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Efficacy of the PlasmaShield®, a Non-Thermal, Plasma-Based Air Purification Device, in Removing Airborne Microorganisms

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Abstract: Airborne microorganisms play a significant role in the transmission of infectious diseases. As such, improving indoor microbial air quality can enhance infection control in numerous settings. This study examined the efficacy of the PlasmaShield® air purification device to remove airborne microorganisms under laboratory conditions. Pure cultures of model microorganisms at varying concentrations were aerosolized using a 1-jet Collison nebulizer through stainless-steel removable piping prior to reaching the PlasmaShield® device. The surviving microorganisms were captured using the Staplex® MBS-6 Six Stage Microbial Air Sampler and enumerated via culture on agar plates. The positive-hole-corrected colony/plaque-forming units were compared with the negative control (microorganisms aerosolized through an empty PlasmaShield® casing). The PlasmaShield® statistically significantly ($p < 0.05$) reduced airborne *Escherichia coli*, *Staphylococcus epidermidis*, Bacteriophage MS2 and *Cladosporium* sp. compared with the negative control. The maximum removal achieved was estimated to be 4 × $\log_{10}$ *E. coli* (99.99% removal), 4 × $\log_{10}$ *S. epidermidis* (99.97% removal), 7 × $\log_{10}$ MS2 (99.99998% removal) and 5 × $\log_{10}$ *Cladosporium* sp. (99.999% removal). Scanning electron microscope images of the surviving microorganisms showed that the PlasmaShield® damaged the cell membrane of these model microorganisms. This study provides proof-of-concept evidence to support the use of this technology to improve indoor microbial air quality.

Keywords: air purification; infection control; indoor air; microbial air quality

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1. Introduction

Indoor air quality is recognized as a significant factor affecting human health [1]. Within clinical and non-clinical settings, it is recognized that inadequate air ventilation is associated with increased risk of transmission of infectious diseases [2–4]. Recent studies during the current COVID-19 pandemic have also shown that overcrowding and poor ventilation promote the airborne transmission of SARS-CoV-2 [5–7].

There are a range of engineering solutions available to improve microbial air quality through air purification technologies. This includes devices utilizing air filtration, ultraviolet irradiation, electrostatic precipitation, negative air ionization, photocatalytic oxidation, air ozonation, passive solar exposure, pulsed light and plasma-based disinfection [8]. This study examines the efficacy of the PlasmaShield®, a novel air purification device utilizing electron beam irradiation and non-thermal plasma-based disinfection, to remove four airborne microorganisms.

Escherichia coli, *Staphylococcus epidermidis*, bacteriophage MS2 and *Cladosporium* sp. were chosen as the model microorganisms. *E. coli* is a Gram-negative bacterium found in the intestines of humans and animals and is recognized as an indicator of fecal contamination. It is the most commonly used model bacteria [9–12]. *S. epidermidis* is a coagulase-negative,

Gram-positive bacteria that is part of the skin's natural flora. It does not cause disease in healthy individuals, but is increasingly associated with hospital acquired infections and indwelling medical device-associated infections [13]. This is further complicated by the emergence of antibiotic resistant strains [14]. Its significance as an opportunistic pathogen is linked to its biofilm forming capabilities [15], which protects it from disinfection methods enabling long term persistence in the environment [16]. MS2 is a single stranded RNA virus that infects *E. coli* and other members of the Enterobacteriaceae. It is a recognized indicator organism for enteric viruses and fecal contamination [17]. It is a suitable surrogate for viral pathogens and has been used as a model organism in virus inactivation studies [18–20] and to investigate virus survival during aerosol dispersion [21,22]. *Cladosporium* sp. is one of the most common fungi found in air samples [23]. It has been isolated from a range of indoor environments including hospitals [24], aged care facilities [25], schools [26] and office buildings [27].

