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Ultraviolet germicidal irradiation is effective against SARS-CoV-2 in contaminated makeup powder and lipstick

Karina Bispo-dos-Santos [a,1], Priscilla P. Barbosa [a,1], Fabiana Granja [a,b], Matheus Cavalheiro Martini [a], Camila Flavia Schettino Oliveira [c], Desiree Cigaran Schuck [c], Carla Abdo Brohem [c], Clarice Weis Arns [d], Sylvio Jorge Hares Junior [e], Caetano Padial Sabino [e,1,*], Jose Luiz Proenca-Modena [a,f,1,*]

[a] Laboratory of Emerging Viruses, Department of Genetics, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, SP, Brazil
[b] Biodiversity Research Center, Federal University of Roraima, Boa Vista, RR, Brazil
[c] The Boticário Group, Research & Development, São José dos Pinhais, PR, Brazil
[d] Animal Virology Laboratory, Department of Genetics, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, SP, Brazil
[e] BioLambda, Scientific and Commercial LTD, São Paulo, SP, Brazil
[f] Experimental Medicine Research Cluster, University of Campinas, Campinas, SP, Brazil

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is mainly transmitted by airborne droplets generated by infected individuals. Since this and many other pathogens are able to remain viable on inert surfaces for extended periods of time, contaminated surfaces play an important role in SARS-CoV-2 fomite transmission. Cosmetic products are destined to be applied on infection-sensitive sites, such as the lips and eyelids. Therefore, special biosafety precautions should be incorporated into the routine procedures of beauty parlors and shops. Indeed, innovative cosmetics companies are currently searching for disinfection protocols that ensure the customers' safety in makeup testing. Here, we propose an ultraviolet germicidal irradiation (UVGI) strategy that can be used to reduce the odds of COVID-19 fomite transmission by makeup testers. It is well-known that UVGI effectively inactivates pathogens on flat surfaces and clear fluids. However, ultraviolet-C (UVC) radiation at 254 nm penetrates poorly in turbid and porous materials, such as makeup and lipstick formulations. Thus, we investigated the virucidal effect of UVGI against SARS-CoV-2 deposited on such substrates and compared their performance to that of flat polystyrene surfaces, used as controls. Concentrated infectious SARS-CoV-2 inoculum (10^6 PFU/mL) deposited on lipstick and makeup powder was completely inactivated (>5$\log_{10}$ reduction) following UVC exposures at 1,260 mJ/cm^2, while flat plastic surfaces required 10 times less exposure (126 mJ/cm^2) to reach the same microbicidal performance. We conclude that UVGI comprises an effective disinfection strategy to promote biosafety for cosmetics testers. However, appropriate UVC dosimetry must be implemented to overcome inefficiencies caused by the optical properties of turbid materials in lipsticks and makeup powders.

Introduction

Coronavirus disease 2019 (COVID-19) is a disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). So far, it has affected more than 200 million people and caused more than 4 million deaths worldwide [1,2]. The high transmission rate and disease evolution to severe pulmonary symptoms and/or extrapulmonary manifestations [3–6] has led healthcare systems almost to collapse in several countries [7,8]. In addition, social distancing measures and lockdown have led to devastating economic losses around the world, mainly in areas affected by severe income inequality and political instability, such as Brazil [9].

SARS-CoV-2 is primarily spread by aerosols and droplets of various sizes from infected individuals during coughing, sneezing, talking, or even breathing. While droplets measuring approximately 10 μm can remain airborne for minutes or hours and are easily inhaled by

* Corresponding authors.
E-mail addresses: caetano@biolambda.com (C.P. Sabino), jlmodena@unicamp.br (J.L. Proenca-Modena).
[1] These authors contributed equally to this work

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susceptible hosts, those with 100−200 μm settle faster and are important sources of surface contamination [10]. In fact, although SARS-CoV-2 transmission through inanimate surfaces is still a controversial issue in the literature [11,12], other respiratory and enteric viruses, such as Influenza, Rhinovirus, and Norovirus, are frequently transmitted by fomites [13–16].

