Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 241 - 242 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.115
Short communication

Validation of the efficacy of disinfectants used in pharmaceutical cleanrooms

Hristina Sekuloska*, Marina Petreska, Keti Shapkovska, Emilija Radevska

Bionika Pharmaceuticals, Str. Skupi 57, 1000 Skopje, North Macedonia

Introduction

Contamination control in pharmaceutical cleanrooms relies on validated cleaning and disinfection procedures designed to maintain microbiological quality and product safety. Effective disinfection is a critical part of the contamination control strategy, particularly in controlled production areas where microbial contamination must be reduced to an acceptable level.

Disinfectants used in these environments are expected to demonstrate consistent activity against representative microorganisms and on materials that reflect routine cleanroom surfaces. Regulatory guidance, including EU GMP Annex 1 and USP <1072>, emphasises that disinfectant efficacy should be demonstrated under conditions that are relevant to routine use, including defined surface materials, contact times, application procedures and appropriate neutralisation of residual disinfectant activity (European Commission, 2022; United States Pharmacopeia, current edition). Disinfectant validation is an integral part of the Contamination Control Strategy (CCS) as required by EU GMP Annex 1 (2022).

The aim of this study was to evaluate the efficacy of two routinely used disinfectants, Klercide Low Residue Quat and Incidin Pro, on representative cleanroom surfaces in Grade D pharmaceutical production areas.

Materials and methods

Disinfectants and surfaces

The study was performed on representative cleanroom surface materials, including polyvinyl chloride (PVC) flooring and aluminium (ALU) wall panels. Prior to testing, the surfaces were cleaned according to established operational procedures in order to simulate real-use conditions. Klercide Low Residue Quat was tested at 1%, while Incidin Pro was tested at 0.25%. Both disinfectants were evaluated using the routine application approach and a contact time of 15-20 minutes.

Test microorganisms and procedure

The test panel included Staphylococcus aureus ATCC 6538, Candida albicans ATCC 10231, Aspergillus brasiliensis ATCC 16404 and Bacillus subtilis ATCC 6633. These microorganisms were selected to represent vegetative bacteria, yeasts, moulds and spore-forming microorganisms relevant for cleanroom contamination control.

Defined surface areas of 25 cm² were inoculated with 100 µL of microbial suspension and uniformly spread. Each test was performed in duplicate. Following the defined contact time, samples were collected using tryptic soy agar (TSA) contact plates containing suitable neutralisers. Incubation was performed at 20-25 °C followed by 30-35 °C to support the recovery of fungi and bacteria, respectively. Microbial recovery was expressed as

*hristina.sekuloska@bionikapharm.com

colony-forming units (CFU) before and after disinfection.

Acceptance criteria were defined according to USP <1072>, with a minimum 3-log reduction required for bacteria and a minimum 2-log reduction required for fungi and spores (United States Pharmacopeia, current edition).

Results and discussion

Both disinfectants demonstrated high antimicrobial efficacy across all tested microorganisms and evaluated surface types. At lower inoculum levels, approximately 10^2 CFU, no microbial growth was detected after disinfection, with results recorded as 0 CFU/25 cm^2. Although this level of initial contamination does not allow reliable logarithmic reduction calculation, the absence of recoverable microorganisms indicates strong disinfectant performance under low-bioburden conditions typically expected in routine cleanroom environments.

At higher contamination levels, above 10^5 CFU, both disinfectants achieved microbial reductions exceeding the predefined acceptance criteria. The observed reductions corresponded to at least 5-log reduction for bacteria and at least 3-log reduction for fungi and spores. These results demonstrate effective activity even under a worst-case microbial challenge and confirm that both disinfectants maintained efficacy across a broad range of microbial loads.

Comparable performance was observed on PVC flooring and ALU wall panels, suggesting that the efficacy of the tested disinfectants was not significantly affected by the evaluated surface material under the applied conditions. This finding supports their suitability for use on different construction materials present in Grade D cleanroom areas.

The use of TSA contact plates containing neutralisers was important for reliable recovery and enumeration of surviving microorganisms, as it minimised the risk of continued disinfectant activity during incubation.

Overall, the results are consistent with expectations for disinfectant validation studies performed under simulated real-use conditions and support the inclusion of these products within a routine cleaning and disinfection programme (Parenteral Drug Association, 2015). These results confirm robust disinfectant performance under both routine low-bioburden conditions and worst-case contamination scenarios

Conclusion

The study confirmed that Klercide Low Residue Quat and Incidin Pro are effective disinfectants for use in pharmaceutical cleanroom environments. Both products achieved microbial reductions exceeding the acceptance criteria defined in USP <1072>, including activity against representative bacteria, fungi and spore-forming microorganisms. The study design incorporated representative surface materials, relevant microbial challenges, defined contact times and appropriate neutralisation, which reflects the expectations of contemporary contamination control guidance. The consistent efficacy observed across different surface types and microbial loads supports the use of both disinfectants as part of a routine contamination control strategy in Grade D cleanrooms.

References

European Commission, 2022. EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, Annex 1: Manufacture of Sterile Medicinal Products. Brussels: European Commission. Available at: https://health.ec.europa.eu/system/files/2022-08/20220825_gmp-an1_en_0.pdf

Parenteral Drug Association (PDA), 2015. PDA technical report No. 70: Fundamentals of cleaning and disinfection programs for aseptic manufacturing facilities. Bethesda, MD: Parenteral Drug Association.

United States Pharmacopeia, 2024. USP <1072> Disinfectants and antiseptics. Rockville, MD: United States Pharmacopeial Convention.