

**AVALIAÇÃO DA EFICÁCIA DO SANEANTE DIVOSAN EM SEIS TIPOS DE
SUPERFÍCIES CONTAMINADAS COM O FUNGO FILAMENTOSO *Penicillium sp***

**EVALUATION OF THE DISINFECTANT DIVOSAN EFFECTIVENESS ON SIX
TYPES OF SURFACES CONTAMINATED WITH THE FILAMENTOUS FUNGUS
*Penicillium sp***

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ARTIGO CIENTÍFICO
Submetido em: 17.06.2026
Aprovado em: 03.07.2026

RESUMO

As indústrias farmacêuticas, produtoras de imunobiológicos ou drogas parenterais precisam manter um critério de limpeza extremamente rigoroso, principalmente em áreas produtivas classificadas. Diferentes guias descrevem que a sanitização inadequada de superfícies representa um risco significativo para a garantia da esterilidade e para a conformidade regulatória, sinalizando que um procedimento de limpeza e descontaminação robusto é fulcral para as estratégias de controle de contaminação (ECC) serem implementadas com maior assertividade e eficiência. O princípio ativo do saneante, sua concentração e o tempo de contato são critérios que devem ser avaliados e validados nas etapas de implementação desses procedimentos de limpeza. Neste estudo, foi utilizado um saneante esporicida a base de ácido peracético ($\text{CH}_3\text{CO}_3\text{H}$) denominado comercialmente Divosan, em concentrações de 0,5% e 2,0% para avaliação de sua eficácia perante o fungo filamentoso *Penicillium sp.*, com intuito de ampliar as possibilidades de saneantes com atividade esporicida e fungicida. Superfícies de aço inoxidável, papel cirúrgico, plástico filme, superfície vinílica, teflon (politetrafluoretileno ou PTFE) e vidro foram contaminadas com uma suspensão de esporos de, aproximadamente, 10000 unidades formadoras de colônia (UFC), após, imersas nas soluções dos saneantes, posteriormente foram adicionadas em um tubo com solução salina de fosfato (PBS) para neutralização do ácido peracético. Essa solução foi filtrada e a membrana dessa filtração foi acoplada em placas de Ágar Sabouraud Dextrose (SDA) e incubada nas condições de melhor desenvolvimento do microrganismo. As duas concentrações se mostraram eficazes frente ao *Penicillium sp.*, com redução logarítmica superior a 4,0 $\log_{10}$ UFC da carga inoculada inicialmente.

Palavras-Chave: Fungo filamentoso. Esporicida. Indústria farmacêutica. Sanitização.

ABSTRACT

Pharmaceutical industries, producers of immunobiologicals or parenteral drugs, need to maintain extremely rigorous cleaning criteria, especially in classified production areas. Different guidelines describe that inadequate surface sanitization represents a significant risk to ensuring sterility and regulatory compliance, indicating that a robust cleaning and decontamination procedure is crucial for contamination control strategies (CCS) to be implemented with greater assertiveness and efficiency. The active ingredient of the sanitizer, its concentration, and the contact time are criteria that must be evaluated and validated in the implementation stages of these cleaning procedures. In this study, a peracetic acid ($\text{CH}_3\text{CO}_3\text{H}$)-based sporicidal sanitizer commercially known as Divosan was used at concentrations of 0.5% and 2.0% to evaluate its effectiveness against the filamentous fungus *Penicillium sp.*, in order to expand the possibilities of sanitizers with sporicidal and fungicidal activity. Stainless steel, surgical paper, plastic film, vinyl, Teflon (polytetrafluoroethylene or PTFE), and glass surfaces were contaminated with a spore suspension of approximately 10,000 colony-forming units (CFU). After immersion in the sanitizing solutions, the spore was added to a tube containing phosphate-based saline (PBS) to neutralize the peracetic acid. This solution was filtered, and the filtration membrane was attached to Sabouraud Dextrose Agar (SDA) plates and incubated under conditions conducive to optimal microorganism growth. Both concentrations proved effective against *Penicillium sp.*, with a logarithmic reduction greater than 4.0 $\log_{10}$ CFU of the initially inoculated load.

