#### Title: Risk Assessment of SARS-CoV-2 transmission by re-using of glasses in bars and restaurants

## Running title: Risk Assessment of SARS-CoV-2 transmission via glasses

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## Abstract

In many countries, bars and restaurants have reopened after a period of COVID-19 lockdown, which has resulted in COVID-19 outbreaks related to visiting bars and restaurants. Presence of intact SARS-CoV-2 on contaminated glasses may result in direct exposure of mucosa to the virus during drinking and indirect via droplets produced during rinsing and cleaning. To assess the risk of infection for people consuming drinks in hospitality settings, and staff and visitors sitting close to the glass rinsing reservoir, we studied the likelihood of SARS-CoV-2 transmission via glasses in bars and restaurants. For this risk assessment we used realistic scenario studies based on literature data, experimental data and expert opinion in case no data were available. Depending on the chosen scenarios, it was concluded that once glasses become contaminated, extensive cleaning of glasses by either rinsing or

using a dishwasher is required to prevent transmission of the virus. In contrast, the risk of indirect transmission through droplets originating from rinsing water is negligible.

#### Importance

It is currently not clear which potential transmission routes attributed to the transmission of SARS-CoV-2 in reported COVID-19 outbreaks related to visiting bars and restaurants. We demonstrate that in order to prevent transmission of virus between visitors, it is essential to extensively clean glasses by either thorough rinsing or the use of a mechanical dishwasher.

#### Introduction

In most countries, persons with COVID-19 (respiratory) symptoms are requested to stay at home and not to visit bars and restaurants. Another general guideline is to comply with social distancing by keeping distance (mostly between 1 and 2 m). In many countries, bars and restaurants have reopened after a COVID-19 lockdown period while personnel and visitors still have to keep distance. Since reopening, COVID-19 outbreaks related to bars and restaurants have already been reported (1, 2). SARS-CoV-2 is easily detectable in saliva, including from pre- and asymptomatic shedders (3, 4). We developed customer-to-customer transmission scenarios involving re-use and cleaning of glasses, in order to get a more complete picture of possible transmission routes of the virus in a "1.5 meter (social distancing) society".

#### **Results and Discussion**

We assumed that a volume of 100  $\mu$ L saliva remains on the rim of a glass after it is emptied, and a viral load in saliva of  $3.3 \times 10^6$  (range 990 -  $1.2 \times 10^8$ ) genome copies per milliliter (gc/mL) (4). We also assumed that when a glass (perimeter of 18-28 cm and contact area where the lips touch the

glass of 2-4 cm) is emptied in 5 sips, about 50% of the glass rim could contain saliva. Based on these assumptions, a glass used by an infected consumer was estimated to contain  $3.3 \times 10^5$  (99 -  $1.2 \times 10^7$ ) genome copies of SARS-CoV-2 distributed on 50% of the glass rim. Due to difficulties titrating SARS-CoV-2 from clinical materials such as saliva, it is currently unknown how many infectious particles this represents. Based on estimates that vary between 1 infectious particle for >10<sup>4</sup> genome copies in clinical samples (5, 6) to 1:55 for virus isolates, we used a ratio of 1:100 infectious particles versus genome copies for further calculations (7). This results in an estimated contamination level of  $3.3 \times 10^3$  (1 –  $1.2 \times 10^5$ ) infectious viruses per used glass. Transfer rates and subsequent exposure of the next consumer via re-use of glasses was estimated based on three different scenarios (Table 1).

Table 1: Scenarios for transfer rates and subsequent exposure to infectious viruses (average and range) of the next consumer.

scenario	reduction by cleaning	transfer	exposure infectious viruses
1: no rinsing, high transfer	0%	100%	3300 (1 – 1.2 x 10 <sup>5</sup> )
2: no rinsing, realistic transfer	0%	0.1 - 10%	330 (0 - 1.2 × 10 <sup>4</sup> )
3: rinsing and realistic transfer	99%	0.1 - 10%	3 (0-120)

In worst case scenario 1, a glass is re-used without any cleaning or rinsing, resulting in 100% virus transfer. The amount of SARS-CoV-2 transferred to the mouth of the next user is then estimated to be as high as 3300 infectious viruses. Assuming an infectious dose of between 10 and 1000 infectious viruses (8), the chance of infecting the next consumer would be very high. In a more realistic scenario (scenario 2), we assumed a virus transfer from the glass to the lips of the next user of between 0.1-10% (based on 9). When assuming the same virus transfer of between 0.1-10% and a proper rinsing of the glasses (99% virus reduction: based on (10) (scenario 3), the number of transmitted viruses is

reduced to 0-120 infectious viruses per emptied glass. In all scenarios we assume that the chance of lips not touching the contaminated half of the glass rim during 5 sips is negligible ((0.5)<sup>5</sup>). Assuming an infectious dose of between 10 and 1000 infectious viruses (8), the risk of virus transmission leading to infection per re-used glass in this scenario is low but possible. For the Netherlands, the average consumption is estimated at 3 glasses for the first hour and 2 glasses for the following hours (https://www.eventplanner.nl/nieuws/4480\_tip-hoeveel-drank-en-hapjes-moet-je-voorzien-op-eenfeest-of-evenement.html). This means that one infected guest could contaminate on average 5 glasses during one night out.

