Imperial College London Projects

Environmental Research Group

Transport For London SARS-CoV-2 RNA Sampling Study

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1. Executive Summary

In December 2019, a pneumonia outbreak was reported in Wuhan, China, and traced to a novel strain of coronavirus named SARS-CoV-2; as of 27th March 2020, there have been over 2 million deaths and over 105 million confirmed cases of COVID-19 worldwide. To prevent the spread of this disease, national governments have restricted local, national and international travel, and introduced social distancing which has significantly impacted economic activity.

This study was commissioned by Transport for London in August 2020 to investigate whether SARS-CoV-2 could be detected in the Transport for London network of stations, underground trains and buses between September and December 2020. Frequently touched surfaces and air samples were taken at three London Underground stations (London Waterloo, London Euston and London Paddington), on a Northern Line train between Waterloo and Euston and on the #21 bus between Euston and Paddington. All results were negative - no traces of SARS-CoV-2 RNA were found.

It provides a baseline for ongoing sampling campaigns to assess the presence of viral contamination on the network over time as well as the efficacy of cleaning and improved ventilation approaches being adopted by the industry.

These uniform negative results reflect the enhanced cleaning approaches adopted by the industry, the widespread wearing of face coverings by passengers and the lower passenger numbers on the network at the time of sampling.

2. Literature Review

A summary of the current scientific understanding of viral transmission in the context of public transport and the emerging evidence of SARS-CoV-2 in these environments is presented here.

a) Understanding viral transmission

Respiratory viruses are transmitted in three ways [1]:

- Surface transmission, where someone comes into direct contact with an infected person or touches a surface that has been contaminated.
- Droplet transmission of both large and small respiratory droplets that contain the virus, which would occur when near an infected person.
- Airborne transmission of smaller droplets and particles that are suspended in the air over longer distances and time than droplet transmission.

In the early stages of the pandemic surface transmission was the primary concern. However, recent research[2] concludes that, although the virus can be detected on surfaces for days, attempts to culture the virus have been unsuccessful and it is unlikely to be a major route of transmission. Consequently, the World Health Organisation (WHO) states [3] that the SARS-CoV-2 virus spreads primarily via droplets and aerosols generated when an infected person coughs, sneezes or speaks. Nevertheless, you can become infected by touching a surface contaminated with the virus by then touching your eyes, nose or mouth.

b) COVID-19 and public transport environments

Public transport environments (trains, buses, trams) are typically characterised by confined, environments where large numbers of people mix for extended periods of time and are associated with surfaces which are frequently touched (buttons, handles). Microbial infections have therefore been studied in train environments internationally [2, 4-6] and in the UK [7]. Early in the pandemic, before mask wearing was adopted, transmission was identified on a bus journey in China [8]. A more recent study [9] examined the COVID-19 outbreaks in China involving three or more cases (1245 cases, 318 outbreaks across 120 cities) found that all were associated with indoor environments and that transport based outbreaks were the second most dominant category (behind homes). The importance of train travel as a transmission route was also confirmed by an epidemiological and modelling study [10] which analysed data from 2334 index patients and 72 093 close contacts who had co-travel times. The transmission risk showed significant differences with co-travel time and seat location demonstrating the importance of social distancing.

c) Detecting the virus

SARS-CoV-2 in the environment is detected using Reverse-Transcription Polymerase Chain Reaction (RT-qPCR); the same technique used for human testing. Surface samples are typically taken using swabs (flocked swabs) which are wiped over the area of interest, these are then immersed in virus transportation medium to maintain virus prior to analysis. Air samples can be taken using filters, which can then be immersed in the cell culture fluid when completed. Alternatively, droplets and aerosols can be impinged directly into the cell culture fluid or collected onto filters or surfaces impregnated with cell culture medium. The RT-qPCR technique is used to target regions of the genome unique to the SARS-CoV-2 virus and amplifies the amount of RNA in the sample to a level at which it can be detected. Very small amounts of individual viral RNA fragments can be detected but their presence does not necessarily indicate that viable virus is present. Large quantities of multiple genetic fragments would indicate high viral loads.

