Persistence of SARS-CoV-2 virus and viral RNA on hydrophobic and hydrophilic surfaces

and investigating contamination concentration

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Running title: Recovery of SARS-CoV-2 virus and RNA from surfaces

Abstract (229 of 250)

The transmission of SARS-CoV-2 is likely to occur through a number of routes, including

contact with contaminated surfaces. Many studies have used RT-PCR analysis to detect

SARS-CoV-2 RNA on surfaces but seldom has viable virus been detected. This paper

investigates the viability over time of SARS-CoV-2 dried onto a range of materials and

compares viability of the virus to RNA copies recovered, and whether virus viability is

concentration dependant.

Viable virus persisted for the longest time on surgical mask material and stainless steel with

a 99.9% reduction in viability by 124 and 113 hours respectively. Viability of SARS-CoV-2

reduced the fastest on a polyester shirt, with a 99.9% reduction within 2.5 hours. Viability on

cotton was reduced second fastest, with 99.9% reduction in 72 hours. RNA on all the

surfaces exhibited a one log reduction in genome copy recovery over 21 days.

The findings show that SARS-CoV-2 is most stable on non-porous hydrophobic surfaces. RNA

is highly stable when dried on surfaces with only one log reduction in recovery over three

weeks. In comparison, SARS-CoV-2 viability reduced more rapidly, but this loss in viability

was found to be independent of starting concentration. Expected levels of SARS-CoV-2 viable

environmental surface contamination would lead to undetectable levels within two days.

Therefore, when RNA is detected on surfaces it does not directly indicate presence of viable

virus even at high CT values.

Importance (81 of 150)

This study shows the impact of material type on the viability of SARS-CoV-2 on surfaces. It

demonstrates that the decay rate of viable SARS-CoV-2 is independent of starting

concentration. However, RNA shows high stability on surfaces over extended periods. This

has implications for interpretation of surface sampling results using RT-PCR to determine the

possibility of viable virus from a surface. Unless sampled immediately after contamination it

is difficult to align RNA copy numbers to quantity of viable virus on a surface.

Keywords: SARS-CoV-2, Environmental persistence, COVID-19, Transmission, Surface

sampling

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease

2019 (COVID-19) has spread globally and many countries are experiencing ongoing local

transmission despite varying levels of control efforts. SARS-CoV-2 is primarily transmitted via

respiratory droplets from an infected host (1). Studies have confirmed aerosol viral

transmission (2-4) with SARS-CoV-2 being shown to remain viable in aerosols for between 90

minutes and 3 hours in laboratory studies (5, 6). Infections from direct person to person

transmission have been confirmed as well as indirect transmission through close contacts

after tracing of case clusters (7, 8). It is suspected that contaminated surfaces or fomites may

also have a role in transmission. Studies detailing SARS-CoV-2 viability on surfaces have

contributed to this (6).

SARS-CoV-2 RNA has been detected on environmental surfaces, potentially indicating the

presence of the viable virus (9, 10). Current environmental sampling of surfaces using swabs,

primarily uses real-time PCR (RT-PCR) to detect viral genome in samples. Few studies have

been able to isolate viable virus from environmental surface sampling, even where RT-PCR

indicates a high level of SARS-CoV-2 RNA is present (11). Recent manuscripts have identified

survivability ranges for SARS-CoV-2 on surfaces in the laboratory, with only one

demonstrating the relationship between viable recovered virus and RNA on the surface (12,

13). As the risk of infection from of virus-contaminated surfaces is difficult to predict (14),

further investigation is required to enhance understanding on the survivability of SARS-CoV-

2 on surfaces.

The three aims of this study were; (i) measure the persistence of viable SARS-CoV-2 virus on

common personal protective equipment (PPE) materials (both hospital-grade and reusable

fabrics), high-touch surface materials and commonly worn fabrics; (ii) to investigate the

relationship between recoverable viable virus from these surfaces and the levels of SARS-

CoV-2 RNA in the same sample; (iii) determine the relationship between inactivation rate

and initial viral titre load on surfaces.

