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Perspectives on Quality

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Evaluation of ultraviolet irradiation efficacy in an automated system for the aseptic compounding using challenge test

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Abstract

Objective: Ultraviolet (UV) irradiation efficacy in the intravenous compounding robot APOTECAchemo was evaluated to define the best operative conditions in terms of sterility and time optimization.
Design: The challenge test was used against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* spores and *Candida albicans*. Inoculated plates were placed inside the robot and irradiated for different times. Microbial air and surface quality inside the equipment were monitored utilizing settle and contact plates, swabs.
Results: After 4 h, no microorganisms were viable with killing rates ranging from 5- to 7-log for different microorganisms after 1 h of exposition. In confirmation of the efficacy of the UV irradiation program adopted, the microbial monitoring inside the equipment always gave negative results.
Conclusions: This is the first exhaustive investigation of UV irradiation efficacy in the aseptic pharmaceutical production. We demonstrated that UV irradiation plays an essential role in maintaining the sterility condition of the workplace inside the APOTECAchemo and assuring the standards for aseptic manufacturing of medicinal drugs, as required for Class A clean areas. A 4-h UV irradiation also ensures sterility in the case of very resistant microorganisms and in the presence of high microbial charge (10^8 CFU/ml), but a killing rate of 5 or more is already recorded after the first hour of exposition. The results provide useful information for the best operative conditions in terms of both sterility and time optimization, not only for the automated compounding, but also for the traditional aseptic manufacturing processes.

Key words: UV efficiency, challenge test, aseptic drug compounding

Introduction

A substantial progress in genomic medicine has led to advances in oncology with creation of new therapeutic regimens and implementation of personalized care. This has resulted in an improved quality of life, and an increased number of patients able to survive longer to cancer disorders. However, in spite of the undoubted benefits to patients, antineoplastic drugs pose risks because of their intrinsic toxicity and small therapeutic index. In addition, the preparation of anticancer drugs is a complex process and consists of several working steps that may pose further risks for potential harmful medication errors such as incorrect dose, concentration or storage of drugs [1]. To this regard, several surveys reveal medication errors in cancer patients

[2–4] and a study referred that antineoplastic therapy was the second most common cause of fatal medication errors during 1993–98 [5]. Antineoplastic preparation may also show toxicity risks for worker's health [6–9]. Automated systems, realized in recent years, may provide advantages in terms of operator safety, quality of preparation and productivity compared with the manual process. In particular, studies evidenced that robotic antineoplastic compounding environments may improve safety to staff and accuracy of medication preparation compared with standard pharmacy practices [10–12].

Sterility is one of the prerequisite for parenteral preparations. Ready-to-administer cytostatic solutions are prepared starting from sterile materials (drug substance, solvent container and disposables) that are brought together. Since it is not possible to sterilize the product in its final container, all working steps must be performed in aseptic environments to avoid end-product microbial contamination. A common sanitizing agent used in the equipment is ultraviolet (UV) irradiation. Electromagnetic radiation in wavelengths ranging from 240 to 280 nm inactivates microorganisms, being the wavelength of 254 nm the most effective [13]. However, the effectiveness of UV disinfection depends on a number of factors such as power of UV source, exposure time, distance of microorganisms from the source, microorganism resistance, the presence of particulate and shadow zones etc. Microorganisms are variously susceptible to UV irradiation; in general, vegetative bacterial cells are the most sensitive, followed by yeasts and spores [14].

However, to the best of our knowledge, literature data regarding the real UV sterilizing efficacy in aseptic drug compounding are lacking. Therefore, in the present study, we intended to evaluate the efficacy of UV irradiation against different microorganisms inside the aseptic compounding room of a robotic system for intravenous (I.V.) drug admixture. The results will provide useful information for the best operative conditions in terms of both sterility and time optimization, not only for the automated compounding, but also for the traditional aseptic manufacturing processes.