2. Materials and Methods

2.1. PlasmaShield Design

The PlasmaShield® is a non-thermal plasma-based air purification device (U.S. Patent No. 10,744,515). Briefly, it contains a discharge electrode located centrally within a cylindrical ground electrode, and a power supply electrically connected to the discharge and the ground electrode. This produces a uniform electric field which destroys microorganisms in the air by electroporation [28] and a low-energy electron beam irradiation which inactivates microorganisms by damaging their nucleic acid [29]. In addition, a corona discharge from the discharge electrode generates ions (excited N and NO molecules) and free electrons, which potentially provide a secondary disinfection mechanism. These ions are produced within a narrow region (100 µm) around the discharge electrode tip inside the plasma reactor. The nature of these excited species suggests that they react with other molecules and are therefore unable to travel a noticeable distance from their generation point [28]. Independent testing has shown that the ozone emissions generated by the device do not exceed 2 ppb [30]. It is also certified by the California Air Resources Board (CARB) as an air cleaning device, meeting the ozone regulation of less than 50 ppb [31].

2.2. Experimental Setup

The PlasmaShield® device in its commercial configuration was provided by PlasmaShield LTD (Keswick, SA, Australia). The efficacy of the device to remove model microorganisms was tested using the experimental setup shown in Figure 1. Briefly, each microorganism was aerosolized (at a range of concentrations) using a 1-jet Collison nebulizer, (CH Technologies: Westwood, NJ, USA) through stainless steel removable piping (0.254 m (10 inch) diameter × 1 m in length) prior to reaching the PlasmaShield® device. The remaining microorganisms were captured 1 m after the device using the Staplex® MBS-6 Six Stage Microbial Air Sampler (The Staplex Company: Brooklyn, NY, USA) for 60 m at a sampling flow rate of 28.3 L/min (1 CFM). Each stage of the air sampler contained a different filter size with 400 holes. Stage 6 contained the finest filter and collected particles of 0.65 to 1.1 µm in size. Stages 5, 4, 3, 2 and 1 contained 1.1 µm to 2.1 µm, 2.1 µm to 3.3 µm, 3.3 to 4.7 µm, 4.7 to 7.0 µm and 7.0 µm and above, respectively. Surviving microorganisms were enumerated via culture on agar plates. After incubation, the colonies/plaques present on each agar plate were counted and recorded as positive hole corrected using the correction described by Macher [32]. The positive hole corrected counts for each of the six stages were added together and the total from the three trials averaged. For each microorganism/concentration tested a negative control, or baseline, was determined by replacing the PlasmaShield® with an empty casing. The air flow rate was controlled at 1 m/s, which was confirmed using an anemometer (Pocket ProTM + Multi 2, HACH Company: Melbourne, Australia), this gave a volume flow rate of approximately 51 L/s (the PlasmaShield® rated capacity is 86 L/s). The air pressure released from the air cylinder

was set to 10 psi. The pressure drop at 1 m/s flow rate was 0.007 psi. All experiments were conducted in triplicate.