Results

SARS-CoV-2 viability decreased on all materials during the 2.5 hour drying period, on average

by 1.01 $\log_{10}$ (between -0.18 $\log_{10}$ for disposable gown to -3.66 $\log_{10}$ for polyester sports

shirt, standard deviation 1.06 $\log_{10}$) from a high starting inoculum of approximately 4×10^5

pfu per material. Viable virus could be recovered from the surgical mask and stainless steel

coupons for the longest periods of time ($\log_{10}$ reductions of 4.91 and 4.99, respectively, over

7 days). The recovery time for cotton t-shirt and polyester sports shirt materials was shorter

($\log_{10}$ reductions of 5.15 over 5 days and 3.9 within 1 day). RNA copy number was recovered

at higher concentrations in all samples compared to the levels of viable virus, decreasing by

~1.5 $\log_{10}$ (non-porous, hydrophobic) and ~1 $\log_{10}$ (porous, hydrophilic) over the initial 7

days, then stabilised at around $10^{7.5}$ copies per coupon from day 7. The ratio of viable virus

recovered ranged from 10^3 to 10^8 times less when compared to viral RNA assayed, from start

to finish of the study period (Fig 1).

Linear regression analysis was completed using the recoverable virus data to calculate time

for percentage reduction (Table 2). Regressions were calculated from t=2.5 hours

onwards. Calculated decay rate is fastest on cotton with a 99.9% reduction in recovery

within 72 hours. The longest survival of virus is observed on surgical mask material, where

124 hours is required for a reduction of 99.9%. For the polyester sports shirt a >3 $\log_{10}$

reduction was detected during the initial 2.5 hour timepoint (Table 2).

The results from the comparative study involving two viral titres revealed an initial rapid

decrease in the recovery of viable virus from the surface during the drying period, with the

inactivation rate decreasing after drying. Virus was recovered after four days for the low

inoculum and up to seven days for the high inoculum (Figure 2). Parallel survival rates of high

and low inocula demonstrate that the decay rate of SARS-CoV-2 is independent of

concentration when applied to a stainless steel surface (P>0.05).

Discussion

Contact with SARS-CoV-2 contaminated surfaces is thought to be a route of transmission in

the current pandemic (8, 15). Surfaces can be contaminated by virus-containing droplets

generated from an infected individual or contact with contaminated hands, with potential

onwards transmission via direct surface contact (16). With contamination events likely to

occur in a range of materials, this study investigated the survival of SARS-CoV-2 UK isolate

hCoV-19/England/2/2020 and associated viral RNA on a range of surfaces that are at risk

from droplet and touch contamination.

Existing studies examining time-based viability of SARS-CoV-2 on different surfaces have

focused on a single virus titre (6, 12, 13, 17). Our work investigated the persistence of high

titres of SARS-CoV-2 on various surfaces, and at two different titres on stainless steel

coupons with identical conditions.

When inoculated onto stainless steel, a five-log reduction in viability of the UK SARS-CoV-2

isolate England 02/2020 HCM/V/052 was observed over a 7 day period (Figure 1). Riddell *et*

al showed a similar $\log_{10}$ reduction at 28 days and Kasloff at 14 days, using the Australian

isolate Betacoronavirus/Australia/SA01/2020 and Canadian isolate hCoV-19/Canada/ON-

VIDO-01/2020 respectively (12, 17). In both studies, the viral propagate included additives

such as serum and mucin to mimic bodily secretions (12, 17). Where in this current study the

virus stock suspension was centrifuged to remove the majority of the cell debris, but

left salts and proteins from the growth media used to propagate the virus. The initial starting

inoculum concentration (between 10^5-10^6 pfu per surface) and environmental conditions of

temperature and RH are similar for the two previous studies and this study, demonstrating it

is likely that the differences observed in survival results could primarily arise from a

protective effect afforded by added serum/mucin and/or the different isolates used. Whilst

potentially artificially high, the starting inoculum in this current study provides the ability to

determine the inactivation characteristics of the virus on the different materials which a

lower starting inoculum may not.