Hygienic-sanitary measures are necessary to contain airborne and surface SARS-CoV-2 transmission [17], especially in scenarios where services and facilities are reopening [18–21]. In fact, it has been more than a century since the first ultraviolet germicidal irradiation (UVGI) systems were developed to inactivate pathogens to avoid infection transmission in different situations [22–30]. Ultraviolet-C (UVC) radiation is the most effective and commonly used germicidal wavelength range of the ultraviolet spectrum [26]. It induces the formation of photoproducts, such as pyrimidine dimers, that lead to structural distortions of DNA and interrupt RNA/DNA transcription/replication processes [31]. Since UVGI-generating devices are easy to implement, sustainable, and cost-effective for the disinfection of gasses, liquids, and solid surfaces [23], governmental agencies from different countries have indicated UVGI as a promising alternative to inactivate SARS-CoV-2, mainly in shared indoor spaces, such as workplaces, schools, and stores [23,32,33].

Consumer needs have been changing throughout the COVID-19 pandemic. The consumption of cosmetic items, for example, is closely related to the need to maintain personal care, which has increased in some regions due to longer periods of confinement at home [34–36]. Cosmetic products are destined for application on infection-sensitive sites, such as the lips and eyelids. Since SARS-CoV-2 and several other pathogens are able to remain viable on inert surfaces for extended periods of time, contaminated makeup products could play an important role in fomite transmission of infectious diseases. Therefore, special biosafety precautions should be taken into account in the scenarios of beauty parlors and shops.

Despite the pandemic situation, some countries did not apply lockdown for political and economic purposes [37], including low adherence by the population to government policies related to closure and containment [38–40]. According to the 2019 Euromonitor International report, Brazil is the fourth largest market of beauty and personal care products in the world [41]. Sales in this sector presented a 5.8% growth in 2020 compared to 2019, remaining stable in the first trimester of 2021, as reported by the Brazilian Association of the Personal Hygiene, Perfumery and Cosmetics Industry [42]. These numbers reflect the well-established culture of beauty customers in Brazil and the impact of this sector on the national economy.

In this scenario, innovative cosmetics companies are currently searching for disinfection protocols that can ensure customer safety in makeup testing. The contact of multiple people with shared makeup products in beauty parlors and shops is being avoided by the use of disposable samples and augmented reality to provide sensory experiences to the customer [43,44]. The viral contamination of cosmetic accessories and products is a cause for concern, although currently there are no published studies that evaluate how long SARS-CoV-2 remains viable on the surfaces of frequently-used cosmetic products. UVGI decontamination of everyday items, such as smartphones, keys, coins, and credit cards, has been assessed [29], but, to our knowledge, data has not been made available regarding cosmetic products. In fact, it is well-known that UVGI effectively kills pathogens on flat surfaces and clear fluids. However, UVC radiation at 254 nm penetrates poorly through turbid and porous materials, such as N95 masks. Nevertheless, UVGI dosimetry can be adapted accordingly to promote effective microbicidal activity in porous materials [45,46].

Cosmetic products, such as makeup and lipstick formulations, also present porous surfaces, a fact that should reduce the pathogen inactivation efficiency induced by UVGI. Therefore, in the present study, we investigated the virucidal effect of UVGI against SARS-CoV-2 deposited on such surfaces and compared their performance with that observed on flat polystyrene surface controls. To this end, the amount of viable viral particles was determined after cosmetic products were contaminated with 100 μL of a highly concentrated (10^6 PFU/mL) inoculum of SARS-CoV-2. The UVGI procedure was carried out using a custom-designed spectral-calibrated device capable of delivering 4.2 mW/cm^2 of absolute irradiance (254 nm) over the samples' surface to test 8 different exposure times. As a consequence, we validated a UVGI disinfection protocol that can be applied in cosmetics businesses to allow for the safe reuse of makeup products, thus promoting safer conditions for consumers. Even in a post-pandemic scenario, this technology could still be applied in the disinfection of other pathogens, such as herpes simplex, influenza, and rhinoviruses, which can be potentially transmitted by makeup testers.