Materials and methods

Compounding robot

APOTECAchemo is a robotic system for the compounding of hazardous drugs, with the aim of reducing the operator exposure and the possibility of human errors. The equipment is already in use in several hospitals worldwide, e.g. Cleveland Clinic (USA), Wake Forest Baptist Medical Center (USA), University Hospital of Mainz (Germany), Capital Region Pharmacy Rigshospitalet (Denmark), Mie University Hospital (Japan) and the University Hospital of Ancona (Italy), to mention a few. The robotic system comprises a compounding area, where a six-axis robot admixtures drugs into a closed and microbiologically controlled area, with a laminar vertical airflow and, above all, without any human interaction (Fig. 1a). The system receives the prescription directly from the integrated Electronic Heath Record and then starts preparing the treatment. The technological solutions of the system allow us to continuously verify the entire process of the preparation. All the production steps and in-coming/out-going materials are checked and registered by means of technological controls such as sensors, photocells, vision system and barcode readers. The automatic identification of drugs, weight checking system and barcode labeling are used to guarantee complete traceability of the process.

APOTECAchemo has six UV lamps distributed in internal chambers. The characteristics of such UV lamps are: peak emission 254 nm; irradiance at 1 m 22 mW/cm^2; radiant flux 2.4 W. The manufacturer suggests to apply UV irradiation at the end of the production activity, with the system fully empty. The system is sealed from the external environment, the irradiation time is set and the UV lamps are turned on. Afterwards, the system automatically shuts down. No further irradiation is expected before a new compounding begins. In the absence of specific studies, the manufacturer suggests a default time of irradiation of 4 h.

Challenge test: UV efficacy

UV efficacy was tested using the microbiological challenge test [15], which was carried out simulating exactly the real operative condition. In fact, due to the high photosentitivity of most of the drugs, this procedure cannot be applied during production, but carried out at the end of the production activity, with the empty system.

The strains used were *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633 spores and *Candida albicans* ATCC 10231.

Bacteria were cultured on Tryptic Soy Agar (TSA) and *C. albicans* on Sabouraud dextrose agar (SDA). Overnight culture of the microorganisms was harvested in sterile 0.85% saline. Densities of inocula were spectrophotometrically read and adjusted to the 0.5 McFarland standard (~1.5×10^8 CFU/ml for bacteria and 5×10^6 CFU/ml for *C. albicans*). The high amount of inoculated microorganisms may provide a valid indicator of UV lamp efficacy even in the case of elevated microbial contamination conditions.

Bacillus subtilis was sporulated [16] and the spore suspension was heated at 70°C for 30 min to inactivate vegetative forms. For assessing the quality of the spores, the suspension was examined with a phase-contrast microscope. The spore concentration was determined by plating serial dilutions in sterile distilled water using pour plates with TSA at 36 ± 1°C for 24 ± 2 h. A titer of 1–5 × 10^8 CFU/ml was adequate to achieve the test inoculum. The spore suspension was stored at 2–5°C until the use [17].

For each microorganism, 10-fold serial dilutions (from 10^{-1} to 10^{-8} for bacteria and from 10^{-1} to 10^{-6} for the yeast) were carried out in sterile 0.85% saline. For all microorganisms, 1 ml of the whole suspension and 0.1 ml of each dilution were uniformly spread on the surface of TSA plates yielding concentrations of 10^7–1 CFU/ml for each bacterium and of 10^5–1 CFU/ml for *Candida*. For bacterial endospores, 10 µl of each dilution was spotted onto a sterile cover slip. The cover slips were allowed to air dry in dark at 37°C and then put on the surface of plates filled with a solid medium in order to avoid shadow zones inside the plate.

Preliminary test

A preliminary challenge test was performed on the UV efficacy in the shadow zones and on the influence of the plate distance from UV source. Among the microorganisms, *S. aureus* (ATCC 6538) had been selected due to its wide distribution and high environmental resistance.

Three main shadow zones inside the equipment were identified: around the internal warehouse, behind the vial stirrer and beneath the scale (Fig. 1b–d). Five plates, with concentrations ranging from 10^8 to 10^4 CFU/ml, were located in the areas mentioned above for different irradiation times until no microbial growth was recorded for every dilution.

The influence of the distance from UV lamp was verified locating nine plates of each concentration (from 10^8 to 10^0) in two different positions inside the compounding room of the system, at 30 and 60 cm from the UV lamps (Fig. 2). The latter represents the farthest

Figure 1 The APOTECAchemo robotic system (**a**). Plate location in the three shadow zones inside APOTECAchemo: (**b**) beneath the scale; (**c**) behind the vial stirrer; (**d**) around the internal warehouse.

