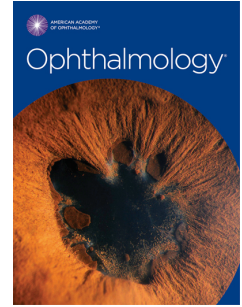


# Journal Pre-proof



Assessing Viral Shedding and Infectivity of Tears in Coronavirus Disease 2019 (COVID-19) Patients

Ivan Seah Yu Jun, MBBS, Danielle E. Anderson, PhD, Adrian Eng Zheng Kang, BSc, Lin-Fa Wang, PhD, Pooja Rao, MBBS, Barnaby Edward Young, MB, BChir, David Chien Lye, MBBS, Rupesh Agrawal, MD

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1 **Title Page**

2 **Title: Assessing Viral Shedding and Infectivity of Tears in Coronavirus Disease 2019**

3 **(COVID-19) Patients**

4 **Authors (In Order):** Ivan Seah Yu Jun, MBBS<sup>1</sup>; Danielle E. Anderson, PhD<sup>2</sup>; Adrian Eng Zheng  
5 Kang, BSc<sup>2</sup>; Lin-Fa Wang, PhD<sup>2</sup>; Pooja Rao, MBBS<sup>4</sup>, Barnaby Edward Young, MB, BChir<sup>3,4,5</sup>;  
6 David Chien Lye, MBBS<sup>3,4,5,6</sup>; Rupesh Agrawal, MD<sup>2,4,5,6,7</sup>

7 **Affiliations**

8 <sup>1</sup>National University Hospital, Singapore

9 <sup>2</sup>Duke-NUS Medical School, Singapore

10 <sup>3</sup>National Centre for Infectious Diseases, Singapore

11 <sup>4</sup>Tan Tock Seng Hospital, Singapore

12 <sup>5</sup>Lee Kong Chian School of Medicine, Singapore

13 <sup>6</sup>Yong Loo Lin School of Medicine, Singapore

14 <sup>7</sup>Moorfields Eye Hospital, NHS Foundation Trust, London, United Kingdom

15 **Corresponding Author**

16 Associate Professor (Dr) Rupesh Agrawal

17 Address: 11 Jalan Tan Tock Seng, Singapore 308 433

18 Email: Rupesh\_agrawal@ttsh.com.sg

19 Contact Number: +65 9061 3202

20 Fax: + 65 6357 1000

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32

33 **Abstract**

34 Ocular transmission of COVID-19 is uncertain. 64 tear samples were collected from 17  
35 COVID-19 patients between Day 3 to Day 20 from initial symptoms. Neither viral culture nor  
36 reverse transcription polymerase chain reaction (RT-PCR) detected the virus, suggesting a  
37 low risk of ocular transmission.

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**39 Main Manuscript**

40 The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has rapidly spread  
41 across the globe to cause a pandemic. While it is known to be transmitted via droplets,  
42 alternative modes of transmission remain unknown. Transmission through infected ocular  
43 tissue or fluid has been a controversy<sup>1,2</sup>. It is hypothesized that the nasolacrimal system can  
44 act as a conduit for viruses to travel from the upper respiratory tract to the eye. Hence,  
45 ocular tissue and fluid may represent a potential source of SARS-CoV-2. In this study, we  
46 attempted to determine the possibility of transmission through tears by assessing for the  
47 presence of SARS-CoV-2 with viral isolation and quantitative reverse transcription  
48 polymerase chain reaction (RT-PCR). As patients were being monitored clinically via routine  
49 nasopharyngeal swabs (NP), they were compared with tears to further understand patterns  
50 of viral shedding.

51 17 COVID-19 patients were recruited for this prospective study in Singapore after obtaining  
52 informed consent. This study was carried out in accord with the Declaration of Helsinki and  
53 ethics approved by the Domain Specific Review Board of the National Healthcare Group  
54 (NHG) Singapore. NPs were collected routinely for clinical monitoring of patient's condition  
55 while tear samples were collected purely for research purposes. On some days, both tears  
56 and NPs were collected at the same time. These samples were delivered to different labs for  
57 processing.