Materials and methods

SARS-CoV-2 strain and Vero cell culture

All experiments were conducted in a Biosafety Level 3 (BSL3) facility located in the Emerging Viruses Laboratory of the University of Campinas (UNICAMP), in Campinas-SP, Brazil. The SARS-CoV-2 strain used (HIAE-02 SARS-CoV-2/SP02/human/2020/BRA) was kindly provided by Professor Edison Luiz Durigon of the University of São Paulo (USP), in São Paulo-SP, Brazil. The virus was inoculated in Vero CCL81 cell monolayers and harvested at 46 h post-inoculation or when 80% cytopathic effect (CPE) was visible. The supernatant was clarified by centrifugation, and titration was performed by plaque assay and expressed as plaque-forming units (PFU). Vero CCL81 was purchased from the American Type Culture Collection (ATCC). The cell line was cultured in Dulbecco's Modified Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco®) and penicillin-streptomycin solution (10,000 U/mL penicillin and 200 mM streptomycin) at 37 °C in 5% CO_2.

Samples

The cosmetics were manufactured by The Boticário Group (São José dos Pinhais, Paraná, Brazil). The lipstick formula consisted basically of emollients, film formers, viscosity regulators, pigments, absorbents, and fragrance, whereas the compact powder formula included anti-static agents, emollients, pigments, and opacifiers. Products from both categories were selected based on the information contained in their formulas, picking those with the largest powder content in their composition. In addition, they were obtained from official brand stores, therefore in compliance with all the manufacturing criteria for products marketed by the company. They had not been used at the time of purchase and were within the validity period (mandatory in Brazil). The formulations of these products are described in Supplementary Table 1.

UVC device

The UVC testing device (UVC prototype 1, BioLambda, Brazil) consisted of three low-pressure Mercury-vapor (LP-Hg) lamps, with 18 Watts each (OSRAM, Germany), placed side by side at a distance of 30 cm from the samples (6-well plates). Since hot-cathode low-pressure Mercury-vapor (LP-Hg) lamps were used, we allowed the irradiation system to stabilize its optical output for 3 min prior to beginning the sample exposure to UVGI treatment. After stabilization, the system constantly delivered 4.2 mW/cm^2 of irradiance over the samples, with more than 90% beam uniformity. The entire irradiation system was operated inside a laminar flow cabinet to avoid environmental contamination by infective SARS-CoV-2 particles. An illustrative scheme of the prototype device used in this study is shown in Fig. 1. The BSL3 laboratory staff was equipped with the following PPEs: polycarbonate glasses and face shields, double nitrile gloves and facial masks, thick clothing with long sleeves and pants, and closed shoes.

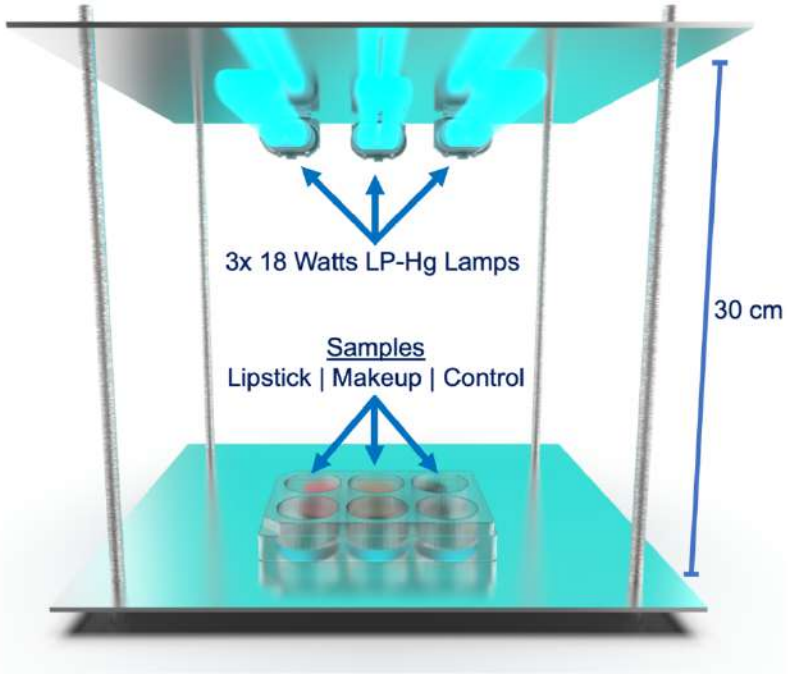

Fig. 1. Custom-designed UVC device. Schematic of the UVGI prototype device used in this study.

