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Efficacy of personal protective equipment against coronavirus transmission by dental handpieces

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ABSTRACT

Background. This in vitro study evaluated the efficacy of personal protective equipment (PPE) and high-volume evacuation (HVE) against human coronavirus (HCoV) 229E spreading by a standard dental procedure.

Methods. Phantoms for both patient and operator were used to recreate a dental setting inside a custom-built class III cabinet-like chamber. The patient's phantom mouth was inoculated with an HCoV-229E suspension having viral load similar to SARS-CoV-2 asymptomatic subjects. The dental procedure was performed using an air-turbine and HVE for 10 seconds. The efficacy of surgical masks, FFP2/N95 and FFP3 respirators, and face shields was tested using quantitative real-time PCR.

Results. The wide surface on which the inoculum was spread caused low contamination. Over the masks and respirators' external surfaces when a face shield was not worn, viral loads ranged 1.2 to 1.4 log₁₀ mean gene copies/cm². When the shield was on, viral loads dropped below detection limit (<0.317 log₁₀ gene copies/cm²) for all PPEs. On the operator's forehead, viral loads were 0.6 to 0.8 log₁₀ gene copies/cm². Inside the operator's mouth, viral loads were under the detection limit using any PPE, with or without the shield. HVE did not significantly change viral loads.

Conclusions. All PPE combinations significantly reduced viral loads in the operator's mouth below the detection limit, but HVE did not decrease viral contamination.

Practical implications. While extreme caution is suggested when removing and disposing of PPEs to avoid self-contamination, the combination of PPE and face shield drastically decreases the risk of human coronavirus transmission during aerosol-generating dental procedures.

Key Words. SARS-CoV-2; Aerosols; Masks; Respirators; Face shields; PPE; High-volume evacuation; Communicable Disease Control; Dental Equipment.

INTRODUCTION

As of early December 2020, the SARS-CoV-2 pandemic has caused more than 1,500,000 deaths worldwide¹. The second wave of COVID-19 is currently spreading and further threatening health, economic development, and social stability worldwide. A third wave is expected, similarly to the Spanish flu diffusion in 1918-1920^{2,3}.

SARS-CoV-2 can be found in the secretions - including saliva, which is an important reservoir⁴- of both symptomatic and asymptomatic infected patients⁵. Since dental practitioners are potentially exposed to the saliva of asymptomatic subjects, especially during aerosol-generating procedures, they have been included among the professionals carrying the highest risk of SARS-CoV-2 infection ⁶⁻⁸.

Several studies found that spatter and aerosol-generating procedures in the dental operatory can spread contamination over virtually any surface, including walls and ceiling 9,10 . While spatters, having a relatively large particle size ($100+\mu m$), are too large to be inhaled, aerosol particles have a smaller diameter ($<50 \mu m$) and can remain suspended for a long time (up to 1,5 h) after the end of an operative procedure 11 . Aerosols act as carriers of infective agents, including those reaching high saliva concentrations such as SARS-CoV- 2^{12} , which may remain viable and infectious on surfaces for days 13 . Thus, data on the efficacy of personal protective equipment (PPE) to reduce the risk of coronavirus infection by dental aerosols and spatter are urgently needed.

Data have been gathered on the efficacy of masks, respirators, and face shields against influenza viruses, and several studies indicated the importance of devices such as face shields in blocking spatters and in preventing eye contamination^{7,14,15}. Many Dental associations and Councils worldwide and several publications recommend using non-valved N95/FFP2-standard respirators or FFP3 respirators^{7,8,16}. They consider the conventional, three-layered surgical masks as providing insufficient protection during aerosol-generating procedures or when SARS-CoV-2 positive patients are treated^{17,18}. Nevertheless, no study assessed the efficacy of masks, respirators, and face shields against coronaviruses to date. Available data have been gathered on influenza viruses only and not in the aerosol-generating dental setting. In particular, in vitro and clinical studies showed that FFP2/N95 respirators are significantly more effective than medical masks at reducing exposure to aerosols^{19,20}. However, the results of most studies on this topic are mixed, not allowing for reliable clinical confirmation²¹.

Furthermore, using a high-vacuum evacuation system (HVE) is considered one of the main possibilities to reduce the contamination of the dental operatory by spatter and aerosol^{22,23}. However, no study evaluated PPE efficacy against coronaviruses, and no data have been collected in the aerosol-generating dental setting. We designed this *in vitro* study to evaluate the efficacy of different types of PPEs, and the use of HVE in reducing human coronavirus contamination on operators during conventional dental procedures. The null hypothesis was that all tested PPE and the use of HVE would significantly reduce viral concentrations.