Surface and air contamination measurements have been undertaken widely in hospital environments using similar approaches to those used in this study (e.g. [11]) to understand and control hospital acquired infections. In a London-based hospital study [11], public spaces and wards with COVID-19 infected patients present were studied. Viral RNA was detected on 52% of surface samples and in 39% of air samples. In this context wider environmental surface and air contamination in public spaces has been of concern. Peer reviewed literature on the surface and air contamination of SARS-CoV-2 on public transport systems, similar to that undertaken here, is currently limited to a single study on buses and subway trains in Barcelona [12]. Of the 82 sampled in Barcelona, 30 showed evidence for the presence viral RNA but this was considered fragmentary, generally weak, and that the chances of infectivity were extremely low. Although they found a decrease in the prevalence of the virus after routine bleach cleaning, the researchers could not distinguish this from environmental decay.

3. Method

Viral RNA detection and absolute quantification was performed using quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR). RT-qPCR assays targeting the envelop (E) and ORF1a genes are used to detect SARS-CoV2 in the samples. A sample is considered positive when both E gene and ORF1a assays give Ct values less than 40. RNAaseP is used to indicate if the sample does contain human RNA.

a) Sampling and Analytical Methodology

The full sampling and analytical methods are included in the Appendix.

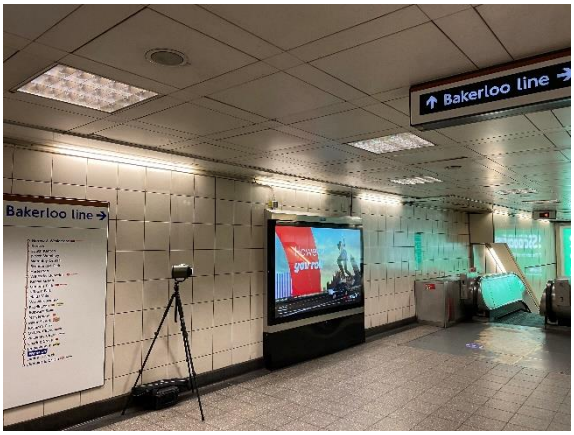
- Surface testing - Swabs were dipped in virus transportation medium and an area of approximately 25 cm^2 is swabbed before the swabs are placed in the vial containing virus transportation medium.
- Air samples – Filter samples were taken using a high-volume sampler (SASS 3100 Dry Air Sampler, Research International, USA) operated at 300 l/min for 1 hour, or the duration of the train or bus journey.
- After sampling the filters were placed in a vial containing virus transportation medium. Samples were kept cold following sampling and transported via refrigerated transport to the laboratory overnight.
- Viral RNA detection and absolute quantification was performed using RT-qPCR.