UVC virucidal activity assay

A layer of lipstick or compact makeup powder was disposed onto 6-well polystyrene cell culture plates simulating a makeup surface that could be contaminated by infected customers testing these products in a cosmetics store. We used a highly concentrated aliquot of 0.1 mL of SARS-CoV-2 at 10^6 PFU/mL to experimentally contaminate each product or plastic surface (Fig. 2A). The 6-well plates were placed 30 cm below the UVC lamps and were submitted to eight different UVC exposure times (0, 5, 30, 60, 120, 300, 600, and 900 s), corresponding to 0, 21, 126, 252, 504,1260, 2520, and 3780 mJ/cm^2 (Fig. 2B). After exposure, the inocula were resuspended in 0.9 mL of cold cell culture medium, filtered, and diluted serially at ten-fold dilutions (Fig. 2C). Next, 0.3 mL of each dilution was inoculated into 90% confluent Vero cell monolayers in 24-well plates for one hour for viral adsorption. The inoculum was removed, and semi-solid medium was added for viral quantification to verify decreases in infectivity (Fig. 2D). For the negative control, 0.1 mL of culture medium without virus was disposed onto the three tested surfaces and exposed to UVC light as described above. Duplicate assays were conducted in samples obtained in two independent experiments.

Plaque-forming units (PFU) assay

The PFU assay was performed according to a previously described protocol [47] with some modifications. Twenty-four-well plates containing Vero cell monolayers were inoculated with 0.3 mL of each ten-fold dilution of samples in DMEM without FBS, and placed for 1 hour in a homogenizing platform for viral adsorption. Subsequently, the inocula were removed, and 1 mL of a semi-solid medium (1% w/v carboxymethylcellulose in DMEM supplemented with 5% FBS and 1% penicillin-streptomycin solution) was added. After 72 h, the overlay medium was removed, and the cell monolayers were fixed for 1 hour with 10% w/v paraformaldehyde, and stained with a 1% w/v methylene blue solution. At the end of the experiment, viral lysis plaques were counted, the average quantities observed in replicates were calculated, and the titers were expressed as plaque-forming units per mL.

Statistical analysis

Data and statistical analyses were performed using the Prism 8.0 software (Graphpad, USA). Quantitative data are presented as means and standard deviation of the means. Data on inactivation kinetics were fitted by a power-law (Eq. (1)), as suggested by Sabino et al. [48]. In brief, the fitted datasets yielded the constant of lethal dose for 90% burden (LD_{90}) and the tolerance factor (T). These constants allow for continuous comparison of the tendency of all points of each group, which can be compared among each other using multiple unpaired T-tests, with Welch's post-test for the correction of variable standard deviations. Statistical results were considered significant when $p < 0.05$ and are presented in the supplementary information section.

$$log_{10}\left(\frac{N_0}{N}\right) = \left(\frac{Dose}{LD_{90}}\right)^T \quad (1)$$

where:

N_0 = initial microbial burden; N = final microbial burden; Dose = light exposure (mJ/cm^2); LD_{90} = lethal dose for 90% of microbial burden (mJ/cm^2); T = tolerance factor.

Results and discussion

Herein, we report the effect of UVC radiation (LP-Hg, 254 nm) on SARS-CoV-2 inactivation on makeup powder and lipstick surfaces. Although light-emitting diodes (LEDs), excimer plasma discharge lamps, and pulsed Xenon lamps can also be used to emit UVC radiation, the centenary LP-Hg lamps remain the most commonly utilized type of UV light source for germicidal applications due to their extensive cost- and energy-efficiency [49,50]. In the present study, the experiments were conducted using a custom-designed spectral-calibrated device equipped with an assortment of LP-Hg lamps to guarantee further experimental reproducibility (Fig. 2B) [51]. In order to simulate a heavy contamination burden over a previously pressed layer of makeup powder and lipstick, we added a suspension of viable SARS-CoV-2 particles in a similar concentration to that found in the sputum of COVID-19 patients (Fig. 2A) [52].