METHODS

Surgical masks were purchased from Conception et Fabrication de Produits Médicaux et Paramédicaux (CFPM, Tremblay-en-France, France). As the vast majority of surgical masks, they are a Class I disposable medical device compliant with the Typical standard IIR IN 14683: protective masks against biological liquids projections. They show bacterial filtration efficiency of 99%, respiratory resistance: < 49 Pa/cm² and splash resistance: ≥ 16 kPa. KN95 - FFP2 respirators were obtained from Jinlu (Filtering Half Mask, Jiangsu Jinlu Group Medical Device Co., LTD, Jinfeng Town, Jiangsu, China). This PPE is a disposable Class I medical device (Category III of PPE) compliant with EN 149/2001+A1/2009 and PPE (EU) 2016/425 regulations. FFP3 respirators were obtained from BLS (BLS Zer0 30 NV FFP3 R D, BLS, Cormano, Italy). They are Class I reusable medical devices (Category III of PPE) compliant with EN 149/2001+A1/2009 and PPE (EU) 2016/425 regulations. They show bacterial filtration efficiency of 99,9%, respiratory resistance: 120 Pa/cm² inspiration, 200 Pa/cm² expiration, and splash resistance. The Galaxy face shield reusable visor (Dental world s.r.l., Molfetta, Italy) was tested. The chosen model did not include soft foam pads to prevent possible contamination sources during re-use due to incomplete disinfection. A confined environment was used that reproduced the dental operating unit and allowed a viral tracer to be used safely. The environment was equipped with phantom heads simulating the operator and the patient and was connected to vacuum pumps to maintain negative pressure and an HVE line. An air turbine operated for 10 s was used as the source of contamination by spatter and aerosol. The patient's phantom mouth was inoculated with a human coronavirus (HCoV) 229E suspension, having viral load similar to SARS-CoV-2 asymptomatic subjects. The efficacy of surgical masks, FFP2/N95 and FFP3 respirators, face shield, and use of HVE were then assessed through the quantification, by real-time PCR, of the viral load on PPEs surfaces, operator's phantom forehead and mouth.

Experimental dental setting. A polycarbonate pressure-tight chamber (100x40x40 cm) was custom-built. A total of three circular holes (10 cm diameter) and a door (35x25 cm) were obtained on the front panel. Three 50 cm-long latex gloves were fitted to the circular apertures. The chamber was connected through air-tight tubing to two laboratory vacuum pumps, a dental high-vacuum system (HVE, flow rate: 1700 l/min; maximum operating head: 280 mbar, TurboSmart 2v, Cattani S.P.A., Parma, Italy), and a handpiece tubing providing a connection for an air turbine. Two digital manometers (measuring range 200±0.1 mbar) were fixed to the front panel's outer part. Two air leak valves were mounted to set a constant negative pressure of 15 mbar inside the chamber to avoid contamination of the surrounding area. The first valve allowed for fine-tuning of the pressure while the other allowed to compensate for a higher air intake due to HVE operation. When HVE was not operating, the second valve was closed, and a non-return valve on the HVE line ensured back-contamination prevention. All holes, the panel, and tubing connections were sealed to make the chamber air-tight and bear a constant negative operating pressure of 15 mbar. (Figure 1)

A dental portable turbine unit (Zhengzhou Kongsin Trading Co. Ltd, Zhengzhou City, Henan, China) was connected to a dental air compressor and used to operate the air turbine handpiece inside the chamber (Bora, Bien-Air Dental SA, Bienne, Switzerland). The latter was equipped with a cylindrical diamond bur (835KR.314.016, Komet Italia s.r.l., Milan, Italy). The air pressure was set to 3.3 atm, and the speed was 320.000 r.p.m. The system was provided with a 1000 ml water tank for the air-water spray (Figure 2). Running tap water was used to fill the tank.

Two phantom heads (operator and patient) were fixed to custom made holders in a vertical position inside the chamber at a conventional working distance of 25 cm (Figure 3). The patient's phantom was equipped with resin teeth (Columbia Dentoform Corp., Long Island City, NYC, USA). The air turbine and the HVE were oriented to simulate the position of a right-handed dentist during the preparation of the occlusal surface of the lower right first molar. A universal laboratory holder was used to hold the air turbine and the HVE in the same position for all experimental runs. The air turbine was positioned 2 mm away from the tooth surface and oriented towards the inner part of the dental arch, while the HVE tip was positioned on the opposite side of the tooth (Figure 4). The distance between the HVE tip and the turbine tip was 2 cm.