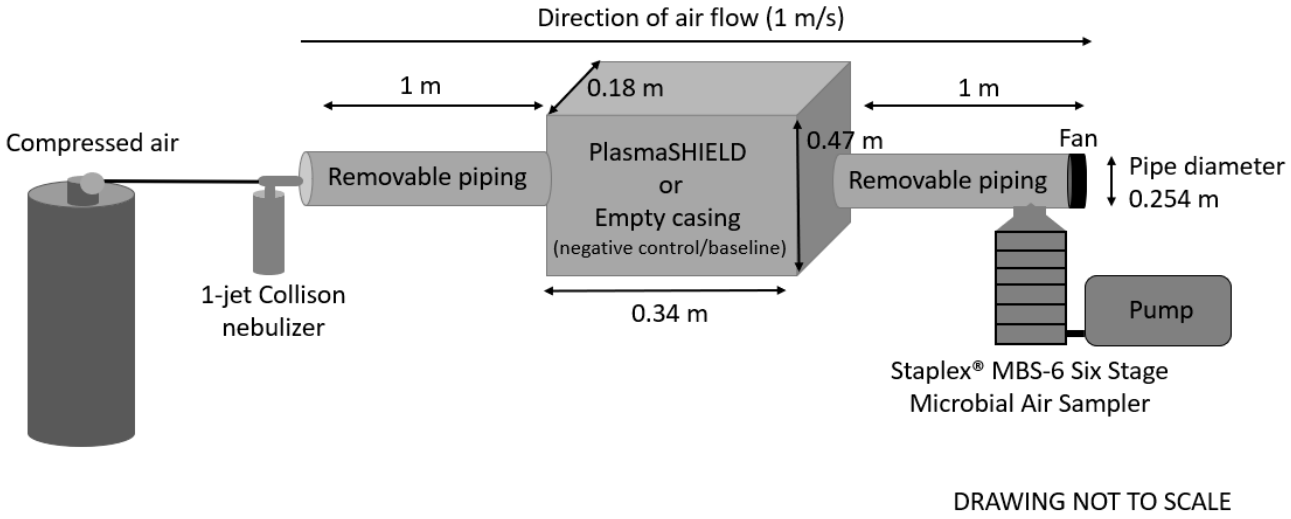

Figure 1. Schematic of experimental setup.

2.3. Escherichia coli

E. coli (American Type Culture Collection (ATCC) 700891) culture was incubated at 37 °C for 12 h in nutrient broth (Oxoid®: Adelaide, Australia). The broth culture was then centrifuged at 3000 rpm for 15 min and the pellet was washed with phosphate-buffered saline (PBS) (Fisher BioReagents®: Adelaide, Australia). The washed pellet was resuspended in PBS and the optical density of the culture adjusted to 1 (10^9 colony forming units (CFU)/mL) at 600 nm (spectrophotometer OD600) using PBS. The culture was then serially diluted with PBS to obtain the desired concentrations (10^7 CFU/mL, 10^8 CFU/mL or 10^9 CFU/mL). After each experiment *E. coli* were captured using the Staplex® MBS-6 Six Stage Microbial Air Sampler on plate count agar (PCA) (Oxoid®) and incubated overnight at 37 °C.

2.4. Staphylococcus epidermidis

S. epidermidis (ATCC 14990) culture was incubated at 37 °C for 12 h in nutrient broth. The broth culture was then centrifuged at 3000 rpm for 15 min and the pellet was washed with PBS. The washed pellet was resuspended in PBS and the optical density of the culture was adjusted to 1 (10^9 CFU/mL) at 600 nm (spectrophotometer OD600) using PBS. The culture was then serially diluted with PBS to obtain the desired concentration (10^7 CFU/mL, 10^8 CFU/mL, 10^9 CFU/mL or 10^9 CFU/mL). For each replicate trial and concentration, 60 mL of the culture was added to a 1-jet Collison nebulizer to produce aerosols (Figure 1). After each experiment, surviving *S. epidermidis* were captured using the Staplex® MBS-6 Six Stage Microbial Air Sampler on PCA and incubated overnight at 37 °C.

2.5. Bacteriophage MS2

MS2 (ATCC 15597-B1) was propagated using the double agar layer method. The bottom layer (of the tryptone soya agar (TSA)-*E. coli* agar plates) consisted of TSA (Oxoid®) and the top layer consisted of 4.5 mL of soft TSA mixed with 500 µL of overnight *E. coli* (ATCC 700891) culture (which had been incubated overnight at 37 °C in typtone soya broth (Oxoid®)) and 200 µL of MS2 culture. The plates were then incubated overnight at 37 °C. The plaques were harvested in peptone water (Oxoid®) and purified by centrifugation at 3000 rpm for 15 min to separate the host cell debris and the MS2. The supernatant was filtered through a 0.22 µm Millex-GP Syringe Filter Unit (Millipore, catalog number SLGP033RS, Ireland) and used as a stock solution. This stock was serially diluted in sterile water to achieve desired concentrations (10^7 plaque forming units (PFU)/mL,

10^{10} PFU/mL, 10^{12} PFU/mL and 10^{15} PFU/mL) which were confirmed by plating and counting plaques using the double agar layer method described above. After each experiment, surviving MS2 were captured using the Staplex® MBS-6 Six Stage Microbial Air Sampler on TSA-*E. coli* agar plates, which were incubated overnight at 37 °C.