b) Sampling Dates and Locations

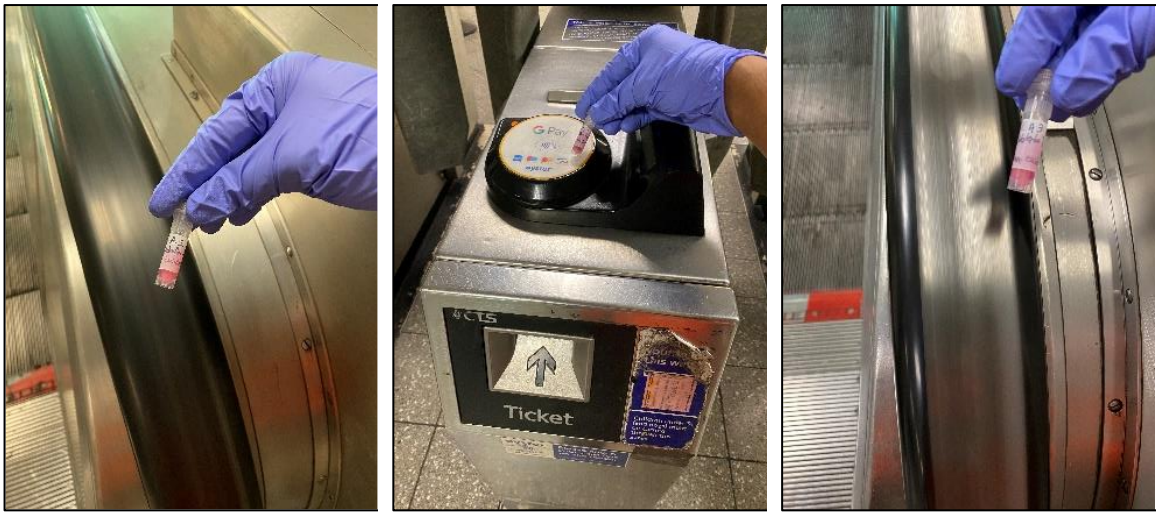
Sampling was undertaken on at three London Underground stations: London Waterloo, London Euston and London Paddington on the 23rd September, 21st October, 25th November and 23rd December 2020. Frequently touched surfaces were chosen in each location (e.g. escalators, buttons, handles) and an air sample was taken for 60mins in each station concourse and for the duration of the train or bus journey.

SAMPLE 1 – Waterloo underground tube station

The air sample was taken on the concourse outside the main LU station master's office located between the Bakerloo and Northern lines for 1 hour.



Swab A1 on the up escalator (either of the escalators) between Northern and Bakerloo lines, swab A2 on the oyster/contactless tap in/out system and swab A3 on the down escalator.



SAMPLE 2 – Northern line from Waterloo to Euston

The air sample was started before boarding the last carriage of the tube and sampled for the duration it takes to get from Waterloo to Euston.

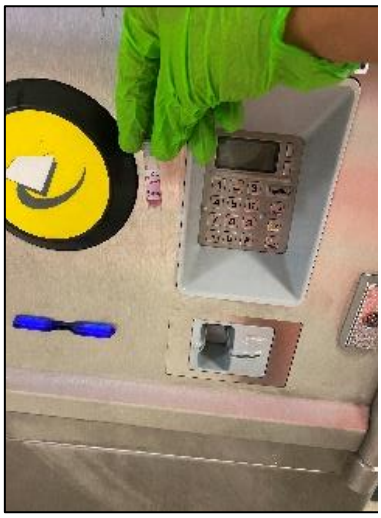
Swab B1 on the main vertical handlebar of the last carriage, swab B2 on a side handle bar near the door of the tube and swab B3 on the top main handle bar near the door.



SAMPLE 3 – Euston underground tube station

The air sample was taken on the station concourse for 1 hour.

Swab C1 on the oyster top-up machine keypad, swab C2 on the oyster tap in/out system, and swab C3 on the escalator (either of the escalators).



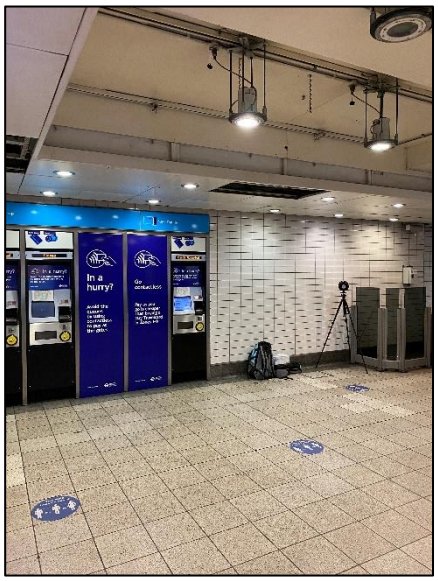
SAMPLE 4 – Bus 205 from Euston to Paddington

The air sample was taken on the top deck of the bus at the very end, with the sampler facing the gangway for the duration of the journey from Euston to Paddington.

Swab D1 on the stop push button of any of the handrails, swab D2 on the vertical handrail at the main exit of the bus, swab D3 on the exit button facing the side exit doors.