58 COVID-19 patients were tested positive by RT-PCR of NPs in a clinical diagnostic laboratory.  
59 NPs were collected in universal viral transport media and RNA extraction done using  
60 NucliSENS® easyMAG® system (bioMérieux). 55µl of the elute was then used to perform RT-  
61 PCR as per manufacturer's instructions using the A\*STAR FORTITUDE kit (Accelerate

62 Technologies Pte .Ltd, Singapore ). The limit of detection was estimated to be <25copies of  
63 RNA.

64 Tears were sampled by a senior consultant ophthalmologist using Schirmer's test strip at  
65 varying timepoints between Day 3 and 20 after the initial development of symptoms.  
66 Caution was taken to prevent contamination of samples. The Schirmer's strip tear collection  
67 method was previously validated in other studies<sup>3</sup>. Samples from both eyes were taken and  
68 analysed separately. Collected strips were placed into individual falcon tubes of universal  
69 viral transport media. Samples were delivered to a research laboratory for processing.  
70 Samples were used to inoculate Vero-E6 cells (ATCC®CRL-1586TM). After 4 days of  
71 incubation, cells were observed for the presence of cytopathic effect (CPE). Total RNA was  
72 extracted from all samples using E.Z.N.A. Total RNA Kit I (Omega Bio-tek) according to the  
73 manufacturer's instructions and samples were analysed by real-time quantitative reverse  
74 transcription-PCR (RT-qPCR) for the detection of SARS-CoV-2 as previously described<sup>4</sup>.

75 Clinical data including age, sex, symptoms, nasopharyngeal swab results were collected  
76 from electronic health records and correlated with RT-PCR results. Ocular symptoms which  
77 were assessed include red eye, tearing, blurring of vision, discharge and colour desaturation.  
78 These symptoms were chosen based on the ocular manifestations of other coronaviruses  
79 known to infect humans and animals<sup>2</sup>. Other symptoms of COVID-19 assessed include fever,  
80 cough, shortness of breath, rhinorrhea and sore throat.

81 Of the 17 patients recruited, none presented with ocular symptoms. However, 1 patient  
82 developed conjunctival injection and chemosis during the stay in the hospital (**Table 1**  
83 **available at [www.aaojournal.org](http://www.aaojournal.org)**). 14 patients presented with upper respiratory tract  
84 symptoms including cough, rhinorrhea and sore throat.

85 A total of 64 samples were taken over the study period, with 12, 28 and 24 samples taken  
86 from first, second and third week of initial symptoms respectively. All were tested negative  
87 for the SARS-CoV-2 on viral isolation and RT-PCR. Tear results were compared with NP  
88 results as shown in **Figure 1**. Ct values of NP swabs were featured.

89 To our knowledge, this is the first study comparing viral shedding in tears with NP results  
90 during the course of COVID-19 infection. A previous study showed positive SARS-CoV-2 RT-  
91 PCR results from a patient's tears, but isolation of the virus was unsuccessful<sup>5</sup>. In this study,  
92 there was no evidence of SARS-CoV-2 shedding in tears through the course of the disease.  
93 Viral load detected in nasal and throat swabs are elevated for a period of approximately 2  
94 weeks from the onset of COVID-19 symptoms<sup>6</sup>. In this study, the tear sampling timepoints  
95 cover these 2 weeks of active infection, providing a good representation of the full disease  
96 course. All tear samples tested negative even when NPs continued to test positive.  
97 Furthermore, patients with symptoms of upper respiratory tract infections did not  
98 demonstrate any viral shedding in tears, suggesting the hypothesis of the lacrimal duct as a  
99 viral conduit may not be true. Most importantly, only one patient developed ocular  
100 symptoms during the disease course and no evidence of SARS-CoV-2 could be found in the  
101 tear samples. This suggests that transmission through tears regardless of the phase of  
102 infection is likely to be low.