Currently there are no published studies investigating inter-isolate differences in

environmental surface stability of SARS-CoV-2. A study investigating the stability of SARS-

CoV-1 (AY274119.3) and SARS-CoV-2 (nCoV-WA1-2020) by van Doremalen *et al* showed that

both were similar under their experimental test conditions (6). Chin *et al* have reported

similar findings to the work presented in our study using a comparable starting

inoculum, also without additional protein (13). Their results showed that infectious SARS-

CoV-2 was recovered from a banknote and stainless steel on days 4 and 7

respectively, compared to our results of recoverable virus on day 5 from the banknote and

recovery on day 7 from stainless steel. With little evidence of difference in environmental

stability between isolates of SARS-CoV-2 the addition of bovine serum albumin and mucin to

the inoculating suspensions indicates that additional protein provided a protective effect to

the virus during and after drying onto the surfaces (12, 13, 17).

Droplets on non-porous or hydrophobic surfaces dry in a beaded shape, giving a high

volume to surface area ratio. In such an environment, these droplets can produce a core-

shell structure (18), which can concentrate the virus particles, salts and organic material into

smaller clumps (19). Such clumping is seen often in nature due to association with cellular

matter or protein (20, 21). These closely associated virus particles are protected from

environmental pressures such as desiccation, UV and heat, which cause inactivation (20).

However, on porous or hydrophilic surfaces the droplets are absorbed into

the material across a larger surface area, which will lead to less clumping and to

the presentation of individual viral particles; this may confer less protection from the drying

effects of the environment, leading to a reduction in the viability of the virus.

Whilst other studies have designated materials as porous and non-porous, this may be an

oversimplification of the surfaces studied (12, 17). The surface of a surgical mask is porous

but is made up of overlapping hydrophobic fibres; similarly, Tyvek material is produced

with non-woven fibres of high density hydrophobic polyethylene, but presents microscopic

pores on the surface. In the context of our study, relatively low amounts of liquid are being

added to these surfaces. Thus, these small droplets of liquid cannot penetrate into the

materials, as their hydrophobicity ensures the droplets of liquids remain on the surface of

the material during the drying process; making the surfaces behave more like a non-

porous one. Our results show that the porous but hydrophobic surfaces of the surgical mask,

disposable gown and Tyvek coverall produce similar decay rates when compared to the non-

porous hydrophobic surfaces of stainless steel with a five $\log_{10}$ reduction in recovered

infectivity over 7 days. Viable SARS-CoV-2 was recovered from these surface materials over

longer periods of time compared to the truly porous and hydrophilic surfaces tested,

cotton and woven polyester. An exception was the hydrophobic polymer bank note, from

which viable virus was recovered at the limit of detection for days 5 and 7 of the study. This

is different to the study of Riddell *et al* where the recovery from the Australian bank note

was similar to the other non-porous surfaces tested (17). At present it is not clear why this

surface had decreased viability compared to the other non-porous hydrophilic surface used

in this study, although there may be antiviral properties from some of the dyes used in the

bank note.

Following a 4.73 $\log_{10}$ decrease, infectious virus was recovered from cotton material up to 3

days after inoculation; matching previous studies, reporting more rapid inactivation of virus

particles on cotton surfaces compared to others (12, 17). These results may be attributed to

two factors unrelated to any potential anti-viral activity of the material: retention of virus

within the cotton fibre matrix, or losses during the inoculum application due to wicking. Due

to the cotton's hydrophilic, woven nature, the liquid inoculum rapidly absorbs and

penetrates into the fibres which, when dried, might cause interactive forces, limiting the

release of virus particles, which is shown by a greater than 1 $\log_{10}$ reduction in recovery of

viable virus after the drying period. This decrease in detection of viable virus may therefore

be attributable to inefficient recovery from this specific type of material rather than

increased inactivation. This result indicates that viral particles may remain in cotton fibres

after contamination posing a forward transmission risk, but they will likely not be released

from the substrate to cause infection. To counteract the materials inherent absorbent

nature, during the inoculation and drying steps we suspended the cotton in strips across an

open box. Whilst this exposed the virus inoculated coupon to the environmental conditions

on both sides of the coupon it reduced any potential losses of the virus due to wicking on to

container surface from the coupon as seen in a previous study (12).