Keywords: Filamentous fungus. Sporicide. Pharmaceutical industry. Sanitization.

1 INTRODUCTION

The Good Manufacturing Practice (GMP) is a set of rules and advises that guide production processes to its efficiency and efficacy, ensuring robustness and repeatability. These adjectives have great importance in any industry, especially in a complex industry, such as an Immunobiological Production Factory. The GMP and different guides, as European Medicine (EMA) Annex 1, describe the importance of cleaning and sanitization as a crucial step in a process production, and it must be taken according to contamination control strategies (CCS). The sanitization process deals with an important issue in pharmaceutical facilities, mainly due to its different surfaces that a controlled area is composed of and the porosity of the surfaces are important factors that can affect the efficacy of the cleaning processes. Current GMP guides highlight the construction material of buildings and also the need for surfaces to be designed, maintained, and sanitized in a way that minimizes contamination risks and facilitates cleaning and sanitization processes.

2 METHODOLOGY

2.1 CHEMICAL AGENTS TESTED

The efficacy test of the sanitizers was applied according to USP <1072>. Aiming to ensure the expected logarithmical reduction of the suspension tested, it was inoculated with a higher concentration than described in the chapter.

The sanitizer tested was peracetic acid, in consequence to its fungicidal and sporicidal activity. Two concentrations were tested, 0.5% and 2.0% of and the exposure time for both was the same, 10 minutes, as described in literature (Costa *et al.*, 2026).

2.2 CARRIERS PREPARATION

In this study, it was aimed to vary the surfaces used in this study, focusing on comprehending as many scenarios as possible, imagining the different surfaces that could be found in construction materials, equipment materials or input materials in production facilities, especially in an Immunobiological industry factory. For this reason, the surfaces selected for this study were stainless steel (SS), surgical paper (SP), plastic film (PF), vinyl (V), Teflon (polytetrafluoroethylene or PTFE), and glass (G).

All six surfaces with approximately 3 cm x 3 cm size were exposed to a sterilizing cycle by autoclaving at 121 °C for 30 minutes and then packaged in sterile containers until the day of the assay.

2.3 PREPARATION AND ENUMERATION OF SPORE SUSPENSION

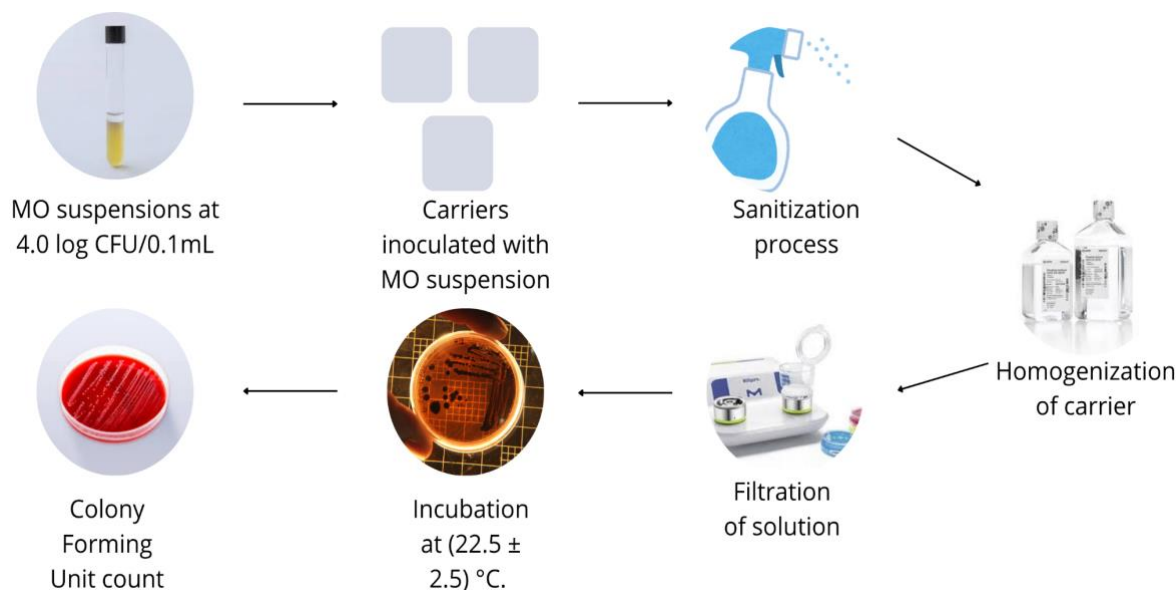
For fungal spore (conidia) suspension preparation, the strain was cultured on SDA and incubated at (22.5 ± 2.5) °C for 6 - 8 days. All fungi grown was suspended in a tube with 10 mL of PBS containing 0.05% (v/v) polysorbate 80 and sterile silica beads. The suspension was intensely homogenized and filtered through a glass funnel containing hydrophobic cotton. The number of conidia was determined using a Neubauer chamber and the stock suspension was stored at (5 ± 3) °C for one day. The methodology was adapted from Costa et al., 2026. On the day of the essay, the suspension was serially diluted in PBS to a final concentration of, approximately, $4 \log_{10}$ CFU/0.1mL in PBS for the efficacy essay and to $2 \log_{10}$ CFU/0.1mL for the residual effect test (RET). From the second dilution, 0.1 mL was spread on SDA and incubated at (22.5 ± 2.5) °C for 5 to 7 days for inoculum size confirmation (ISC).

