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Application Note

Review of the Neutralization Efficacy of ICR Contact Plates against ANIOS disinfectants

This review summarizes the results of studies into the neutralization of the four disinfectants *Surfanios Premium*, *Aniosurf ND Premium, ANIOXY-SPORE TWIN* and *ANIOSPRAY QUICK*, all manufactured by *Laboratoires Anios*[1] Inactivation of the selected active ingredients was evaluated using TSA based ICR portfolio plates containing different neutralizers.

Introduction

The FDA Aseptic Guide (2004) states the reason for adding neutralizers to culture media, explaining that "inactivating agents should be used to prevent inhibition of growth by cleanroom disinfectants or product residuals (e.g. antibiotics)". ISO 14698-1 instructs: "Appropriate additives shall be included to overcome, or minimize, the effects when residual antimicrobial activity at the sampling point is expected", and the new EN 17141 standard stipulates in its part Culture Media and Incubation the "use of neutralizers in media used for surface sampling to ensure that disinfectant residues on surfaces do not suppress the growth of the microorganisms sampled".[2,3,4]

Antimicrobial activities can be effectuated by disinfectant or sanitizer residues on surfaces, vaporized hydrogen peroxide (VHP) residues after decontamination procedures and antibiotics in the production environment.

The activity of dried sanitizers can be reactivated by the humidity in contact plates. To overcome the antimicrobial properties and facilitate the growth of microorganisms, culture media are supplemented with appropriate neutralizers and enzymes.

The concentration of ingredients like alcohol or hydrogen peroxide tends to drop significantly due to evaporation or to chemical disproportionation that leaves only non-toxic residues behind, whereas active substances such as quaternary ammonium compounds, aldehydes or biguanides can be deposited as stable residues on surfaces after desiccation, where they can be reactivated.

Recommended neutralizers for several active ingredients are listed below.[5]

Table 1. Suitable neutralizers for different disinfectants

Product	Product Description
Disinfectant	Suitable Neutralizer
Alcohol (e.g. IPA, ethanol) (Volatile)	Tween® 80 or dilution
Aldehydes	Sodium hydrogen sulfite, sodium thiosulfate, glycine, histidine
Sodium hypochlorite	Sodium thiosulfate
Biguanides (e.g. chlorhexidine) (polyhexamethylene biguanides not included)	Lecithin
Quaternary ammonium compounds (QAC)	Tween® 80
Phenolics	Tween® 80, lecithin
Peracetic acid	Buffer (e.g. phosphate buffer)
Hydrogen peroxide (VHP) (nontoxic degradation products)	Pyruvate, catalase
Antibiotics, e.g. beta-lactam antibiotics	Enzymes, e.g. beta-lactamases

For more information see also USP: <61> and <1227>; EP: 2.6.12, and ISO 18593

Materials and Methods

We evaluated the efficacy of three contact plate media with a worst case "Direct Plating Test" whereby the disinfectants are spread onto the agar surface of the contact plates directly. This was done with volumes of 25 µL, which is well-suitable for 55 mm plates

The treated plates and control plates (without disinfectants) were inoculated with the test strains after a standardized 20 ± 5 minutes of exposure time.

ICR Contact Plates

The neutralizing efficacy of Neutralizer A Contact–ICR+, Tryptic Soy Contact Agar (TSA) +LT –ICR+ and TSA w LTHThio cont.-ICR+ were tested **(Table 2)**.

Table 2. Contact plates tested in this study

Article No.	Product	Product Description
146697	Neutralizer A Contact – ICR+	Lockable contact plate for total viable count with Neutralizer A (mixture
146552	Tryptic Soy Contact Agar +LT – ICR+	Lockable contact plate for total viable count with lecithin and Tween® * 80 (LT)
146783	TSA w LTHThio cont.-ICR+ **	Lockable contact plate for total viable count with lecithin, Tween® *, histidine and thiosulfate

*Tween®[6] = polysorbate

**TSA w LTHThio cont.-ICR+ (146783) only tested with ANIOSPRAY QUICK

Test Strains

The strains and incubation conditions used for determining the recovery rate are listed in **Table 3**.

Table 3. List of test strains (ATCC®[7]) and incubation conditions

Test Strain	Incubation temperature (°C)	Incubation time (days)
Bacillus subtilis **(spores) (ATCC® 6633)**	30-35	≤ 3
Pseudomonas aeruginosa **(ATCC® 9027)**		
Staphylococcus aureus **(ATCC® 6538)**		
Staphylococcus epidermidis **(ATCC® 14990)***		
Aspergillus brasiliensis **(ATCC® 16404)**	20-25	≤ 5
Candida albicans **(ATCC® 10231)**		

*S. epidermidis not tested with TSA w LTHThio cont.-ICR+ (146783)

Disinfectants

The following disinfectants **(Table 4)** to assess the neutralization efficiency of test plates were used.