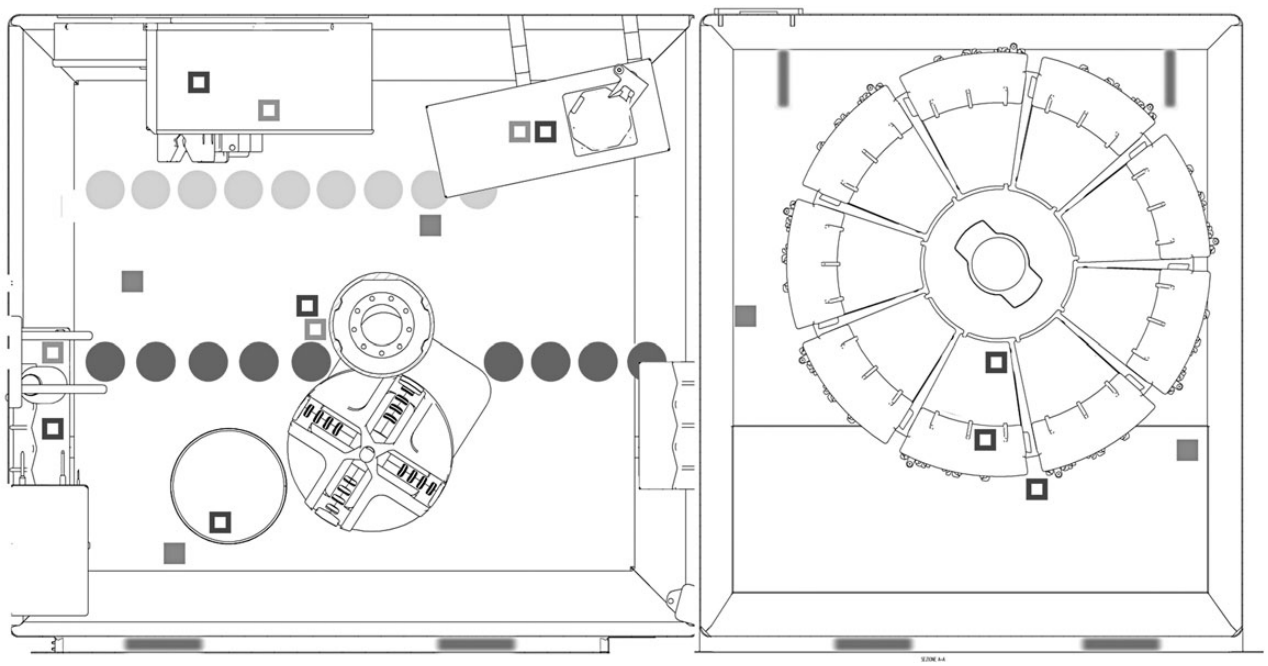

Figure 2 Sampling areas inside the APOTECA chemo. Inoculated plates (light grey dots: at a 60 cm distance from UV lamp; dark grey dots: at a 30 cm distance), sedimentation plates (full squares), swabs (dark grey empty squares), contact plates (light grey empty squares) and UV lamp location (segments).

position from UV source. Different irradiation times were applied until no microbial growth was recorded. The plates were incubated at 36 ± 1°C for 24–48 h, after exposure to UV. Counts referred to as CFU/ml were carried out multiplying the numbers of colonies by the dilution factors.

Effect of UV irradiation on microorganisms

According to the preliminary test results, the plates of the not diluted microbial suspension and every dilution of each microorganism were located into the compounding room positioned at a distance of 60 cm from the UV lamps for irradiation time from 1 to 4 h. Therefore, cultures of non-spore-forming bacteria and *C. albicans* were incubated at 36 ± 1° C for 24–48 h. Bacterial spores were recovered from the cover slips by the method of Lindberg and Horneck [18] and spread on TSA plates and incubated at 36 ± 1°C for 24–48 h. Controls were performed keeping a plate of each microorganism (not diluted sample and the same dilutions as the irradiated ones) in a not-exposed location for the whole irradiation time and then incubated under the same conditions of the UV-irradiated plates. Counts were performed as described earlier.