2.4 EFFICACY ESSAY AND RESIDUAL EFFECT EVALUATION

Three carriers of all six surfaces were placed in Petri dishes and the sanitizers were added until complete covering the carriers. After the exposure time of 10 minutes, all carriers were transferred to other dishes and were inoculated with 0.1 mL of a suspension approximately at $2 \log_{10}$ CFU charge. After, the carriers were homogenized in tubes containing 40 mL of PBS and filtered in a 0.45 µm membrane with a vacuum pump. Later, this membrane was added to a culture medium plate and incubated for 5 to 7 days at (22.5 ± 2.5) °C. The same process was conducted in three carriers of each surface, except the contact time with the sanitizer, these carriers were considered the inoculum control. The sanitizer is considered as not having residual effect whether the viable count of the plates of the process described above was between 50% and 200% of the inoculum control result.

For the efficacy essay, three carriers of each surface were positioned in Petri dishes and inoculated with 0.1 mL of a suspension of approximately $4 \log_{10}$ CFU, they were left until dry, after, the sanitizers were added in all dishes until covering the carriers inside. After the exposure time, the carriers were added in tubes containing 40 mL of PBS and it followed as described in the last paragraph. The sanitizer is considered efficient when the logarithmic reduction of the viable count on the plates that refers to the post exposure carriers is equal or greater than $3 \log_{10}$ CFU.

Figure 1 – Schematic diagram for Efficacy essay.



3 RESULTS AND DISCUSSION

3.1 EFFICACY ESSAY

After the incubation period, the plates were counted to determine the real microorganism quantity of the inoculum, which was calculated using the ISC result, according to the following equation 1.

$$\text{Microorganism inoculated charge (CFU/0.1mL)} = \frac{\text{average of ISC} \times \text{dilution factor to ISC}}{\text{dilution factor to microorganism inoculated charge}}$$

Substituting the valours used in the essay, we have equation 2, below:

$$\text{Microorganism inoculated charge (CFU/0.1mL)} = \frac{25.33 \times 10^{5.78}}{10^3}$$

The result of the equation is 15264 CFU/0.1mL, which means in logarithmic scale, 4.18 $\log_{10}$ CFU/0.1 mL.

The efficacy of the sanitizers was assessed according to two references, the USP <1072> and PDA TR No 70. Table 1 summarizes the reference, its application areas and the recommended reduction, such as the microorganisms cited in the documents.

Table 1 – Summary of reference, application and recommended reduction for each reference

Reference	Application	Recommended Reduction (log ₁₀ CFU)
Parenteral Drug Association – Technical Report No. 70	Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities	> 1.0 (non-spore formers, Mycoplasma, Mold spores, Bacterial spores)
United States Pharmacopeia	Cleaning and sanitization program	≥ 3.0 (for vegetative bacteria) ≥ 2.0 (for bacterial spore suspension)

CFU – Colony forming unit.