Though polyester, produced from polyethylene terephthalate, is hydrophobic, when spun

into fine fibres, aligned in the same orientation and woven into fabric, it behaved like the

other woven fabric tested, cotton. It is possible that the aligned polyester fibres which are

close together, but not fused, causes capillary action to draw the liquid into the interstitial

spaces between the fibres and trap the virus particles. Virus that was inoculated onto

polyester sports shirt was rapidly inactivated to unrecoverable levels in one day; there may

also be interaction between the chemicals used to process/colour the fabric and the virus

(22).

SARS-CoV-2 RNA has been detected on surfaces in different environments, but there have

been few reports of viable virus recovery from these surfaces. The use of RT-PCR to

determine the presence of SARS-CoV-2 on surfaces has advantages, increased sensitivity (RT-

PCR can detect small amounts of target RNA) and rapid high throughput of samples

compared to culture-based methods. The limitation of the use of RT-PCR in such studies is its

inability to distinguish between viable and non-viable virus.

The detection of SARS-CoV-2 RNA from surface samples is used to indicate that virus (viable

or non-viable) has been present on that surface at some point previously. Lower cycle

threshold (CT) values from the RT-PCR assay indicates that more copy numbers of target

RNA are present in that sample. Our study determined that a CT value of 18 equates to

approximately 5×10^8 copy numbers of the RNA target. The initial recovery of infectious

virus from the materials (Figure 1), excluding the polyester sports shirt, is approximately 3.1

$\log_{10}$ (SD 0.13 $\log_{10}$) lower than the copy number, showing that there is a large amount of

RNA exogenous to infection-competent viral particles in the inoculum. This

difference between infectious virus and RNA copy number was also reported by Kasloff *et al*

(12). The ratio between copy number and viable virus changes with time with recoverable

infectious virus rates reducing more rapidly than copy numbers recovered in the same

sample over time. The RNA is therefore more persistent in the environment compared with

infectious virus. It has been shown that SARS-CoV-2 RNA was detected on cruise ship

surfaces 17 days after cabins had been vacated (23).

Thus, it is not possible to draw conclusions on the viability of surface contamination from

genome copy number of RNA detected after the initial contaminating event. In addition,

the comparative persistence of RNA on the surfaces in comparison to infectivity makes it

difficult to relate copy number to the date when the contamination may have occurred.

Although our laboratory-based study used a concentration of infectious virus that may not

reflect the contamination load present in the environment, we demonstrated recovery of

viable virus at high and low concentrations.

Patient nasal and throat swab samples have produced CT values below 18 (24, 25), even to a

CT value of 10 (26), but reported surface samples have produced CT values above 28 (9, 27-

29). Using the results from our study to provide a calculation of the initial viable load on the

surface, a CT of 28 would provide an approximate infectious virus titre of 10^2 viral particles.

Using the recovery results from stainless steel as a representative surface in this study

(Figure 2), infectious virus from an initial recoverable inoculum of 10^2 viral particles would be

unrecoverable within 2 days. This is based on the vortex mixing recovery method used and

the detection would be thought to reduce further using direct surface sampling methods.

Future studies could address this limitation of knowledge where the copy number is

determined for much lower concentrations of SARS-CoV-2 on surfaces which will help to

further identify if the persistence of RNA is independent of concentration and addressing the

relationship to viable virus recovery.

Conclusions:

This study shows that the UK SARS-CoV-2 isolate, hCoV-19/England/2/2020, remains viable

on hydrophobic surfaces for up to 7 days, with recoverable viability on hydrophilic surfaces

reduced to 3 days at ambient temperature and relative humidity. Indicating that some

common surfaces could pose an infection risk if contaminated with high concentrations of

virus, although viable virus contamination levels of environmental surfaces are likely to be at

a low concentration. In contrast, recovery of RNA from the same samples shows little

reduction in copy numbers over the same period. The data presented also indicates that the

inactivation rate on environmental surfaces is independent of initial loading for SARS-CoV-2

and varies depending on surface type.