Interestingly, SARS-CoV-2 showed high susceptibility to UV

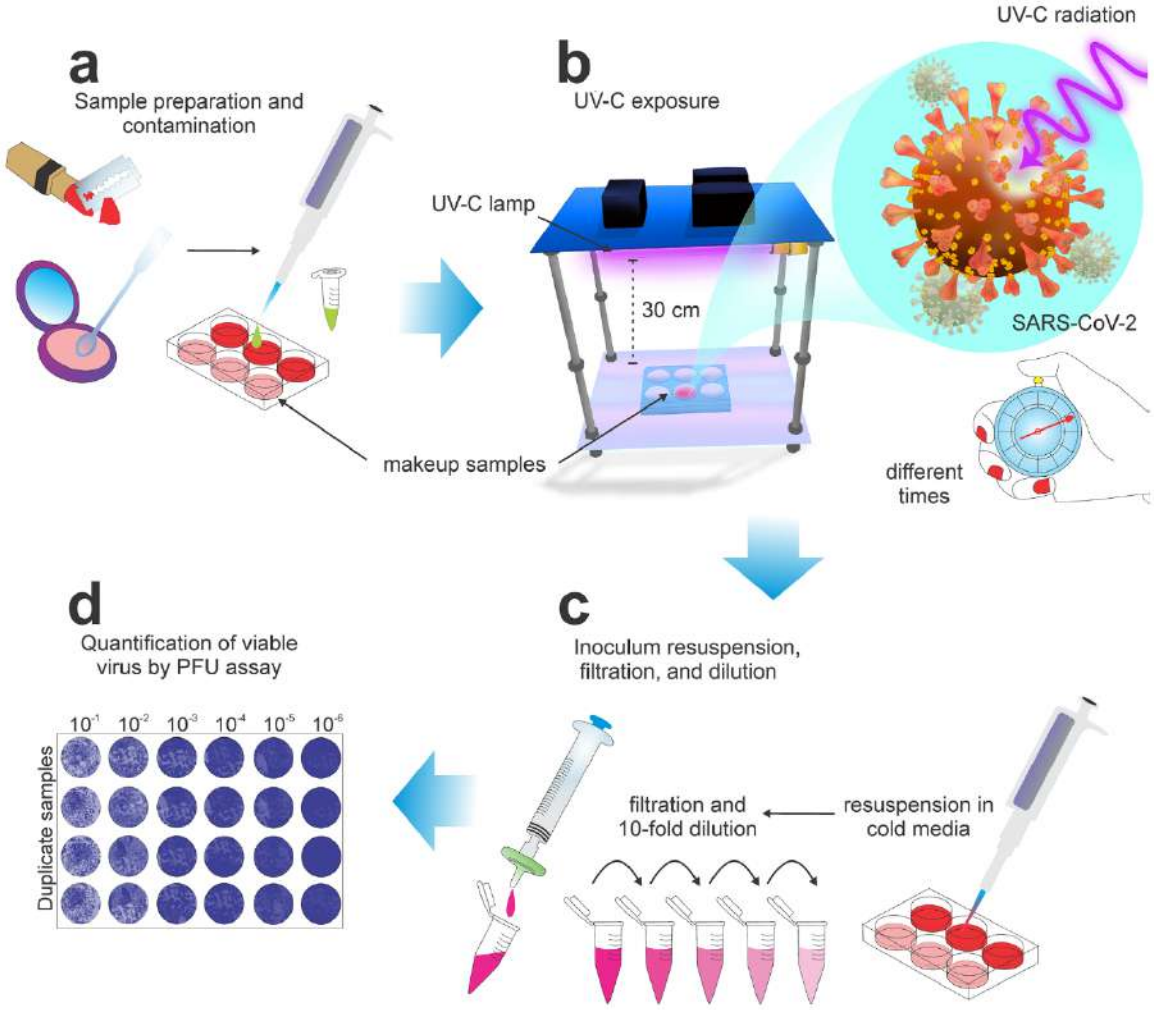

Fig. 2. Scheme of the UVC 254 nm virucidal activity assay against SARS-CoV-2. Illustration of the experimental setup of the UVC (254 nm) virucidal assay described in the Methods section. (A) A layer of compact makeup powder and lipstick was disposed onto 6-well cell culture plates and infected with 10^5 PFU of SARS-CoV-2. As controls, 0.1 mL of DMEM containing 10^5 PFU of SARS-CoV-2 or DMEM without virus were added to the 6-well polystyrene cell culture plates. (B) All samples were submitted to different UVC doses using eight different UVC exposure times in a custom-designed device. (C) After UVC exposure, the samples were resuspended, filtered, serial-diluted, and inoculated in Vero cells for virus titration. (D) SARS-CoV-2 were titrated using the Plaque Forming Units (PFU) Assay.

irradiation, even in the presence of the makeup powder and lipstick. Total inactivation (>5$\log_{10}$) of SARS-CoV-2 on the polystyrene surface was achieved after 30 s of UVC exposure, or 126 mJ/cm^2 (Fig. 3A). Such level of UVC sensitivity on flat surfaces is compatible with previous results reported by Bormann et al. [29], that 1$\log_{10}$ (LD_{90}) of SARS-CoV-2 was inactivated by exposure to 11.7 mJ/cm^2 of UVC-LED (250 and 280 nm); by Gidari et al. [53], that 20.06 and 23.71 mJ/cm^2 induced a reduction of 4$\log_{10}$ in the viral titer, and Sabino et al., that a 5$\log_{10}$ reduction was achieved with 108.7 mJ/cm^2 [54]. However, these data contrast with those reported by Heilingloh et al. [28], that complete virus inactivation was only obtained with 1048 mJ/cm^2 of UVC exposure at 254 nm. This divergence could be related to the different experimental conditions, such as inocula concentration, sample volume (*i.e.*, dimension of the optical path), irradiation medium (*e.g.*, culture media, saline solution, dried surface, surface porosity and roughness, etc.), spectral output, light homogeneity, and even the method of viral quantification.