The operator phantom was sealed, making it pressure-tight, save for the mouth opening. The phantom was connected to one of the manometers and a low-vacuum pump to simulate the air-flow during inspiration. The operator phantom was provided with a custom-made tray (7x7 cm) to accommodate a 4-well plate (Nunc IVF, Merck, Darmstadt, Germany) inside its mouth. A site was identified and marked on the phantom's forehead to position another 4-well plate using double-sided adhesive tape. In this way, data about viral surface contamination were gathered on three sites, namely the operator's forehead, the mask surface, and the inside of the mouth of the operator phantom behind the mask (Figure 5). Finally, a sprayer containing absolute ethanol to be used during operation procedures was inserted on the chamber's side opposite the phantoms.

Preparation of viral solutions. A coronavirus (Human coronavirus 229E, ATCC® VR-740) was used as a biological tracer instead of SARS-CoV-2 for safety reasons. A suspension of HCoV-229E with a viral load of 6.03±0.04 log₁₀ gene copies/ml, that resembles SARS-CoV-2 saliva levels of asymptomatic infected subjects^{24,25} was prepared in an artificial saliva solution. The artificial saliva simulated the average electrolyte and mucin composition of human whole saliva and was prepared from 0.1 L of 150 mM KHCO₃, 0.1 L of 100 mM NaCl, 0.1 L of 25 mM K₂HPO₄, 0.1 L of24 mM Na₂HPO₄, 0.1 L of 15 mM CaCl₂, 0.1 L of 1.5 mM MgCl₂, and 0.06 L of 25 mM citric acid. Distilled water was added to obtain 1 L of solution, a total of 2.5 g mucin (type II, porcine gastric) was added, and the pH was adjusted to 7.0 by pipetting 4 M NaOH or 4 M HCl solutions under vigorous stirring²⁶. Cryo-vials, each containing 1 ml of stock viral suspension, were prepared and frozen at -80 °C. On the day of testing, stock suspensions were thawed and stored in an ice bath. Experiments were performed in triplicate.

Operation procedures. All personnel operating the chamber wore protective equipment, including gloves, FFP3 respirators and face shields, and disposable gowns. Before starting each experimental run, two 4-well target plates were placed in their corresponding locations (inside the operator phantom's mouth and on its forehead) with their lid closed. A surgical mask was positioned on the operator phantom, taking care to adapt it over the nose and the mouth openings and removing the 4-well lid just before positioning of the mask. A 1.9 cm² square was marked in a central position on the external part of each mask. A sterile, leakproof plastic bag was positioned in the chamber to collect specimens after the experimental run. A

micropipette with its sterile tips was also inserted into the chamber. Then, one vial containing the viral suspension was positioned on a stand inside the chamber.

Vacuum pumps and HVE were turned on to reach operating pressure conditions inside the chamber. After that, all procedures inside the chamber were performed using air-tight gloves. The micropipette was used to transfer the viral solution (1 ml) on the bottom of the lower arch inside the patient phantom, mimicking saliva drain from the submandibular glands. The air turbine was then operated for 10 s to generate an aerosol containing the viral particles. After that, HVE was turned off, and a 60 s time was allowed for aerosol dispersion. Then, the mask was removed, and the forehead and the mouth plates were covered with their lid. The mask and the two plates were positioned in the plastic bag and hermetically sealed. The bag was marked with the corresponding code of the experimental run. The sprayer was then used on all chamber surfaces. A total of 60 s was allowed for ethanol disinfection and evaporation, then HVE was turned on again to remove residual ethanol completely. The chamber's negative pressure was equalized to the atmospheric pressure; the door was opened to remove the specimen-containing bag and discard the equipment used during the run safely.

The experimental conditions tested were the followings:

- 1) Surgical mask PPE, No HVE;
- 2) Surgical mask PPE, HVE;
- 3) FFP2 respirator PPE, HVE;
- 4) FFP3 respirator PPE, HVE;
- 5) Surgical mask and face shield PPE, HVE (Figure 6);
- 6) FFP2 respirator and face shield PPE, HVE;

When the face shield was used, the forehead target was placed on the uppermost part of the shield (Figure 6). Each experimental run was performed in triplicate.