Materials and methods

Viral isolate

The SARS-CoV-2 isolate, England 02/2020 (EPI_ISL_407073) passage 3 (P3) used in this study

was propagated by the High Containment Microbiology Department at PHE, Porton Down.

The virus was isolated from a clinical sample taken during acute phase illness, using Vero E6

cells (ECACC, 85020206). A P2 master bank was produced using Vero E6 cells (BEI Resources,

NR-596) and a P3 working bank produced in Vero/hSLAM cells (ECACC 04091501). Cell lines

were infected at 95% confluence with an of MOI 0.0005 – 0.01 and maintained in 1x Minimal

Essential Medium GlutaMax, 4% heat-treated foetal bovine serum (Gibco), 1x non-essential

amino acids (Gibco), 25mM HEPES buffer (Gibco); additionally, Vero/hSLAM cells were also

maintained in the presence of 0.4mg/ml geneticin (ThermoFisher Scientific, Gibco). Virus

was harvested 3-6 days post-infection and supernatant clarified by centrifugation (3000 rpm,

10 min), virus was aliquoted and stored at -80oC. The titre of the P3 virus stock was

determined to be 2.0 x 107 pfu/ml by plaque assay. All work handling SARS-CoV-2 was

performed within a containment level 3 laboratory.

Preparation of test surfaces

The surfaces used in this study are representative of non-porous hand-touch sites (stainless

steel, 316 grade) and bank note (English polymer £10 note); PPE items used in the hospital

and wider environments (Multiple layered surgical mask, Tyvek coverall, disposable plastic

gown (37310 Breathable Impervious Gown)); and materials representing clothing items;

cotton t-shirt (Fruit of the Loom) and polyester sports shirt (85% polyester, 15% elastane,

Activewear SFP5-M02). 1cm x 1cm coupons of each material were prepared. Once prepared,

non-porous coupons (stainless steel and bank note) were cleaned with Neutracon (NEU5,

Scientific Laboratory Supplies, Nottingham, UK) detergent followed by rinsing with 70% IPA.

Additionally, stainless steel was sterilised by autoclaving. The surfaces not suitable for

cleaning (disposable plastic gown, cotton t-shirt, polyester sports shirt and Tyvek

coverall) were purchased new and handled aseptically. Materials were subdivided

depending on their surface properties, Table 1.

Inoculation of test surfaces

Viral aliquots were thawed to room temperature immediately prior to inoculation of the

materials. The stock suspension was diluted with cMEM to give a starting concentration of 4

$x\ 10^7$ and $4\ x\ 10^5$ pfu/ml for high and low loading, respectively. Coupons were inoculated by

the addition of 2 x 10μl droplets of virus culture within a negative pressure flexible film

isolator (FFI) and were left uncovered in plastic petri dishes in the FFI for the duration of the

study at a temperature of 21.5°C (±1°C) and an average relative humidity of 45%. Three

biological replicate coupons were prepared and inoculated for each time point (hours; 0, 2.5,

24, 48, 72, 96, 144, 168, 336 and 504) and each material. Triplicate coupons exposed but not

inoculated with the virus acted as negative controls.

Recovery of SARS-CoV-2 from test surfaces

Recovery of virus from the coupons at each specified timepoint was performed by

transferring a coupon into 1ml of cMEM in a 7ml bijou tube with 4 glass beads (3mm

diameter), followed by vortex mixing for 1 minute at maximum (Heidolph Multireax vortex).

The resulting suspension was transferred to a cryotube for storage at -80°C before analysis.

Storage of samples at -80°C was not found to affect viability of the virus or denature the RNA

(results not shown). Timepoint zero (t=0) coupons were recovered within 5 minutes of being

inoculated, before any drying occurred.