Our results showed that the UVC effect on SARS-CoV-2-contaminated makeup powder and lipstick caused complete SARS-CoV-2 inactivation only after 300 s of UVC exposure, or 1260 mJ/cm^2, for both types of cosmetic substrates (Fig. 3A). UVC irradiation doses of 21 mJ/cm^2 caused a titer reduction of approximately 1$\log_{10}$ for the compact makeup powder and 2$\log_{10}$ for the lipstick, corresponding to a decrease of 71.3% and 97%, respectively (Fig. 3B). Using a dose of 126 mJ/cm^2, we obtained a titer reduction of nearly 3$\log_{10}$, or 99.7% and 99.6% for the compact makeup powder and the lipstick, respectively (Fig. 3B). Finally, UVC exposure for 60 and 120 s (252 and 504 mJ/cm^2, respectively) was capable of suppressing the SARS-CoV-2 titers in 3$\log_{10}$, or a reduction of 99.9% (Fig. 3B). Based on the outputs of our data fitting method (Table 1), the inactivation curves (Fig. 3C) were statistically identical between the makeup powder and the lipstick, whereas both groups presented significant tolerance factor (T) differences with the polystyrene surface (Supplementary Tables 2 and 3). Collectively, these findings demonstrate that the inactivation of SARS-CoV-2 on the surface of contaminated makeup powders and lipsticks by UVC (254 nm) exposure is experimentally feasible when the protocol is adapted for porous substrates, such as the two cosmetic products analyzed. Despite the fact that UVC radiation may not penetrate deeply into the makeup, our method of sample resuspension in medium ensures that any viable viruses located in the innermost parts of the sample can also be retrieved and detected through cytopathic effect in later titration.