Source: Adapted from Costa et al. (2026).

The plates referred to the carriers exposed to the sanitizer (Test plates) were counted and its concentration is described in table 2, in addition to it there are the logarithmic reduction results, using the microorganism inoculated charge described in equation 2. The reduction is calculated according to the equation.

Logarithmic Reduction (log₁₀ CFU) = Microorganism inoculated charge (log₁₀CFU) – Viable average count of Test plates (log₁₀ CFU)

Table 2 – Efficacy assay results

Peracetic Acid Concentration	Surfaces	Average count (log ₁₀ CFU)	Microorganism inoculated charge (log ₁₀ CFU)	Logarithmic Reduction (log ₁₀ CFU)
0.5%	SS	0.0	4.18	4.18
	SP	0.0		4.18
	PF	0.9		3.28
	V	0.0		4.18
	PTFE	0.0		4.18
	G	0.0		4.18
2.0%	SS	0.0	4.18	4.18
	SP	0.0		4.18
	PF	0.85		3.34
	S	0.0		4.18
	PTFE	0.0		4.18
	G	0.0		4.18

CFU- Colony forming unit; SS – stainless steel; SP – surgical paper; PF – plastic film; V – vinyl PTFE – polytetrafluoroethylene; G – glass.

The results described in table 2 meet the recommendations in table 1, showing a greater reduction than the two reference standards analysed in this assay. It is observed that five of the six surfaces tested showed absolute reduction, only the PF (plastic film) did not. This can be explained because of its lower density, which made the carrier float above the sanitizer, impacting the contact time with the chemical ingredient, making him present a lower efficiency compared to other surface carriers.

3.2 RESIDUAL EFFECT TEST (RET)

After the incubation period the plates were counted, the viable count on the plates submitted to the sanitizer before inoculation (RET plates) were compared to the viable count of the inoculum control, all the results are described in table 3. The calculations were followed according to the following equation.

$$\text{Recovery rate (\%)} = \frac{(\text{Average of RET plates}) \times 100}{(\text{Average of IC plates})}$$

Table 3 –Results of residual effect test.

Peracetic Acid Concentration	Surfaces	Average count (log ₁₀ CFU)	Average of IC plates (CFU)	Recovery rate (%)
0.5%	SS	35.67	32.00	111
	SP	40.67		127
	PF	43.00		134
	V	51.67		161
	PTFE	40.33		126
	G	47.33		147
2.0%	SS	32.67	32.00	102
	SP	49.67		155
	PF	45.33		141
	S	50.00		156
	PTFE	48.33		151
	G	33.67		105

IC – inoculum control; CFU- Colony forming unit; SS - stainless steel; SP - surgical paper; PF - plastic film; V - vinyl PTFE – polytetrafluoroethylene; G – glass.

The results described in table 3 corroborate that this sanitizer does not have a residual effect, or inhibitory activity, because all the results are within the range of 50-200% of recovery.

4 FINAL CONSIDERATIONS

An industrial cleaning and disinfection program must be elaborated including many different aspects evaluated in this article, but not only them. It should be planned to work as a tool for preventive actions and corrective actions in pharmaceutical facilities contamination investigations. In the scenario of this study it was aimed not to vary a large number of microorganisms, but to vary the greater the possible surfaces tested, contaminated with a crucial strain which is found to be a great issue in an industrial facility.

The most important reference when validating cleaning and sanitizing processes is the USP <1072> because of its large acceptance and its great diffusion in different countries. As an official compendial document it sets the standard for others, such as the PDA Technical Report No. 70. Even though the latest discusses sanitization processes in a context of clean-rooms bioburden levels, the first has a larger spread among the pharmaceutical industries. In this scenario, this article showed that the sporicidal and fungicidal activities of Divosan in 0.5% and 2.0% concentrations are indeed effective against a highly concentrated conidia suspension burden.

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