

MICRONCLEAN
WHITEPAPER

Prepared by
Dr Dave Stokes
Tasmin Crompton

Micronclean Whitepaper

Does your Sterile Cleanroom Disinfectant have a Hidden Problem?

Dr Dave Stokes, Tasmin Crompton

Sterile disinfectants are often gamma irradiated but this can effect their efficacy and have a detrimental effect on the cleanliness of your cleanroom.



As one of the UK's leading providers of sterile cleanroom consumables, Micronclean has developed decades of expertise in cleanrooms, sterilisation, cleaning cleanrooms and cleanroom disinfectants. For many years Micronclean has successfully sold its unique validated rotational disinfectant system Alpha and Beta, to hundreds of cleanrooms around the world. Micronclean is constantly striving for innovation and to *"be the first to introduce new technological solutions that change the shape of the markets we serve"*. To that end, Micronclean's dedicated R&D department has, for several years, been developing new, superior versions of its disinfectant system. During this research and development, within the Micronclean laboratories, a disturbing discovery was made regarding the sterile cleanroom disinfectants on the market. In this whitepaper we describe this dilemma for the cleanroom industry and propose some solutions.

Most cleanroom disinfectants are comprised of one, or two, active biocides and this is used in rotation, in a two-step cleaning process, with another mechanistically orthogonal disinfectant. For best practice, the periodic use of a sporicide can also be part of the rotation, depending on the microbiological and corrosion risks present in the individual cleanroom. For cleanrooms of grade A or B these disinfectants must be sterile.¹ This sterility is almost always achieved via a VD_{max}25 gamma irradiation of the disinfectant product. Aseptic manufacturing of the disinfectant is also an option, however, this is fraught with higher risks, lower sterility assurance and higher validation demands.² The European Pharmacopeia states *"Wherever possible, a process in which the product is sterilised in its final container (terminal sterilisation) is chosen."*³ Aseptically manufactured disinfectants cannot apply a sterility assurance level because accidental contamination, cannot be reliably eliminated. Any manipulation of the sterilised disinfectant containers, prior to, or during, aseptic filling and assembly, poses the risk of microbial contamination. Aseptic processing therefore presents a much higher risk of microbial contamination of the product than terminal gamma sterilisation.

The biocides used in sterile cleanroom disinfectants are drawn from a relatively small number of options (due to various regulatory, commercial and technical reasons)⁴ with ethanol, isopropanol, triamines and quaternary ammonium compounds making up >90% of the non-sporicidal cleanroom disinfectant biocides. Ethanol and isopropanol are often not suitable for the surface disinfection of

cleanrooms due to the evaporative nature of the 70% solutions (and therefore the inherent issues with contact time and workplace exposure limits). This leaves quaternary ammonium and triamine compounds as the most versatile, practical and effective means of disinfecting a cleanroom. Indeed, quaternary ammonium (quat) based disinfectants are the most popular cleanroom biocides for surfaces, as they tend to be less toxic, less corrosive, have broad spectrum efficacy, long shelf lives and convenient and safe contact times, when compared with their alternatives.

In trying to develop new and improved versions of its quaternary ammonium and orthogonal triamine based cleanroom disinfectants (Alpha and Beta), Micronclean's Research and Development department subjected various leading sterile cleanroom disinfectants on the market, to a battery of in-depth tests. This revealed the surprising result that none of those analysed passed the standard EN tests for cleanroom disinfectant efficacy (EN13697, EN1276 and EN1650)⁵⁻⁷ (see Table 1).



Disinfectant	Type	Sterilisation?	EN 13697 Bacteria Surface				EN 1276 Bacteria Suspension				EN 13697 Fungi Surface		EN 1650 Fungi Suspension	
			P.aeruginosa	E.coli	S.aureus	E.hirae	P.aeruginosa	E.coli	S.aureus	E.hirae	A.brasiliensis	C.albicans	A.brasiliensis	C.albicans
Popular Cleanroom Disinfectant A	Quat	Aseptic	FAIL	FAIL	PASS	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS
Popular Cleanroom Disinfectant B	Quat	Gamma	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL
Popular Cleanroom Disinfectant C	Triamine	Gamma	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS
Popular Cleanroom Disinfectant D	Quat	Gamma	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS
Popular Cleanroom Disinfectant E	Quat	Gamma	FAIL	PASS	PASS	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	FAIL
Popular Cleanroom Disinfectant F	Triamine	Gamma	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS

Table 1: EN disinfectant test results for various popular cleanroom disinfectants on the market.

This worrying discovery led to further research and investigations into the possible reasons why this could be, after all these popular cleanroom disinfectants were all claiming PASS efficacy with these EN standards.

A breakthrough in this investigation came when High Performance Liquid Chromatography (HPLC) analysis was performed on the levels of biocidal active contained within these popular cleanroom disinfectants (see Table 2).