2.6. Cladosporium sp.

An environmental culture of *Cladosporium* sp. previously isolated from indoor air [33] was incubated for three days at 25 °C on to malt extract agar (MEA) (Oxoid®). After incubation, a small piece of agar at the edge of the fungal colony (where the mycelium is highly active) was cut using a sterile scalpel and placed on to a fresh MEA plate. The agar was placed upside down to allow the growth to touch the fresh agar plate. The inoculated plates were then incubated for 7 days at 25 °C until the lawn cultures were visible, and spores were produced. The fungal plates were flooded with 10 mL of 0.05% Tween 80 solution (Labchem, AJAX Laboratory Chemicals, Adelaide, Australia) and the spores were separated from the hyphae using a sterile glass slide. The solution was then filtered through sterile absorbent cotton wool pads to remove the hyphal debris. This step was repeated three times. The spore solution was mixed well and serially diluted in 0.05% Tween 80 solution to obtain the desired concentration (10^7 spores/mL, 10^9 spores/mL and 10^{10} spores/mL). The concentration of the spore suspension was determined using a hemocytometer (spores/mL). After each experiment, surviving *Cladosporium* sp. were captured using the Staplex® MBS-6 Six Stage Microbial Air Sampler on MEA and incubated at 25 °C for three days.

2.7. Scanning Electron Micrscopy (SEM)

Experiments were conducted using the setup described in 2.1 (see Figure 1); however, the Staplex® MBS-6 Six Stage Microbial Air Sampler was replaced with a liquid impinger sampler (BioSampler® SKC Inc: Eighty Four, PA, USA). Captured microorganisms were then deposited onto cleaned glass slides (oxygen plasma treated for 5 min). Samples were then fixed by adding to 3% glutaraldehyde solution in PBS (pH 7.2) at 21 °C for one hr. The glutaraldehyde solution was then discarded and samples were washed twice for 5 min with PBS. Samples were then dehydrated using three × 15 min washes of increasing ethanol concentrations (70%, 90% and 100% ethanol). The samples were then immediately dried using a Leica CPD300 Critical Point Dryer. Glass slides were then mounted onto SEM stubs using carbon adhesive tape and coated with 5nm of Platinum using an Emitech K57X sputter coater. SEM analysis was performed using a FEI Inspect F50 SEM, with a beam voltage of (10–30 kV).

3. Results

Efficacy of PlasmaShield® to Remove Airborne Escherichia coli, Staphylococcus epidermidis, Bacteriophage MS2 and Cladosporium sp.

The PlasmaShield® statistically significantly ($p < 0.05$) reduced airborne *E. coli*, *S. epidermidis*, MS2, *Cladosporium* sp. compared with the negative control (Table 1). The negative control for each model microorganism could only be calculated at the lowest concentration tested. As the test concentration increased the number of CFU or PFU per plate was too many to count and as such the negative control for these higher concentrations were estimated by extrapolating from the highest countable concentration (e.g., the negative control from the lowest test concentration). The maximum removal achieved was estimated to be 4 × $\log_{10}$ *E. coli* (99.99% removal), 4 × $\log_{10}$ *S. epidermidis* (99.97% removal), 7 × $\log_{10}$ MS2 (99.99998% removal) and 5 × $\log_{10}$ *Cladosporium* sp. (99.999% removal) (Table 1). The SEM images (Figure 2) demonstrate that the PlasmaShield® damages the cell membrane of these model microorganisms. Figure 2A,C,E,G show intact *E. coli*, *S. epidermidis*, MS2 and *Cladosporium*, respectively. These cells were collected during the baseline trials with the PlasmaShield® turned off. Figure 2B,D,F,H show cell debris of *E. coli*, *S. epidermidis*, MS2 and *Cladosporium*, respectively, collected after treatment with the PlasmaShield®.