An optimized UVC device can also be developed for cosmetics-related businesses to inactivate SARS-CoV-2 within shorter exposure times. This could be achieved by using multiple light sources and reflectors and by sample placement closer to the light emitters. Since the

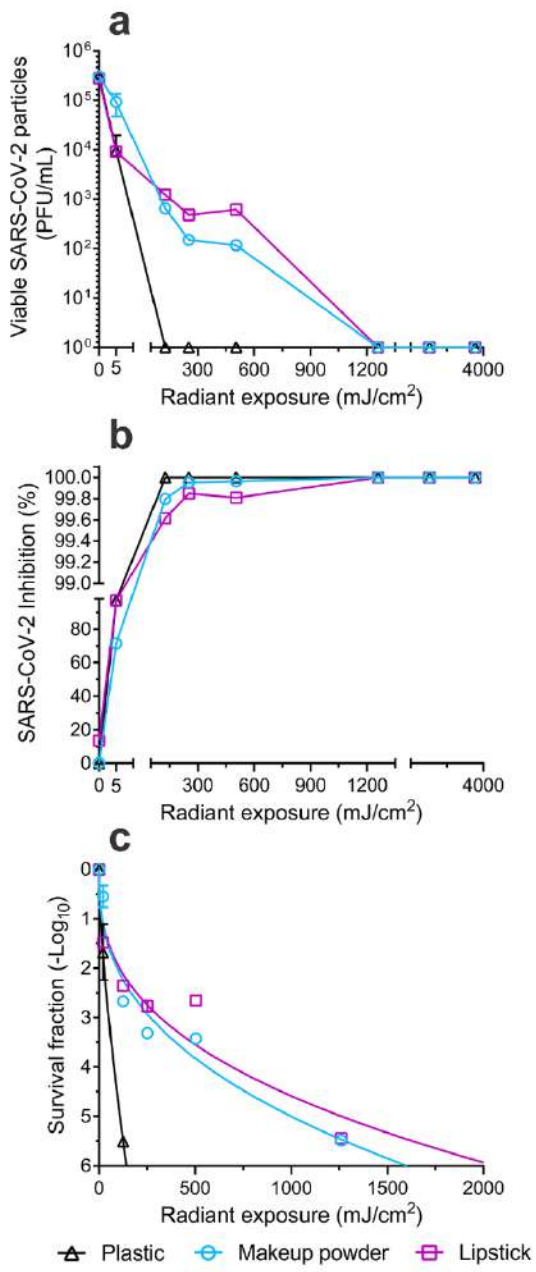

Fig. 3. SARS-CoV-2 inactivation in cosmetic or plastic samples. (A) Viral load of SARS-CoV-2 as a function of the UVC exposure time. (B) Inhibition percentage of viable SARS-CoV-2 as a function of the UVC exposure time. (C) The calculated inactivation kinetics of SARS-CoV-2 in function of the UVC dose (or radiant exposure).

Table 1
Means and standard error of the means (SEM) calculated for lethal UVC doses for 90% viral inactivation (LD_{90}) and the tolerance factor (T).

	LD90 (mJ/cm²)		T	
	Mean	SEM	Mean	SEM
Control	10	2	0.66	0.07
Makeup	16	6	0.39	0.04
Lipstick	17	9	0.37	0.05

UVC dose (or radiant exposure) is linearly correlated to the irradiance and exposure time, a 10-times higher irradiance (*e.g.*, 42 mW/cm² instead of 4.2 mW/cm²) could provide the same dose (*e.g.*, 1260 mJ/cm²) at a tenth of the required exposure time (*e.g.*, 30 s instead of 300 s). Also, it is important to highlight that the device must include safety systems that avoid the operator's exposure to UVC. It should consist of an enclosed system that blocks UVC transmission and also have some type of interlock mechanism that turns off the emitters if the enclosure is opened. Although the UVC-induced photodegradation of some compounds used in cosmetic formulations may occur, no changes in pigment color could be observed by the naked eye following several consecutive exposures to the 1260 mJ/cm² protocol. The pigments used in cosmetic formulations are also required to be photostable in order to last longer under display and when applied. However, formulations claiming to contain any phototoxic ingredient in their formula, including, for example, vitamin A derivatives and fluorescent dyes, might be subjected to significant photodegradation; therefore, we recommend that further studies be carried out to address this photochemical issue.