SAMPLE 5 – Paddington underground tube station

The air sample was taken on the station concourse for 1 hour.



Swab E1 on the oyster tap in/out system, swab E2 on the oyster top up machine keypad, and swab E3 on the escalator (either of the escalators).

4. Results

All RT-qPCR results were negative for both gene fragments – no traces of SARS-CoV-2 RNA were found in any of the sample locations during any of the journeys.

Location	#	sample	September			October			November			December		
			E gene	ORF1a	RNase	E gene	ORF1a	RNase	E gene	ORF1a	RNase	E gene	ORF1a	RNase
Waterloo	A	air	N	N	N	N	N	N	N	N	N	N	N	N
	A1	swab	N	N	36.7	N	N	N	N	N	N	N	N	N
	A2	swab	N	N	31.6	N	N	34.8	N	N	33.8	N	N	38.1
	A3	swab	N	N	37.3	N	N	N	N	N	37.8	N	N	N
Northern Line Train	B	air	N	N	37.3	N	N	N	N	N	37.7	N	N	N
	B1	swab	N	N	34.5	N	N	31.9	N	N	35.2	N	N	37.2
	B2	swab	N	N	35.3	N	N	N	N	N	35.0	N	N	37.5
	B3	swab	N	N	N	N	N	35.2	N	N	36.3	N	N	35.7
Euston	C	air	N	N	37.2	N	N	N	N	N	N	N	N	N
	C1	swab	N	N	33.7	N	N	32.8	N	N	34.2	N	N	N
	C2	swab	N	N	31.8	N	N	30.8	N	N	31.3	N	N	N
	C3	swab	N	N	37.1	N	N	N	N	N	N	N	N	38.0
#21 Bus	D	air	N	N	N	N	N	38.7	N	N	N	N	N	N
	D1	swab	N	N	35.8	N	N	33.5	N	N	35.0	N	N	35.0
	D2	swab	N	N	33.5	N	N	33.8	N	N	33.5	N	N	35.0
	D3	swab	N	N	33.3	N	N	34.2	N	N	34.4	N	N	34.2
Paddington	E	air	N	N	37.4	N	N	38.9	N	N	N	N	N	37.7
	E1	swab	N	N	32.7	N	N	31.7	N	N	33.7	N	N	N
	E2	swab	N	N	35.8	N	N	34.8	N	N	30.6	N	N	N
	E3	swab	N	N	N	N	N	38.8	N	N	N	N	N	36.9

Table 1: Summary of results (N indicates negative response)

5. Conclusions

This study sought to sample surfaces and air for the presence of SARS-CoV-2 RNA in the Transport for London network and whether there was any variation in the presence of virus over time. The sampling and analysis techniques adopted were consistent with the latest research approaches and World Health Organisation protocols.

20 samples were taken each month between September and December 2020 on frequently touched surfaces and air sample. They encompassed three London Underground stations (London Waterloo, London Euston, and London Paddington), onboard a Northern Line train between Waterloo and Euston and on the #21 bus between Euston and Paddington. All results were negative - no traces of SARS-CoV-2 RNA were found.

These uniform negative results reflect the enhanced cleaning approaches adopted by the industry, the widespread wearing of face coverings by passengers and the lower passenger numbers on the network at the time of sampling.

6. References

1. The Lancet Respiratory, M., *COVID-19 transmission—up in the air.* The Lancet Respiratory Medicine, 2020. **8**(12): p. 1159.
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7. Appendix