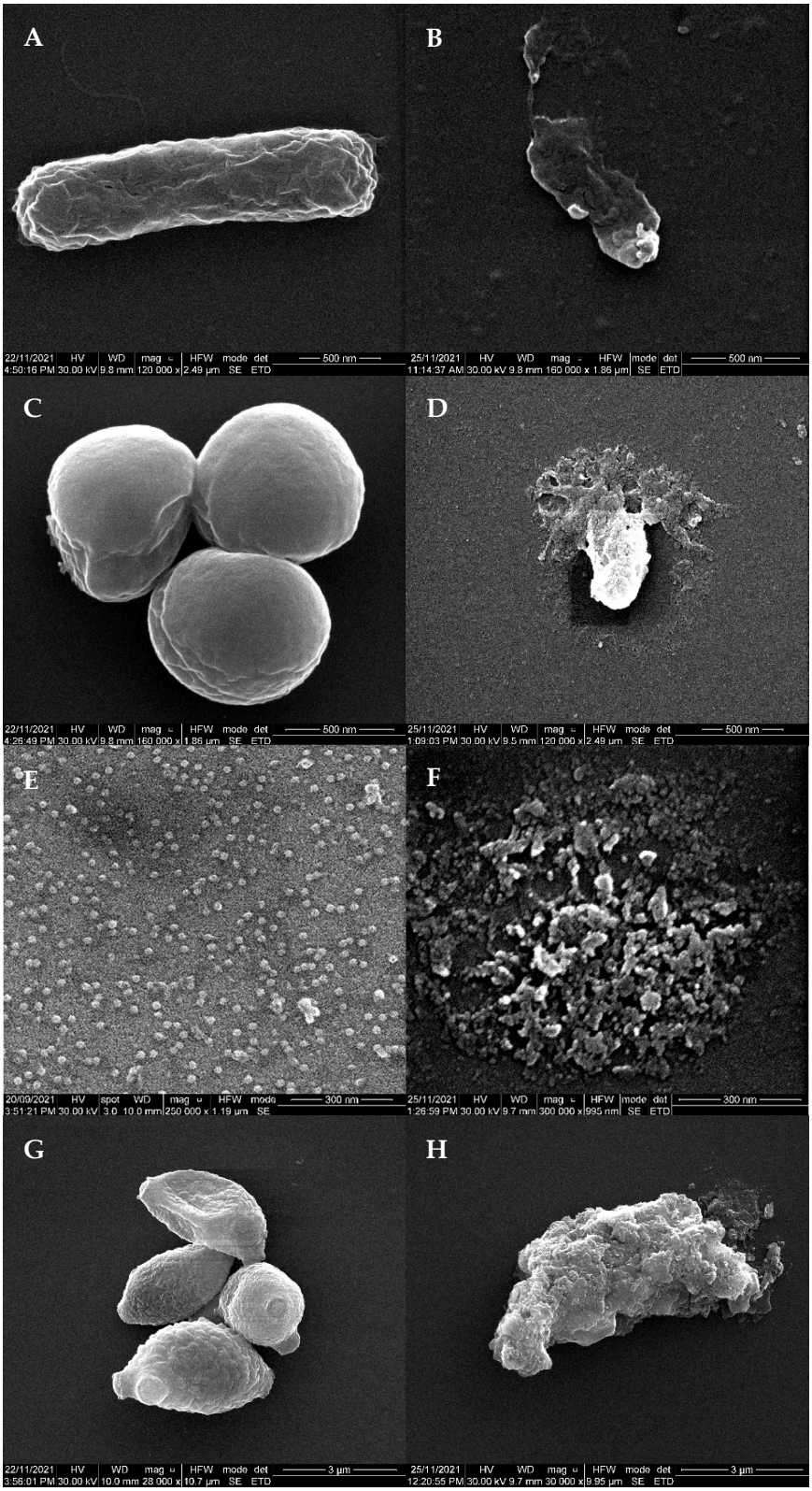

Figure 2. Scanning electron microscope images of baseline/negative control (**A**) *E. coli* (**C**) *S. epidermidis* (**E**) MS2 and (**G**) *Cladosporium* spp. and PlasmaShield® destroyed microorganisms (**B**) *E. coli* (**D**) *S. epidermidis* (**F**) MS2 and (**H**) *Cladosporium* sp.

Table 1. Removal efficacy of the PlasmaShield® compared with the negative control for each microorganism and concentration tested.