Determination of viral loads on the target surfaces. At the end of each experimental run, the target-containing bag was immediately transferred to the virological laboratory in the next room. The wells of each target were washed with 500 μl PBS; the solution was collected in sterile Eppendorf vials and stored at -80 °C until analysis. Samples with the same surface area as the target's wells (1.9 cm²) were cut from the external layer of the mask, inserted in Eppendorf vials containing 500 μl PBS, and stored as previously

described. HCoV-229E presence on targets was performed using quantitative Real-Time PCR. RNA extraction was performed using Purelink viral RNA/DNA kit (ThermoFisher, Milan, Italy). A total of 500 μl of viral suspension was used, and the elution was performed with 10 μl elution buffer. RNA was retrotranscribed with Superscript VILO cDNA synthesis kit (ThermoFisher) and amplified with a HCoV-229E specific Real-Time PCR gene assay (Vi06439671_s1, Catalog number: 4331182, ThermoFisher).

Statistical analysis. All analyses were performed using SAS software (JMP 14.0, SAS Institute, Cary, NC, USA). Real-Time PCR data were analyzed after log-transformation to approach a normal distribution, which was verified using Shapiro-Wilk's test. The limit of detection for quantitative Real-Time PCR in an error-free environment, where only sampling noise contributes to the variation, was calculated to be three viral copies at 95% confidence interval²⁷. Considering the noise due to extraction and reverse transcription, the limit of detection was conservatively determined as four viral copies, according to the methodology proposed by Forootan and coworkers²⁸. Data were expressed in Log₁₀viral copies/cm². Homogeneity of variances was checked using Levène's test, and two way ANOVA model was used considering the site (forehead, mask tissue, and mouth), and the experimental setting (HVE, PPE type and combination) as fixed factors. Tukey's test was used to evaluate significant differences between groups. The significance level was set to a two-sided p<0.05.

RESULTS

When a face shield was not worn, viral loads ranged from a mean of 1.2 to 1.4 log₁₀ gene copies/cm² over the external surfaces of masks and respirators (Figure 7). When the shield was on, the viral loads dropped below the detection limit (<0.317 log₁₀ gene copies/cm²) for all PPEs. On the operator's forehead, the viral loads ranged from 0.6 to 0.8 log₁₀ gene copies/cm² in all experimental runs. Inside the operator's mouth, the viral loads were under the detection limit using any PPE, with or without a face shield, with no significant differences between surgical masks and respirators (all p>0.05). The use of HVE did not significantly modify the viral loads in any experimental run (all p>0.05).

DISCUSSION

Due to the SARS-CoV-2 pandemic, the aerosol generation during dental procedures has regained attention as a high-risk factor for airborne transmission of infectious diseases^{10,29,30}, and procedures for infection control and PPE use in the dental setting were updated to face this new emerging infective disease^{6,7,31}. The data from already-known coronaviruses and other airborne-spread diseases were used^{14,32}, because of the scarcity of information on this agent^{6,7}. In fact, this is the first study assessing the efficacy of masks, respirators, and face shields against coronaviruses in the dental setting

The main findings of the study have direct clinical implications. First, the highest viral loads were found on the masks/respirators' external surfaces, and the virus was detected on the phantom's forehead and outer part of the face shield in all runs. These results confirm a high risk of contamination for dental health-care providers during aerosol-generating procedures. They also outline the importance of carefully removing and disposing of masks and respirators after each patient, as they may concentrate any airborne pathogen on their surface and be a significant source of self-contamination. Interestingly, the viral contamination of masks' outer surfaces was significantly higher than that of control surfaces on the phantom's forehead. This result can be explained considering that a vacuum pump was connected to the operator phantom to simulate inspiration by continuous air intake through the tested PPEs. The air-flow through the masks allowed large amounts of aerosol particles carrying the virus to settle on their external surfaces. The above findings are in agreement with the ones of Prospero and coworkers³³, who found that the outer surfaces of the masks were significantly more contaminated than all other surfaces. Another study evaluated the contamination by respiratory viruses on the outer surfaces of surgical masks used by hospital health-care workers³⁴. The Authors found contamination on a median of 10% of the masks, concluding that respiratory pathogens on the outer surface of the masks might cause self-contamination of the operators.