103 The study had several limitations. Firstly, the samples were analysed in different  
104 laboratories utilising two different assays. As the NPs were utilised in the clinical setting to  
105 monitor disease progression, they were analysed in a clinical diagnostics lab while the tear  
106 samples were analysed in a research lab. While the limit of detection for the research lab  
107 was not assessed due to logistical limitations, it should be noted that the tear samples were

108 incubated with Vero-E6 cells for 4 days prior to obtaining the RNA for RT-PCR. If SARS-CoV-2  
109 existed in the samples, CPE would have been observed even in a false negative RT-PCR  
110 result. We observed neither CPE nor a positive RT-PCR result, thereby the likelihood of  
111 SARS-CoV-2 being found in the tear samples is still low. Secondly, only tears were sampled  
112 rather than conjunctival tissue. In the pandemic setting, COVID-19 patients are already  
113 emotionally distraught with their diagnosis. Hence, conjunctival tissue sampling was avoided  
114 to reduce patient distress. Despite this, we believe that our results do highlight a low risk of  
115 ocular transmission. In the acute infection of conjunctival cells, cells die through viral-  
116 mediated lysis or from immune reactions. Cell death will release viral material into tears  
117 which can still be detected via RT-PCR. Thirdly, the study had a small sample size due to the  
118 logistical limitations of the outbreak response. These patients also usually present a few  
119 days after symptom development, making sampling during early infection difficult. Finally,  
120 only 1 patient had ocular symptoms in our study. However, studying patients with ocular  
121 symptoms can be difficult. In a study of 1099 COVID-19 patients, only 0.8% developed  
122 conjunctival congestion<sup>7</sup>.

123 The results from this study suggests that the risk of SARS-CoV-2 transmission through tears  
124 is low. However, further definitive mechanistic studies are required. SARS-CoV-2 has been  
125 known to infect cells via ACE2 receptors. More studies are required to definitely prove the  
126 presence of ACE2 on corneal and conjunctival cells. Future studies involving more patients  
127 with ocular symptoms should also be considered. Finally, future studies should consider the  
128 association between serum viral load and viral shedding in tears. Unfortunately, no blood  
129 samples were analysed for this experiment as they were not routine clinical investigation in  
130 the management of patients.

131



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149 **Figure Legends**

150 Figure 1: Comparison of Tear Samples and Nasopharyngeal Swab Samples Over Course of  
151 COVID-19 Illness

152 CT results of all nasopharyngeal swabs are displayed. All tear samples were tested  
153 neagative for on both viral isolation & RT-PCR. These results were labelled by a red  
154 coloured box.