Microbial viability was expressed as absolute counts; in addition, viable count reduction after exposure to UV light was expressed as the ratio of the viable count after irradiation (N_t) to viable count before irradiation (N_0) and the optimal time of exposure was determined.

Air and surface monitoring inside the equipment

Air sampling was carried out utilizing settle Petri dishes 90 mm diameter located in five different positions inside the compounding area, the warehouse (scale, dosing device, vial, warehouse) and the loading area (Fig. 2). The plates with TSA were kept open during a simulated production cycle for 4 h. After exposure, the plates were incubated at 36°C for 48 h and at 25°C for 7 days for bacteria and *C. albicans*, respectively, and examined for growth every day.

Surface sampling was performed immediately before and after the simulated production cycle utilizing both swabs and contact plates. Moistened swabs were streaked on eight different positions inside the compounding area, the warehouse and the loading area [handle of warehouse gate, carousel (vial storage), carousel (syringe storage), scale, dosing device, vial warehouse, robot grippers, waste gate] (Fig. 2). Each swab was then inoculated onto plates containing TSA and incubated under the same incubation conditions.

Contact plates filled with TSA were pressed on four diverse positions inside the compounding and the warehouse area (scale, vial warehouse, robot grippers, dosing device) (Fig. 2), and then incubated as described above.

The experiments were repeated three times and the data are presented as mean values for each sample.

Statistical analysis

To evaluate the difference between the microorganisms, we used one-way ANOVA model, followed by the Bonferroni *post-hoc* test. Experiment-wise significance level was fixed at 0.05. All the analyses were performed using Stata 12.1 SE (Stata Corporation, College Station, TX, USA).

Results

Challenge test: UV efficacy

Preliminary tests

Preliminarily, in order to assess UV efficacy in correlation to the shadow zones and to the distance from the UV source, several locations inside the APOTECAchemo were identified and investigated with *S. aureus*. In all shadow zones, the microorganism did not retain viability after 3-hour exposure. No colonies were detected on the plates located around the internal warehouse and the vial stirrer, respectively, after 1- and 2-h exposition. The location beneath the scale resulted in the most resistant to UV exposition and needed 3 h for the detection of no colonies; nevertheless, a significant 10^6-fold reduction was recorded after 2-h irradiation.

When the plates were placed at 30 and 60 cm from the UV lamps, no relevant differences in microorganism inactivation were recorded between the two locations.

Effect of UV irradiation on all microorganisms tested

After 4-h UV exposition, none of the tested microorganisms apparently retained viability. In general, as shown in Figs 3 and 4, a dramatic decrease in viable cells was registered within the first hour exposition with a reduction of 10^6-fold for *E. coli* and *B. subtilis* spores and of 10^7-fold for *P. aeruginosa* and *S. aureus* which corresponded to 6-log inactivation for the first three microorganisms and to 7-log for *S. aureus*; a decrease in an order of 10^5-fold corresponding to 5-log inactivation was observed for *C. albicans*. After 2-h exposition, a further reduction in the order of 10-fold was achieved for *P. aeruginosa*, *E. coli* and *C. albicans* and of 10^2-fold for *B. subtilis* spores; in this case, a 8-log inactivation was registered for *P. aeruginosa and B. subtilis* spores and a 6-log inactivation for *E. coli* and *C. albicans*. *Pseudomonas aeruginosa* was the only microorganism showing a count of 1×10^0 CFU/ml after 3-h irradiation, in any case with a very high inactivation rate (8-log). Statistical analysis evidenced significant differences between *C. albicans* and bacteria after both 1- and 2-h exposition ($P < 0.05$).

Air and surface monitoring inside the equipment

Finally, the monitoring of microorganisms inside the equipment evidenced no microbial growth both in air and surface samples after the simulated production cycle.