a) SAMPLING METHOD

- Travel to St. Mary's and contact Dr. Jie Zhou (at Virology) to pick up the swab sample test kit containing swabs, vials, flasks, ice pack, box.
- Travel to FWB to pick up the air sampler, filters, tweezers, tripod, gloves, masks, permanent marker and orange high-vis.
- SAMPLE 1 – Waterloo underground tube station. Set up the air sampler on the tripod at full length. Tripod to be setup on the concourse between Northern and Bakerloo lines facing the escalators. Sample for 1 hour. Swab 1.a on the up escalator, swab 1.b on the down escalator (either of the escalators) between Northern and Bakerloo lines and swab 1.c on the map display board.
- SAMPLE 2 – Northern line from Waterloo to Euston. Setup the air sampler on the tripod at half length (seat height). Sample at the end of the tube. Sample for the duration it takes to get from Waterloo to Euston. Swab 2.a on a top handrail, swab 2.b on a side handrail and swab 2.c on the door handrail.
- SAMPLE 3 – Euston underground tube station. Set up the air sampler on the tripod at full length. Tripod to be setup on the 'half' concourse, one escalator down from the main train station concourse. Sampler to face the escalators. Sample for 1 hour. Swab 3.a on the up escalator, swab 3.b on the down escalator (either of the escalators) and swab 3.c on the map display board.
- SAMPLE 4 – Bus 205 from Euston to Paddington. Setup the air sampler on the tripod at half length (seat height). Sample on the top deck of the bus at the very end, with the sampler facing the pathway. Sample for the duration of the journey from Euston to Paddington. Swab 4.a on the handrail of the stairs, swab 4.b on the stop button of the side exit doors, swab 4.c on the window handle facing the side exit doors.
- SAMPLE 5 – Paddington underground tube station. Set up the air sampler on the tripod at full length. Tripod to be setup on the concourse, before the ticket tap in system on the Bakerloo line. Sampler to face the escalators. Sample for 1 hour. Swab 5.a on the up escalator, swab 5.b on the down escalator (either of the escalators) and swab 5.c on the map display board.
- Drop off samples at St. Mary's (Dr. Jie Zhou).
- Drop off sampler at FWB.

b) SURFACE SWAB COLLECTION

Objective

Assess the extent and persistence of surface contamination of COVID-19.
Identify environmental surfaces and fomites which may play a role in onwards transmission of COVID-19.

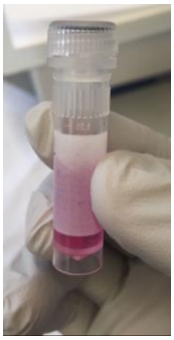
Materials

Virus transportation medium: DMEM with 1% P/S, 1% NEAA and 0.3% BSA.
FLOQSwabs 502CS01 or 519CS01, regular flocked swab

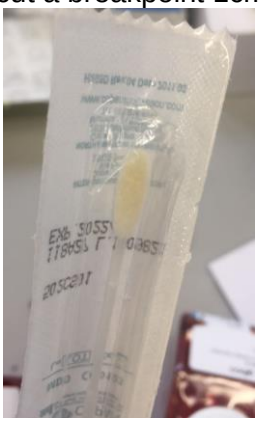
Procedure

In CL2 lab at St Mary's campus

- In a BSC2 hood, aliquot 1ml viral transportation medium into each 2ml screw-cap



- cut a breakpoint 1cm before the tip on the flocked swab.



In field

- Wet the swab with the viral transport medium.
- When applying pressure with the wet swab onto the surface, move in at least two different directions while rotating the swab stick. Avoid letting the swab dry completely. The recommended swab surface area is 25 cm2.
- Put the swab into the vial, break the plastic stick at the breakpoint.
- After labelling the vial, place in a bio-bottle and clean the outside of the bio-bottle with 70% ethanol.
- Put the bio-bottle into a transportation bag with ice packs, then transport back to the lab.

In CL3 lab at St Mary's campus

- Take the bio-bottle out of the transportation bag in the anteroom of CL3 lab. Leave the transportation box in the anteroom.
- Unpack the bio-bottle and container inside the hood.
- The environmental samples will be used for RT-qPCR and virus culture.

c) AIR SAMPLE COLLECTION

Objective

To characterize SARS-CoV-2 in air.