| Patient Serial Number | Days Since Initial COVID-19 Symptoms |       |       |       |       |       |       |       |       |        |        |        |        |        |        |        |        |        |        |        | Discharge Status | Total Duration of Symptoms (As of 12/3/20) |         |
|-----------------------|--------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|------------------|--|---------|
|                       | Day 1                                | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 | Day 15 | Day 16 | Day 17 | Day 18 | Day 19 | Day 20 |                  |  |         |
| 1                     | 24.4                                 | 20.11 | 19.09 | NA    | 21.65 | 24.00 | 23.46 | 23.17 |       |        |        |        |        |        |        |        |        |        |        |        | Still admitted   | 6 Days                                     |         |
| 2                     | 24.3                                 | 37.92 | NA    | NA    | 32.75 | -     | -     | -     | -     | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -      | D9 Discharge     | 5 Days                                     |         |
| 3                     |                                      | 27.06 | 22.28 | NA    | 22.41 | 24.30 | 28.50 | 30.31 | 26.67 | 25.80  | 27.99  | 38.05  | -      | -      | -      | -      | -      | -      | -      | -      | Still admitted   | 5 Days                                     |         |
| 4                     |                                      |       |       |       | 21.20 | 22.04 | NA    | NA    | 24.51 | 26.4   | 28.24  | NA     | 30.54  | NA     | 34.86  | NA     | 36.83  | NA     | 37.17  | NA     | Still admitted   | 18 Days                                    |         |
| 5                     |                                      |       |       |       | 29.48 | 26.19 | NA    | 34.98 | NA    | NA     | NA     | NA     | 34.07  | -      | -      | -      | -      | -      | -      | -      | Still admitted   | Still Symptomatic (23 Days)                |         |
| 6                     | 33.5                                 | -     | 31.18 | -     | 36.28 | -     | -     | -     | -     | -      | -      | -      | -      | -      | -      | -      | -      | 35.48  | *      | NA     | *                | NA   | *       |
| 7                     |                                      |       |       |       |       | 37.70 | NA    | 35.02 | NA    | 34.69  | 35.09  | NA     | -      | -      | -      | -      | -      | -      | -      | -      | D8 Discharge     | 2 Days                                     |         |
| 8                     |                                      |       |       |       |       |       | 26.33 | NA    | NA    | NA     | NA     | NA     | 29.15  | NA     | 37.05  | NA     | 37.1   | NA     | 35.35  | NA     | Still admitted   | 11 Days                                    |         |
| 9                     |                                      |       |       |       |       |       | 31.22 | 33.71 | NA    | 34.17  | NA     | NA     | 34.63  | NA     | NA     | NA     | -      | 34.25  | 29.04  | 35.33  | Still admitted   | 18 Days                                    |         |
| 10                    |                                      |       |       |       |       |       | -     | -     | -     | NA     | 34.10  | -      | -      | -      | -      | -      | -      | -      | -      | -      | D14 Discharge    | 11 Days                                    |         |
| 11                    |                                      |       |       |       |       |       |       |       | 29.19 | NA     | NA     | 33.72  | 36.20  | NA     | 36.71  | NA     | NA     | 33.13  | NA     | 35.14  | NA               | Still admitted                             | 12 Days |
| 12                    |                                      |       |       |       |       |       |       |       | 37.55 | 32.79  | 33.43  | 38.16  | 32.28  | 39.39  | 35.91  | 37.72  | 38.42  | 38.21  | -      | 38.21  | 36               | D25 Discharge                              | 15 Days |
| 13                    |                                      |       |       |       |       |       |       |       |       |        | 29.54  | NA     | NA     | 33.04  | NA     | NA     | NA     | -      | 37.55  | NA     | Still admitted   | 23 Days                                    |         |
| 14                    |                                      |       |       |       |       |       |       |       |       |        | 29.30  | 34.00  | 33.31  | 28.66  | 35.27  | 38.53  | 30.72  | 37.45  | 35.92  | 32.29  | -                | 6 Days                                     |         |
| 15                    |                                      |       |       |       |       |       |       |       |       |        |        |        |        |        |        |        |        |        |        |        | -                | 11 Days                                    |         |
| 16                    |                                      |       |       |       | 22.89 | NA    | 25.40 | NA    | NA    | NA     | 26.38  | 33.06  | 32.26  | 30.20  | 36.21  | 29.48  | -      | -      | -      | -      | D17 Discharge    | 11 Days                                    |         |
| 17                    |                                      |       |       |       | 19.35 | NA    | 20.01 | NA    | 21.97 | 29.06  | 32.19  | 32.27  | 21.31  | 19.22  | 34.10  | 32.05  | 30.43  | -      | 38.81  | 36.34  | -                | D21 Discharge                              | 20 Days |
|                       |                                      |       |       |       |       |       |       |       |       |        |        |        |        |        |        |        |        |        |        |        | -                | D22 Discharge                              | 15 Days |

| Legend |                              |
|--------|------------------------------|
| -      | Negative Nasopharyngeal Swab |
| NA     | No Nasopharyngeal Swab Taken |
| *      | Ocular Symptoms              |
|        | Tears Sampled Negative       |
|        | Tears Sampled Positive       |

Figure 1: Comparison of Tears Samples and Nasopharyngeal Swab Samples Ct Values Over Course of COVID-19 Illness

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