Discussion

Improving patient safety is a major topic in clinical settings and it may be achieved through various interventions [19–21]. In particular, the quality of the workplace is essential to ensure sterility of pharmacological products, derived from the combination of sterilized components, for which a final sterilization is not possible. The conventional manufacturing of cytostatic solutions prepared by aseptic process needs that all working steps must be executed in the so-called clean areas in order to avoid microbiological contamination. Clean areas are classified according to the characteristics of the environment required for sterile medicinal product manufacturing. In fact, each manufacturing operation requires an appropriate environmental cleanliness level in order to minimize the risks of particulate or microbial contamination of products or materials being handled. The APOTECAchemo system belongs, according to GMP lines, to Class A room [22] for which the recommended limits for microbial contamination during operation are the following: air sample: <1 CFU/m^3; settle plates (diameter 90 mm): <1 CFU/4 h; contact plates (diameter 55 mm): <1 CFU/plate.

Because of its ability to inactivate aerosolized microorganisms, UV irradiation is a widely accepted technique for an effective disinfection of the indoor air and surfaces. Nevertheless, this hypothesis is not assessed by literature data and this is the first exhaustive investigation of

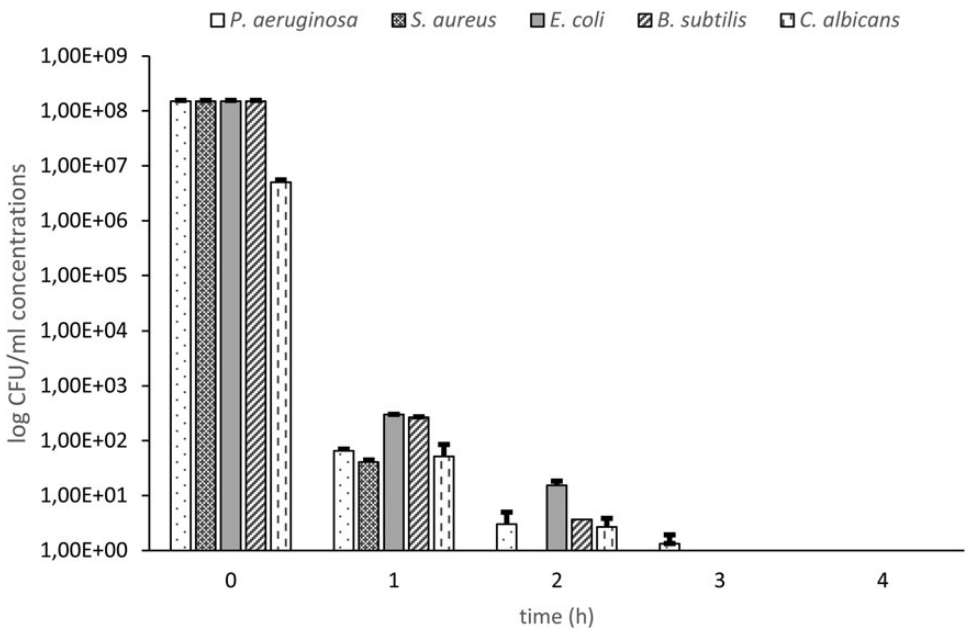

Figure 3 Microbial growth at different UV irradiation times.

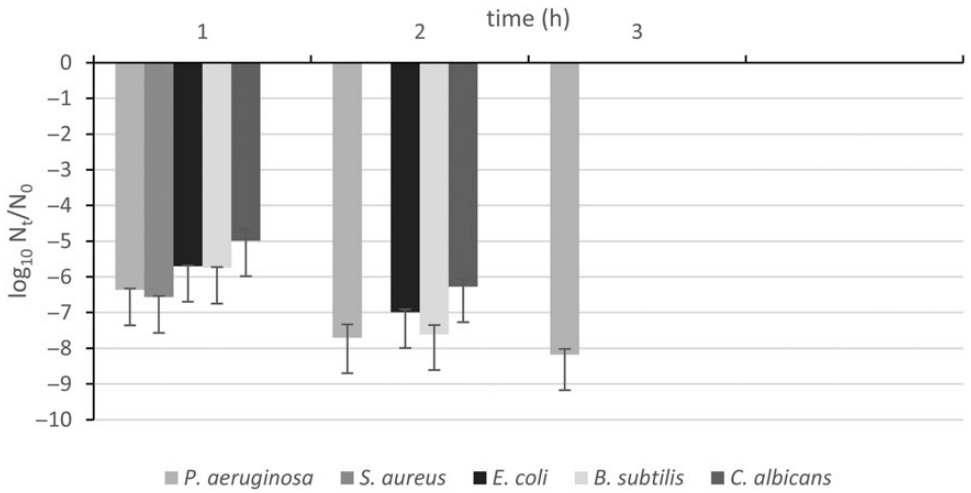

Figure 4 Microbial inactivation rate during 4-h UV irradiation time expresses as the log of the ratio between the viable count after irradiation (N_t) and the viable count before irradiation (N_0).