Materials:

- Sampler (SASS 3100 Dry Air Sampler) with battery installed
- Sampler carry case
- Tripod (in bag)
- Filters

- Gloves
- Pens
- Timer / Watch
- Laptop with USB / serial port connector and software installed
- A boards (2)
- His-vis vest
- Camera
- Cool-bag for filters
- Cool pack
- Paperwork (TfL letter, SOPs)

Operation

Wear gloves and use forceps when handling filters.

- Assemble the air sampling equipment (SASS 3100 electret filter air sampler on camera tripod) at an appropriate sampling location. Mount the SASS 3100 on the camera tripod at a 45° downward facing angle (to avoid direct deposition of larger particles) approximately 1.5 meters above the floor.
- Put on a pair of gloves and clean the filter holder of the SASS 3100 with an alcohol wipe.
- Air dry filter holder for a few seconds.
- Carefully open the filter pouch with gloves still on. Do not tear the pouch all the way down.
- Mount the filter on the SASS 3100 filter holder by only touching the filter frame.
- Start the SASS 3100 air sampler by flipping the ON/OFF switch all the way over (take care not to end up in the middle position).
- Record data in the sample and metadata sheet
- After 60 minutes of sampling – turn the SASS 3100 by flipping the ON/OFF switch all the way over to the OFF position.
- With gloves, take the filter off the SASS 3100 only touching the frame.
- With sterile tweezers – gently push the filter in one corner (from the front). Grab the filter from the back and place in prelabelled screw-cap vial (with 1 ml transportation medium, provided).

d) DETECTION OF 2019 NOVEL CORONAVIRUS (2019-NCOV) BY REAL-TIME RT-PCR

RNA extraction

In the BSC-1 in the CL3

- Add 200 µl sample to 800 µl buffer AVL (with carrier RNA) in a screw-cap tube.
- Incubate at room temperature (15-25C) for 10 min.
- Add 800 µl ethanol to the sample, mix completely.
- Spray the tube with Distel, then the tube can be taken out of the BSC.
- Put tubes into a secondary container, spray the inside of the secondary container and lid with Distel. Screw the lid, spray the outside of the container. Then the inactivated samples can be taken out of the CL3.

The rest RNA extraction steps can be conducted in the RNA room on the 4th floor.

Real-time RT-PCR

Reagent

- AgPath-ID One step RT-PCR (Applied Biosystems, CatLog number 4387391 1000 Reactions)

Primers and probes

E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT
	E_Sarbeco_P	FAM-ACACTAGCCATCCTTACTGCGCTTCG-MGB
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA

ORF1ab	ORF1ab_F	CCCTGTGGGTTTTACACTTAA
	ORF1ab_P	FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-MGB
	ORF1ab_R	ACGATTGTGCATCAGCTGA

RNaseP	RNaseP_F	AGA TTT GGA CCT GCG AGC G
	RNaseP_P	VIC-TTC TGA CCT GAA GGC TCT GCG CG -MGB
	RNaseP_R	GAG CGG CTG TCT CCA CAA GT

- Make the mastermix. (20 ul reaction in the 384 well plate)
  - Make the following mix

	Volume (ul)
2 x buffer	10
Enzyme	0.8
Forward primer (10 µM)	0.8
Reverse primer (10 µM)	0.8
Probe (10 µM)	0.4
H2O	2.2
Total	15

- Add 5 ul RNA to each reaction.
- Dilute standards
  - E gene standards are in the freezer T-rex (use 1e5 copies/ul, 40 ul/tube)

    - Label tubes x 5: 1^4, 1^3, 1^2, 1^1, 1^0 copies/ul
    - Dilute E gene standard curve: 10 ul in 90 ul H2O x 5 for dilution 1^4, 1^3, 1^2, 1^1, 1^0 copies/ul
    - Add 5 ul standards to each reaction (duplicate or triplicate). Standard curve concentration 1^5 – 1^0 (6 dilutions) copies/ul
- Use the following Real-time RT PCR conditions:
    - 450C 10 min + 950C 15 min + (950C 15s + 580C 30s) x 45 cycles
- Samples are defined as positive if both E gene and ORF1ab are positive.