Disinfectant	Type	Sterilisation?	HPLC Analysis					
			DDAC% Claimed	DDAC% Found	ADBAC% Claimed	ADBAC% Found	Triamine% Claimed	Triamine% Found
Popular Cleanroom Disinfectant A	Quat	Aseptic	0.04	0.04				
Popular Cleanroom Disinfectant B	Quat	Gamma	0.30	ND				
Popular Cleanroom Disinfectant C	Triamine	Gamma					1.4	0.01
Popular Cleanroom Disinfectant D	Quat	Gamma	0.12	0.01				
Popular Cleanroom Disinfectant E	Quat	Gamma	0.05	0.02	0.05	ND		
Popular Cleanroom Disinfectant F	Triamine	Gamma					0.15	0.04

Table 2: HPLC analysis of the biocidal actives in popular cleanroom disinfectants.

HPLC analysis clearly showed that most of the biocidal actives were not at the levels claimed by the manufacturer, and some were not present in detectable levels at all. This was an alarming finding with wide ranging consequences for the cleanroom industry. We had to discover the root cause. The first clue came in the form of an aseptically manufactured disinfectant. This, despite also failing the EN testing, did have the claimed quantity of active present, whereas the gamma irradiated disinfectants did not. Could it be that gamma irradiation was responsible?

At approximately this time, Micronclean had also run into difficulties in our attempts to achieve significant performance improvements with our new disinfectant formulations. We wondered if this too was due to gamma irradiation? Therefore, solutions of the common quaternary ammonium biocides (DDAC and ADBAC) as well as the triamine (N-3-Aminopropyl-N-dodecylpropane-1,3-diamine) were subjected to gamma irradiation (25-40kGy) and analysed by HPLC (see Figure 1).

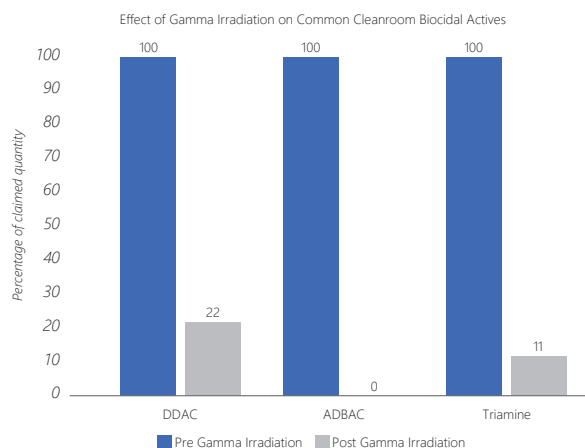


Figure 1: Bar chart showing the effect of gamma sterilisation on biocidal actives.

Figure 1 shows a significant drop in biocidal active concentration between pre and post gamma sterilisation. For the two Quat's (DDAC and ADBAC) the DDAC is reduced to a fifth of its initial concentration whereas the ADBAC is entirely destroyed. This is particularly concerning, as the ADBAC has good activity for the fungal organisms,

especially *Aspergillus* spp. The triamine is also largely destroyed by the gamma irradiation process. Interestingly, whilst the reduced DDAC concentration remains largely constant over time, at approx. 20%, after gamma irradiation, the triamine concentration continues to drop over time, suggesting subtly different mechanisms may be at play.

The conclusion which we were forced to draw is that gamma irradiation is destroying large proportions of the actives in popular cleanroom disinfectants. It is probable that the industry only tests EN efficacy pre-irradiation, and is thus unaware that these disinfectants do not have the same level of performance post-irradiation.

Having identified this problem for the industry, Micronclean set out to study the problem in more detail, and ultimately devise a solution for our new disinfectant development.

Through extensive experimentation, the scientists at Micronclean believe they have determined the mechanism by which the destruction of the actives is occurring (see Figure 2).

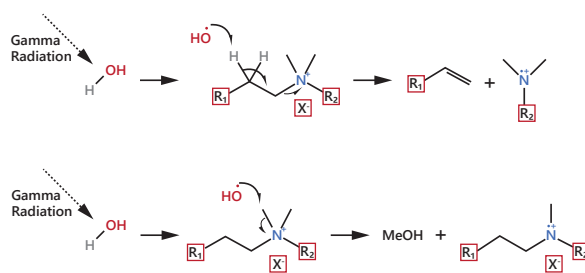


Figure 2: Mechanistic proposal for the destruction of quaternary ammonium actives by gamma irradiation; top = beta hydrogen abstraction
bottom = nucleophilic substitution at an alpha carbon

Gamma irradiation will primarily affect the hydrogen-oxygen bond of water creating hydroxyl radicals. These hydroxyl radicals can attack the quaternary ammonium biocides, either by beta-hydrogen abstraction or by nucleophilic substitution at the alpha-carbon. In both cases the resulting degradation product will have

considerably less/ no antimicrobial activity. This type of radical cascade degradation has been well studied and the mechanism for the degradation of the triamine is likely very similar.⁸

Not only does this gamma induced destruction of the quats and triamines, reduce the active levels, and therefore the disinfectant efficacy, it also produces numerous degradation by-products altering the residue profile of the cleanroom disinfectant. Micronclean scientists have found that the disinfectant residues after gamma irradiation are much more solid and require significantly more mechanical action to remove, compared to pre irradiation.