UV irradiation efficacy in the aseptic pharmaceutical production. We evaluated the effectiveness of UV disinfection inside the aseptic compounding room of APOTECAchemo, against different groups of microorganisms utilizing the microbial challenge test that has been and continue to be a useful tool for the validation of disinfection processes against target organisms.

Initially, results of the preliminary tests demonstrated that UV irradiation was effective also in the shadow zones. This is likely due to the mirror effect of the steel that the equipment is composed of. The absence of no relevant differences between the two examined distances from the UV lamps, despite the intensity of an electromagnetic radiation, is inversely proportional to the square of the distance from the source, indicates that the UV system is well overdimensioned for the size of the internal chambers of APOTECAchemo (around 2 m^3).

Afterwards, to get more information, we extended the study to other microorganisms, keeping into account the different sensitivity of microbial species to UV irradiation [23].

After 4-h irradiation, none of the tested microorganisms apparently retained viability with killing rates ranging from 5- to 7-log for the different microorganisms after the first hour exposition. Interesting are the results concerning *B. subtilis* endospores. Literature data report that *Bacillus* spores are 5–50 times more resistant than growing cells to UV radiation at 254 nm [24]; this is due to the accumulation in their DNA of the photoproduct 5-thymil-5,6-dihydrothymine during dormancy and accurate repair of the photoproduct during spore germination [25]. However, if too high damage to the spore occurs during dormancy, it can overwhelm the repair systems and cause the spore killing [26, 27]. Because treated spores sometimes appear apparently dead when cultured on poor media [28] in our experiment we utilized for vegetative regrowth TSA agar.

Finally, in order to verify the efficacy of the UV irradiation program utilized and to demonstrate the compliance with the microbiological standard required by GMP lines for Class A rooms [22], we monitored the presence of microorganisms inside the equipment. In acknowledgment of the efficacy of the UV irradiation adopted, the results evidenced that no microbial growth took place in any situation.

According to our results, we conclude that a 4-h UV decontamination cycle ensures sterility also in the case of very resistant microorganisms and in the presence of high microbial charge (10^8 CFU/ml).

In any case it is unlikely to find so high microbial charges in the air of chemotherapeutic drug compounding areas also considering that the recommended limit for microbiological contamination in cleanroom classified as Class D is 200 CFU/m^3 for air sample, 100 CFU/4 h (for 90-mm settle plates) and 50 CFU/plate (for 50-mm settle plates). Therefore, taking into consideration the very high killing rate associated with 1-h exposition for all tested microorganisms, we think that a daily irradiation of 1 h may be sufficient to maintain the aseptic condition under ordinary circumstance. A confirmation of our study arises from the negative results of microbiological qualification test (Media Fill) performed by the hospitals equipped with an APOTECAchemo system [29]. Indeed, all the hospitals expose daily the automatic system to UV irradiation and perform periodically the Media Fill test to verify the aseptic processing of the automated production. The negative outcomes of Media Fill test are an evidence of the importance of UV irradiation to maintain the aseptic state under ordinary condition.

In conclusion, we demonstrated that UV irradiation plays an essential role in maintaining the sterility condition of the workplace inside an APOTECAchemo and assuring the standards for aseptic manufacturing of medicinal drugs, as required for Class A clean areas. A 4-h UV irradiation ensures sterility also in the case of very resistant microorganisms and in the presence of high microbial charge (10^8 CFU/ml). The outcomes of this study suggest that, based on the above-mentioned level of contamination expected for the environment, a daily irradiation of 1 h can be considered sufficient to maintain the aseptic condition under ordinary condition. Indeed, the killing rate associated with 1-h exposition was very high (>5) for all tested microorganisms.

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