The urgency of dosimetry studies such as the present assessment is due to the fact that, despite the alarming situation of the ongoing global pandemic, in several countries beauty parlors and shops remained open during most of the pandemic period [37,38]. The state of São Paulo (SP), the third largest economy and consumer market in Latin America [55], and the most populous state in Brazil, has the highest number of confirmed COVID-19 cases in the country [56,57]. According to the São Paulo economic recovery plan, the state was divided into 17 regions where commercial establishments were completely closed only when the intensive care unit (ICU) occupancy was above 80% [58].

In twenty-six São Paulo government reports, the average number of regions that underwent lockdown during the June 2020 to April 2021 period was only 4.6 (27%). In the same period, the daily average of infections, deaths, and social isolation index in São Paulo were 8365, 265, and 43%, respectively [56,59]. Even though this social isolation strategy is relatively mild, it is still the strictest when compared to that applied by the federal government, which declared that beauty parlors are essential businesses in our country [60]. One of the justifications for such policy is the consideration of contextual differences in developing nations during public health emergencies [61].

So far, there are no studies on SARS-CoV-2 viral contamination in cosmetic formulations in general. Nonetheless, it is noteworthy that these products can be easily contaminated by pathogens during utilization by consumers, defined as secondary contamination [62]. Rheinbaben and Heinzel [63] artificially contaminated different types of cosmetic formulations, including makeup and lipstick samples, with enveloped and non-enveloped viruses. They found that nearly all products tested – with the exception of soap – were suitable vehicles for transferring viruses, evidencing that secondary contamination is a potential infection pathway. Meanwhile, bacterial and fungal contamination were previously shown to be possible in market and post-market environments [64,65] in lipstick, makeups, beauty blenders, lip gloss, and eye-makeup [66,67]. Therefore, we believe that the presented protocol is a feasible, fast, sustainable, safe, and cost-effective method that can easily be applied in cosmetics stores and beauty shops to inactivate SARS-CoV-2 and other pathogens that could contaminate makeup testers or makeup applicators.

Conclusion

In the present study, we established that UVGI dosimetry is a basis for the effective disinfection of turbid and porous cosmetic products found in beauty parlors and shops. Even though it is well-known that UVGI effectively kills pathogens, including SARS-CoV-2, on flat surfaces and clear fluids, there is a need to compensate UVGI dosimetry since UVC radiation poorly penetrates turbid materials such as lipstick and makeup formulations. Our study showed that a 10-fold UVC dose increase was required in order to obtain complete microbicidal effectiveness ($> 5\log_{10}$) in the cosmetic products (1260 mJ/cm²) when compared to the flat polystyrene surfaces (126 mJ/cm²). Based on our findings, we concluded that, when applied in adequate doses, UVGI constitutes an effective disinfection strategy to promote biosafety

improvements in cosmetic testing. Thus, we believe that our UVGI protocol represents a feasible, fast, sustainable, safe, and cost-effective method that can easily be applied in cosmetics shops and beauty parlors to inactivate highly relevant pathogens, such as SARS-CoV-2.

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Author contributions

Conception: JLPM, KBS, CPS, FG. Acquisition: KBS, PPB, MCM, SJHJ. Analysis: KBS, CPS, JLPM. Interpretation: KBS, CPS, JLPM, CFSO, DCS. Drafting: KBS. Revising: KBS, PPB, CPS, JLPM, DCS, CAB, CFSO, FG, CWA. All authors have read and approved the final version of the manuscript. Funding: JLPM, CFSO.

Declaration of Competing Interest

This study was supported by The Boticário Group, which also manufactured the lipstick and compact powder. C.P.S. and S.J.H.J. are employed by BioLambda, where the UVC prototype device was developed and produced free of charge.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpap.2021.100072.

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