Second, the use of a face shield reduced viral loads on the external surfaces of masks and respirators under the detection limit. This finding supports the protective effect of face shields against contamination by aerosol, confirming the importance of combined use of face shields with masks or respirators. In the dental practice, especially in times of COVID-19 pandemic, face shields are highly recommended when aerosol-generating procedures are performed^{7,14,15}. However, limited data is available on the efficacy of face shields in blocking contamination by splashes and spatters, ¹⁵ and only one study indirectly evaluated their efficacy in

reducing aerosol diffusion: Akagi and coworkers performed a computational flow simulation that highlighted the relatively low efficacy of a face shield in protecting against aerosols³⁵. They found that aerosols directed towards a shield-wearing subject form ring-shaped vortexes that reach the shield's top and bottom edges and form a high-velocity entrainment flow, quickly reaching the areas behind the shield³⁵. In contrast with these data, the present study results support the protective effect of face shields against contamination by aerosol in addition to the one by spatter.

As a third finding, in the experimental setup of the present study, surgical masks and N95/FFP2 or FFP3 respirators were equally effective in protecting the operator: even in a critical environment such as the aerosol-generating dental setting, the viral loads were below the detection limit wearing both surgical masks and respirators. This finding must be interpreted with caution: given the short duration of the test, the risk of virus transmission may still be high during long-lasting procedures, with the exclusive use of a single PPE. Nevertheless, our results are in line with those of a recent meta-analysis 36 and an RCT 21, which found no significant differences between masks and respirators in preventing laboratory-confirmed viral respiratory infection or clinical respiratory illness, including coronaviruses. Other two older RCTs by another research Group 19,37 found that continuously worn N95/FFP2 respirators provided significantly higher protection than surgical masks. In fact, N95/FFP2 respirators are currently believed to provide better protection than surgical masks against viral respiratory infections, and their use whenever aerosol-generating procedures are performed is still recommended by several Scientific Societies 16-18,38. A recent study by MacIntyre and coworkers demonstrated a significantly lower efficacy of cloth masks compared to surgical masks³⁹. They wrote an update to this study considering the spread of the COVID-19 pandemic in which they urge to consider the use of cloth masks only as a last resort due to their lower protection efficacy³⁹. This conclusion was considered when, in the present study, it was decided not to test cloth masks as protective means for dental operators.

As a fourth outcome, we found no significant influences of HVE on viral contamination. Such system has been proposed as one of the main possibilities to reduce the contamination of dental operatory by spattering and aerosol^{22,23}. The efficacy of several dry-field isolation techniques, including HVE alone, in spatter and aerosol mitigation was recently tested by Ravenel and coworkers²³. In that study, the use of HVE significantly reduced spatter but not aerosol spread. Moreover, a recent Cochrane review by Nagraj and

coworkers concluded that the effects of HVE were only detectable within ≈ 30 cm from the source of contamination (the patient's mouth)²². Our results agree with these data, showing no difference in the aerosol contamination level with or without the use of HVE at a conventional working distance of 25 cm between the operator and the source of infection.

Thus, the null hypothesis must be partly rejected since all PPE combinations significantly reduced viral loads in the operator's mouth below the detection limit, but HVE did not influence viral contamination.

As mentioned, the study's main limitation is the reduced duration of the aerosol-generating procedure (10 s). Such time was selected for each experimental run to achieve the dispersion of the viral inoculum through aerosol and spatters. It is clear that other dental procedures, such as tooth crown preparation, last longer and produce higher amounts of aerosols. However, testing the latter procedure would have requested, in turn, much larger amounts of viral inoculum that would have been difficult to produce and manage. There is an urgent need for further evaluations, with a more prolonged testing time of aerosol-spreading dental procedures, to confirm whether respirators could be replaced by the more tolerated and less expensive surgical masks, maintaining a comparable protection level.

The strengths of the study include the use of artificial saliva containing a viral concentration similar to those of asymptomatic patients', the high number of conditions under evaluation using a human coronavirus, and a methodology designed to reproduce aerosol spreading in the clinical setting as closely as possible, using a custom-built, class-III-like, negative pressure cabinet.

CONCLUSIONS

The first quantitative analysis of human coronavirus viral loads transmitted during aerosol-generating dental procedures showed that large amounts of viral loads are deposited on masks and respirators' outer surface when a face shield is not used, suggesting extreme caution when removing and disposing of PPEs to avoid self-contamination. The combination of mask/respirators and face shield reduced the viral loads below detection limits, thus drastically decreasing the risk of operators' contamination. In the experimental setup of the present study, surgical masks and N95/FFP2 or FFP3 respirators were equally effective in protecting the operator, while HVE did not seem to decrease the risk of aerosol contamination.