Microorganism	Test Concentration (CFU/mL or PFU/mL)	Average Positive Hole Corrected CFU or PFU (±2SD)		Log_{10} Removal	Percentage Removal (%) +
		Negative Control	PlasmaShield®		
Escherichia coli	10^7	2875 (±22)	37 (±22)	2	98
	10^8	28,750 *	16 (±4)	3	99.9
	10^9	287,500 *	15 (±2)	4	99.99
Staphylococcus epidermidis	10^6	2026 (±92)	44 (±25)	2	98
	10^7	20,260 *	34 (±11)	3	99.8
	10^8	202,600 *	165 (±20)	3	99.9
	10^9	2,026,000 *	625 (±153)	4	99.97
Bacteriophage MS2	10^7	117 (±30)	24 (±9)	1	80
	10^{10}	117,000 *	34 (±14)	4	99.97
	10^{12}	11,700,000 *	91 (±30)	5	99.999
	10^{15}	11,700,000,000 *	2757 (±886)	7	99.99998
Cladosporium sp.	10^7	4957 (±1809)	10 (±1)	3	99.8
	10^9	495,700 *	21 (±5)	4	99.99
	10^{10}	4,957,000 *	54 (±27)	5	99.999

* The negative control could not be calculated at this concentration as there were too many CFU or PFU per plate. As such, this is an estimate extrapolated from the highest countable concentration for each microorganism (e.g., the negative control from the lowest test concentration). + The number of decimal points included reflects the log_{10} removal achieved.

4. Discussion

Currently, HEPA ("high efficiency particulate air") filtration is most commonly used method for improving indoor air quality [34,35]. However, there are several limitations with this approach. This includes the need to regularly change and dispose of contaminated filters as well as the energy requirements needed to overcome the high pressure drop that occurs across the filters [35,36]. New air purification technologies provide an alternative to HEPA filtration and can potentially overcome these limitations. Previous studies have shown that plasma-based devices have microbial inactivation/sterilization capabilities [37–39]. This includes a study by Tanaka et al. [38] that demonstrated that a chemical reactor with plasma-assisted catalytic technology (PACT) achieved a 99.99% disinfection efficacy against airborne feline calicivirus (FCV), which is considered an appropriate surrogate for norovirus [38]. Similarly, this current study demonstrated that the PlasmaShield® had disinfection capabilities against airborne model microorganisms *E. coli*, *S. epidermidis*, MS2 and *Cladosporium* sp.

A limitation of this study was that the number of CFU or PFU per plate for the baseline/negative control measurements were often too many to count (Table 1). For these trials, the negative control was estimated by extrapolating from a trial conducted with a lower test concentration. This does not consider the potential for additional loss of microbial cells through physical inactivation during the aerosolization process. This may have resulted in a slight overestimation of the baseline and therefore the disinfection removal efficacy. However, other approaches to either reduce the aerosolization time or to dilute the baseline to a countable level would have required additional handling steps that would have also added additional uncertainty. The manufacturer of the PlasmaShield® states that the device utilizes two modes of action. This includes electron beam irradiation (through multilayer electron beam emitters) and irreversible electroporation (an intense electric field). Previous studies describing the antimicrobial mechanism of action of non-thermal plasma-based devices describe the breaking of bacterial peptidoglycan bonds. As these bonds are structurally important, their breaking leads to cell wall damage, enabling reactive oxygen species to enter through the site of injury [40–42]. However, there is limited research investigating the mechanism of action responsible for the breaking of these bonds.

It is also thought to be dependent on the impinging plasma species [42]. The destruction of the cell wall by the PlasmaShield® was observed in this study through the SEM images and supports these mechanisms of action.

The antimicrobial efficacy of the PlasmaShield® demonstrated in this study was achieved under controlled laboratory conditions at a constant flow rate and air pressure. Future research is needed to determine the influence of environmental factors, such as airflow, humidity, temperature, and particulate matter, on the efficacy of the PlasmaShield® disinfection capabilities.

5. Conclusions

The airborne transport of pathogens represents a significant source of infection in many settings, such as medical facilities, crowded locations, facilities that handle such pathogens, and, recently, quarantine facilities used to control the spread of COVID-19. This study demonstrated that the PlasmaShield® air purification device effectively removed model airborne microorganisms under controlled conditions. This provides proof-of-concept evidence to support the use of this technology to improve indoor microbial air quality.

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