Micronclean has just launched two new sterile rotational disinfectants, specifically designed for cleanrooms, and which do pass the EN efficacy tests post-gamma irradiation. These disinfectants, Alpha Plus and Beta Plus, can withstand the effects of gamma sterilisation via two patent pending solutions to the problem, unique to Micronclean, and which are the result of several years of R&D development.⁹

Figure 3 shows the effect of gamma sterilisation on the biocidal active levels of Alpha Plus and Beta Plus.

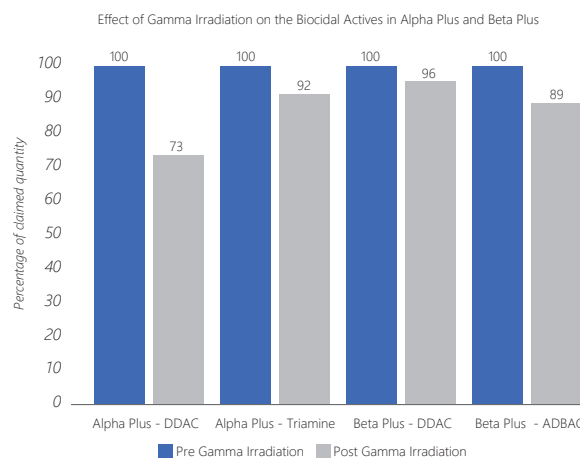


Figure 3: Bar chart showing the effect of gamma sterilisation on the biocidal actives in Alpha Plus and Beta Plus.

As you can see from Figure 3 most of the actives are still present in these cleanroom disinfectants compared to those currently on the market (for example, Beta Plus retains 96% of the DDAC active, whereas most other popular cleanroom disinfectants retain less than 25%, post-gamma sterilisation). This means that Alpha Plus and Beta Plus have sufficient levels of active remaining after gamma sterilisation to pass all the required EN tests (EN13697, EN1276 and EN1650) as well as before gamma irradiation. (see Table 3).

Disinfectant	Type	Sterilisation?	EN 13697 Bacteria Surface				EN 1276 Bacteria Suspension				EN 13697 Fungi Surface		EN 1650 Fungi Suspension	
			P.aeruginosa	E.coli	S.aureus	E.hirae	P.aeruginosa	E.coli	S.aureus	E.hirae	A.brasiliensis	Calbicans	A.brasiliensis	Calbicans
Alpha Plus	Triamine	Gamma	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
Beta Plus	Quat	Gamma	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
Popular Cleanroom Disinfectant A	Quat	Aseptic	FAIL	FAIL	PASS	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS
Popular Cleanroom Disinfectant B	Quat	Gamma	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL
Popular Cleanroom Disinfectant C	Triamine	Gamma	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS
Popular Cleanroom Disinfectant D	Quat	Gamma	PASS	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS
Popular Cleanroom Disinfectant E	Quat	Gamma	FAIL	PASS	PASS	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	FAIL
Popular Cleanroom Disinfectant F	Triamine	Gamma	PASS	PASS	FAIL	PASS	PASS	PASS	PASS	PASS	FAIL	FAIL	FAIL	PASS

Table 3: EN disinfectant test results for Micronclean's Alpha Plus and Beta Plus compared to various popular cleanroom disinfectants on the market.

Unlike other sterile cleanroom disinfectants, Alpha Plus and Beta Plus have been carefully formulated to protect them from the destructive effects of gamma irradiation, allowing the products to both carry the VD_{max}25 surety of gamma sterilisation (versus less robust methods like aseptic manufacture which carries a greater risk of contamination of the customers cleanroom, a higher cost of production, a much higher burden of quality assurance/control and a greater onus on the customer to audit/ assess aseptic manufacturing systems and competence) and ensure that they have the required efficacy to be effective sterile disinfectants in a cleanroom. The positive practical and safety aspects of the quaternary ammonium, and triamine, biocides are retained (versus alcohols or sporicides), by adding substances to the disinfectant which specifically protect the actives from the destructive effects of the hydroxyl radicals, stopping them before they can damage the biocidal actives. The patent pending formulation, even has proprietary technology which results in improved anti-microbial efficacy after gamma sterilisation. This takes advantage of a microscopic physical effect which has been carefully tailored, turning a potential problem for the cleanroom industry into a positive advantage.

Are your cleanroom rotational disinfectants working as well as you want them to? Are the EN test results pre or post gamma sterilisation? Have your sterile disinfectants been specially designed for cleanroom use? Have they been specifically formulated to work after gamma sterilisation? Through this whitepaper we have tried to shine a light on this problem for the cleanroom industry and Micronclean believes Alpha Plus and Beta Plus are the only disinfectants on the market which address this problem, and pass the EN efficacy tests after gamma sterilisation.

Micronclean's patent pending new cleanroom rotational disinfectants Alpha Plus and Beta Plus are now available to supplement your contamination control strategy, in trigger, presaturated wipe, 5L, or presaturated mop, formats.

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Roman Bank / Skegness / Lincolnshire / PE25 1SQ
T: +44 (0)1754 767 377
E: sales@micronclean.co.uk
W: www.micronclean.com