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Figure legends

Figure 1. Setup of the custom-built Class III-like air-tight glove box with chamber pressure control. Three accesses for gloves are shown on the front panel, one created at the center of the door. Between glove apertures, two digital manometers and a backup analog manometer measured the negative pressure inside the chamber (right digital manometer and analog manometer) and the differential pressure inside the mouth of the operator phantom when a mask or respirator covered its mouth and nose (left digital manometer). On the upper right corner of the chamber, the two air leak valves are visible for pressure control. The control apparatus operating the air turbine is located on the right part of the upper panel, having attached the pressurized water tank to generate the air-water cooling spray for the turbine handpiece.

The chamber is still to be connected to an oil-free air compressor, HVE line, and two low-vacuum pumps, here not shown.

Figure 2. Representation of the air turbine and control apparatus used in the present study. The pressurized water tank used to generate the air-water cooling spray for the turbine handpiece is seen on the left. The handpiece cord was afterward inserted inside the chamber and sealed, for the turbine to operate inside the patient phantom.

Figure 3. The two phantoms inside the chamber are shown. On the right the patient phantom can be seen, having the air-turbine and HVE tip fixed in the same position throughout all experimental runs, as if operated by a right-handed dentist and dental assistant. On the left, the operator phantom equipped for the first experimental run, with a surgical mask and the first target fixed with double-sided adhesive tape on its forehead. The external area of the mask to be assessed for viral contamination can be seen marked in red. In all runs, the chamber space between operator and patient was left free of materials and protruding gloves to allow for an even aerosol spread, similarly to clinical conditions.

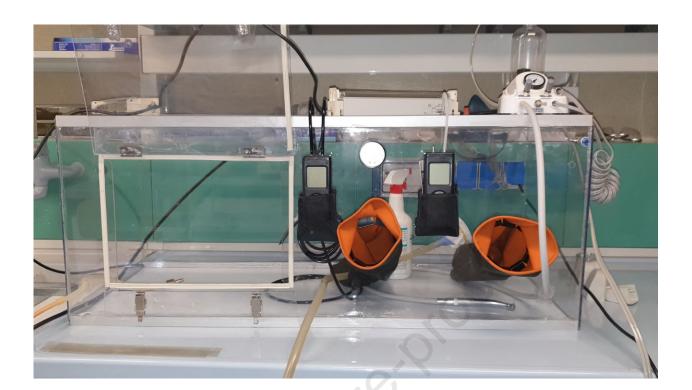
Figure 4. Detail of the patient phantom showing the standardized positions of the air turbine and the HVE inside its mouth.

Figure 5. Detail of the operator phantom as prepared for the first experimental run, equipped with the forehead target and the surgical mask with the red mark for viral load assessment. The decontamination spray containing absolute ethanol can be seen in the background.

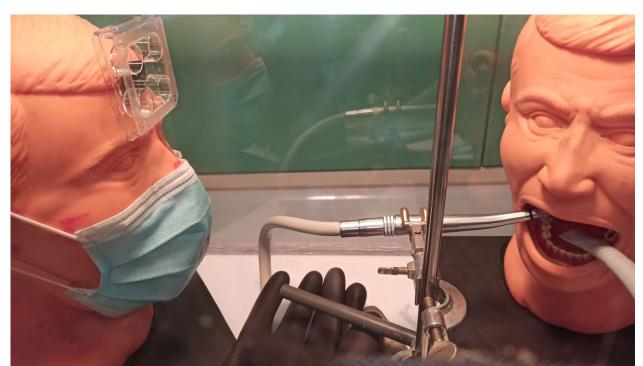
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Figure 6. The setup for the fifth experimental run, testing the association of face shield and surgical mask is shown from the transparent upper panel of the chamber. In this case, the forehead target was fixed to the upper external part of the face shield.

Figure 7. Graph showing the results of the experimental runs (mean Log₁₀ viral copies /cm² ±1SE). Results are divided by the aims of the study, that is to compare the efficacy of masks, respirators and face shield against Human Coronavirus 229E aerosolized by a conventional dental procedure (PPE), or the efficacy of high-volume evacuation (HVE) in mitigating coronavirus spread by aerosol. Blue shades throughout the graph indicate the use of surgical mask to protect the operator, while orange and green stand for N95/FFP2 and FFP3 respirators, respectively. Darker shades indicate the simultaneous use of a face shield. Experimental runs testing HVE (right part of the graph) were performed using surgical masks.







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