 480 Analyzer (Roche Diagnostics, Mannheim, Germany) in a 20 µL mixture containing 10 µL RNA template, 0.5 µL

Figure 1: FTA card before (left) and after (right) the application of 150 μ L NPS sample.

LightMix® SarbecoV E-gene plus EAV control (Cat.-No. 40-0776-96; TIB MOLBIOL, Berlin, Germany), and 4.1 μ L LightCycler® Multiplex RNA Virus Master (Roche). The EAV control target was added to each NPS sample to be extracted thereby also serving as an internal PCR control.

PCR conditions comprised an reverse transcription step at 55 °C for 3 min followed by an initial denaturation step at 95 °C for 30 s and 45 cycles of denaturation at 95 °C for 3 s, and annealing/elongation at 60 °C for 12 s. Each PCR run contained a non-template control with C_t mean values calculated from duplicate reactions. All manipulations were performed in a biological safety level 2 (BSL-2) cabinet. This study was approved by the Institutional Review Board of Sigmund Freud University Vienna, and carried out complying with the latest version of the Declaration of Helsinki.

The effect of different temperatures on the stability of SARS-CoV-2 RNA FTA card spot-prep samples were evaluated over a storage course of three weeks. After week 3 we observed differences between the C_t values obtained for storage temperatures at –20, 4–8 °C, RT, and 37 °C and the rFTA card sample to range from –0.6 to 1.1%, –2.5 to 4.9%, –1.8 to 4.9%, and 0.6 to 6.4%, respectively. C_t determinations obtained after week 1 and 2 were also within this range (data not shown). We did not observe PCR inhibition in our clinical samples judging from the amplification curves obtained for the EAV control target (data not shown).

The mean ΔC_t value between rFTA card sample and respective NPS sample measurements was 3.06 ± 0.31 , indicating an initial RNA loss of about 10-fold. The lower copy number of FTA card-derived RNA may be explained by two reasons: (i) the number of four FTA card punches representing an actual NPS inoculation volume of approximately 35 μ L, and (ii) only three-quarters of the elution volume (i.e., 300 μ L instead of 400 μ L) could be used for the extraction of FTA card-derived RNA, thereby reducing the amount of elutable RNA, an effect already

described for the nucleic acid testing of other viruses archived on FTA cards [10].

Here, we did not observe relevant changes in RNA quantity between FTA card samples stored for up to three weeks at different temperatures (–20, 4–8 °C, RT, and 37 °C). This is in accordance with a former study that could not find a substantial impact of the storage temperature (RT, 4, –20, and –80 °C) on the detectability of SARS-CoV-2 RNA from NPS collected in phosphate-buffered saline (PBS) for up to a month [7].

Although many viruses will become inactivated during FTA card sampling and storage [9], specimens should be considered potentially contagious until proven otherwise. Therefore, SARS-CoV-2 FTA card samples should be handled with care complying with the same safety standards as applicable to NPS or other potentially infectious materials.

In conclusion, our results suggest, that SARS-CoV-2 RNA from NPS collected in 0.9% saline solution is stable on FTA cards for at least three weeks even at elevated storage temperatures.

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