Besides virus transfer through lip-surface-lip contact caused by re-using glasses, viruses could also be transmitted via the water in rinsing tanks. Virus released in the water could potentially crosscontaminate other glasses, or be spread via droplets. To estimate the relevance of such virus transfer through rinsing tank water, the effect of a standard cleaning agent in rinsing water on viral load was tested experimentally by exposing virus to 0.5x, 1x and 4x of the prescribed concentration of beer glass cleaner (of 5 mL beer glass cleaner in 10 liters of water). Two SARS-CoV-2 isolates were exposed to these three beer glass cleaner concentrations at room temperature for up to 2 hours. We found that inactivation of SARS-CoV-2 in rinsing water with the prescribed amount of beer glass cleaner at room temperature resulted in 90-99% reduction of the number of infectious viruses in 30 minutes (see figure 1). This means that when a contaminated glass is washed in the rinsing tank, the SARS-CoV-2 concentration of  $3.3 \times 10^3$  (0 –  $1.2 \times 10^5$ ) infectious viruses per 10 L rinsing water (assuming all virus washed into the rinsing water) will be reduced to a concentration of 0 - 1.2 infectious viruses/mL after 30 minutes. The presence of beer glass cleaner in the rinsing water prevents accumulation of virus in the rinsing reservoir, which further reduces the likelihood of transmission. In conclusion, transmission of infectious virus via the rinsing tank due to cross-contamination from a contaminated glass to another glass or to droplet transmission, is very unlikely because of the low

virus concentration in rinsing water as well as virus inactivation by beer glass cleaner, as was demonstrated experimentally.

The risk of virus transmission when using automatic dishwashers was estimated by evaluation of the washing process based on publicly available information. The contamination reducing potential of dishwashing is determined by mechanical reduction (rinsing) and inactivation (by soap and heat). Household dishwashers operate at a washing temperature of 55-65°C during 20 to 60 minutes, followed by thermal disinfection, where the crockery reaches a surface temperature of 80-85°C. Professional dishwashers with multi-tank machines runs include a pre-wash zone (35-45°C), a wash zone (55-65°C) and a rinse zone (80-85°C) (<u>https://www.rhima.nl/over/haccp-spoelkeuken)</u>. SARS-CoV-2 is an enveloped virus that can remain infectious for up to a few days on non-porous surfaces at room temperature (11), but is quickly inactivated at higher temperatures (>60 °C) and high humidity (humidity or in suspension) (12, 13). The probability of infectious virus remaining on glasses after washing in an automatic dishwasher is therefore negligible.

Obviously, the likelihood of glasses becoming contaminated depends on the prevalence of infected persons and compliance to guidelines such as staying at home when having even mild symptoms. But since asymptomatic and pre-symptomatic shedding does occur, the risk of transmission via glasses in bars and restaurants need to be considered. Moreover, the likelihood of this transmission route per bar or restaurant, with a restricted number of visitors allowed, is limited for each location in itself, but with >60,000 locations in the Netherlands alone, it is potentially a relevant factor in the transmission of SARS-CoV-2.

#### Conclusion

Using literature data on SARS-CoV-2 loads in saliva and estimated virus transfer efficiencies we estimated that re-use of glasses from infected individuals without cleaning will likely result in SARS-CoV-2 transmission, since a person using a non-rinsed contaminated glass is exposed to viral loads

that may cause an infection. We also showed that in a scenario with thorough glass cleaning in a rinsing tank, the level of virus transmission is significantly reduced, and only a low risk remains. An even further risk reduction can be achieved by mechanical washing of glasses. Indirect transmission via droplets coming out of the rinsing reservoirs is very unlikely.

To summarize our findings, the most likely contact transmission of virus in hospitality settings is through mouth-to-surface contact via contaminated glasses. However, such transmission can be prevented by extensive cleaning of glasses by either thorough rinsing using beer glass cleaner or using a an automatic dishwasher.

### Materials and Methods

Cells and viruses: Viruses were isolated from throat swabs on Vero-E6 cells as described (14). The two stocks were titrated on the same cells and were characterized as hCoV-19/Netherlands/ZuidHolland\_10003/2020 at 5.62x10<sup>7</sup> TCID50/mL and hCoV-19/Netherlands/ZuidHolland\_10004/2020 at 2.4\*10<sup>8</sup> TCID50/mL. Prior to use the stocks were diluted 1:10 in MEM with 10% FCS.

To study inactivation of 2 SARS-CoV-2 isolates in beer glass cleaner, the diluted virus stocks were mixed with beer glass cleaner in duplicate to contain 0.5x, 1x and 4x of the prescribed concentration of a commercial beer glass cleaner (Horeca Select, 15-30% anionogenic surface active agents, <5% non-ionic surface active substances, sodium benzoate, potassium sorbate). After the indicated exposure time at room temperature (18-22 °C) the samples were diluted 1:10 in MEM with 10%FCS at 4 - 6 °C and dilutions for titrations were made immediately. Virus inactivation was quantified as described (10).

Figure 1: Inactivation of SARS-CoV-2 isolates in beer glass cleaner at room temperature. Inactivation was determined for two isolates in duplicate.

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