Plaque Assay

Coupon recovery liquid was assayed by thawing samples at room temperature, then serially

diluted (1 in 10) with MEM ((Gibco), 1% L-glutamine (Gibco), 1% Non-Essential Amino Acids

and 2.5% HEPES 1M). 100µl of each dilution was pipetted in duplicate (technical replicates),

up to four replicates for neat dilutions (400µ) onto confluent vero-E6 cells within a 24 well

plate (7.9 x 10^4 cells/cm^2). After one hour of incubation (±15 minutes) at 37°C with plate

rocking every 15 to 20 minutes, 0.5mL CMC overlay was added to each well; (1.5% CMC (3%

(w/v) carboxymethylcellulose solution in sterile distilled water (Sigma C4888)), 1% Antibiotic

Antimycotic Solution (100x) (Sigma Aldrich), 2x Overlay Media (20% 10x MEM (Gibco), 2% L-

glutamine (200mM) (Gibco), 2% Non-essential amino acids (Gibco), 6% Sodium bicarbonate

solution (Gibco), 8% Fetal bovine serum (Sigma Aldrich), 5% HEPES buffer (Gibco) and 57%

Distilled water (Versol). After 3 days incubation at 37°C, cells were fixed with formaldehyde

and stained by addition of approximately 250µl 0.2% crystal violet for 5 minutes, before

washing with water. The number of plaques in each well was determined and expressed as

plaque forming units (pfu).

RNA extraction and RT-PCR Analysis

RNA was extracted from aliquots (140 µL) of the coupon recovery liquid using the QIAamp

Viral RNA Mini Kit (Qiagen Ltd, Manchester, UK). RT-PCR was performed using the VIASURE

SARS-CoV-2 Real Time PCR Detection Kit (Viasure; CerTest Biotec, Zaragoza, Spain), following

the methods provided. Quantification was undertaken using the N target with a standard

curve generated by serial dilution of an *in vitro* transcript (10).

Data Analysis

Each time point for each material coupon had 3 biological replicates (individual coupons)

and 2 technical replicates (plaque assay performed in duplicate). Calculations for the mean

and standard deviations of the assay counts were determined from these 6 replicates using

Microsoft Excel (Office 365). Time to percent reduction values were calculated using linear

regression of pfu/coupon averages, in Minitab 18. High and low loading line slopes were

analysed using linear regression analysis with GraphPad Prism software version 7.

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Conflict of interest

The authors do not have any conflict of interests.

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Table 1. Materials used in this study.

Fig 1. Mean quantities of viable virus recovered (pfu/coupon, orange) and viral RNA detected

(genome copy number/coupon, blue), for 7 materials assessed. Error bars represent the

standard deviation from three replicates. Grey dashed line represents the limit of detection of

the plaque assay for the combined assays from the triplicate coupons (0.8 pfu/ml). For Tyvek

coverall and disposable gown, 21 day coupons were not processed.

Table 2. Time for percentage reduction values for the multi-surface study. Data is sorted in

descending order by time to 99.9% survival rates. N/A = not applicable.

Fig 2. Virus viability results from loading of high (4 x 10^5 pfu added, blue) and low (4 x 10^3 pfu

added, red) SARS-CoV-2 inoculum onto stainless steel coupons (n = 3). Dashed lines show

linear regression based on recovery over time.

Material	Properties
Stainless steel, Tyvek, disposable gown, bank note	Hydrophobic, non-porous
Surgical mask	Hydrophobic, porous
Cotton T-shirt, polyester sports shirt	Hydrophilic, porous

Table 1. Materials used in this study.

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Tyvek coverall and disposable gown, 21 day coupons were not processed.

Material	Log change after 2.5 hour drying	Time for percentage reduction (hours)		
		R^2 value	90%	99%	99.9%
Surgical mask	-0.64	0.954	27.9	76.2	124.4
Stainless steel	-0.56	0.982	30.7	71.6	112.6
Tyvek Coverall	-0.70	0.962	33.3	69.3	105.2
Disposable Gown	-0.18	0.946	23.0	59.7	96.5
Bank note	-1.13	0.956	17.5	45.1	72.7
Cotton t-shirt	-1.34	0.904	17.2	44.6	72.0
Polyester sports shirt	-3.66	N/A	<2.5		

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