Table 4. List of Laboratoires Anios disinfectants tested for this report

Disinfectant	Indication	Active Ingredients
Surfanios Premium IP sterile PAE*	Cleaning and disinfection of floors, walls, medical equipment and non-invasive medical devices.	5% N-(3-Aminopropy) -N-Dodecylpropane-1,3-Diamine, 2. 5% Didecyldimethylammonium chloride, Propan-2-ol
Aniosurf ND Premium IP sterile PAE*	Cleaning and disinfection of floors, walls and medical equipment	2.5 - 10% Didecyldimethylammonium chloride, 2.5 - 10% Propan-2-ol, up to 2.5% D-gluconic acid, compound with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecane diamidine(2:1), up to 2.5% N-C12-14-Alkyltrimethylenedi-Amines
ANIOXY-SPORE TWIN IP Sterile Concentre*	Disinfection of clean or previously cleaned equipment	2.5 - 10% Hydrogen peroxide, 2.5 - 10% Acetic acid, up to 2.5% Peracetic Acid
ANIOSPRAY QUICK	Swift disinfection of previously cleaned, non-immersible medical devices resistant to alcohol (stethoscopes, cables and connectors, pressure sensors, blood sugar testers).	Ethanol, up to 2.5% N,N-DIDECYL-N-METHYL-POLY(OXYETHYL) AMMONIUM-PROPIONATE

*CONCENTRATED DISINFECTANT-DETERGENT PRODUCTS (dilution according to manufacturer's instruction)

Preparation of Test Strains

- Strains were recovered weekly from stock cultures on Columbia Blood Agar (bacteria) or Sabouraud Dextrose Agar (yeasts and molds), except for *Bacillus subtilis* which was used as a spore suspension.
- Sub-cultures were prepared as overnight cultures before testing.
- Dilutions were prepared in NaCl Peptone Buffer to achieve 10 to 100 CFU in the final inoculum

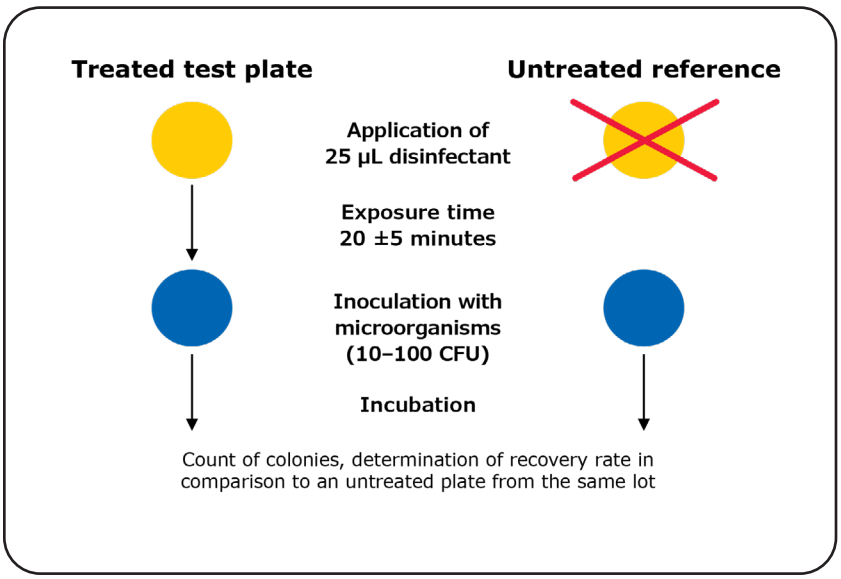
Surface-independent "Direct Plating Test"

- For testing the inactivation efficacy via the "Direct Plating Test", the disinfectant was spread on the agar surface using a Drigalski spatula (glass, 146 x 45mm).
- After an exposure time of 20 ± 5 minutes, the test plates were inoculated with 10 to 100 CFU of the strain, also using a Drigalski spatula.
- Control plates from the same lot were inoculated and incubated in parallel, but without adding disinfectant.
- The plates were incubated as indicated in **table 3**.
- Each experiment was repeated **five** times. The neutralization of disinfectants for the test plates was defined as sufficient if the recovery on test plates with 25 µL of disinfectant was 50–200% of what the corresponding control plates (without disinfectant) achieved.

A volume of 25 µL per 55 mm contact plate corresponds to 10 mL disinfectant per m^2. The simplified workflow is shown in **figure 1**.

ISO 11930 stipulates the interpretation of the results and the assessment of neutralizer efficacy to be evaluated as follows: "the inherent variability in enumeration on agar plates shall be taken into account. Two counts are usually considered different only if their difference exceeds 50%"[8]

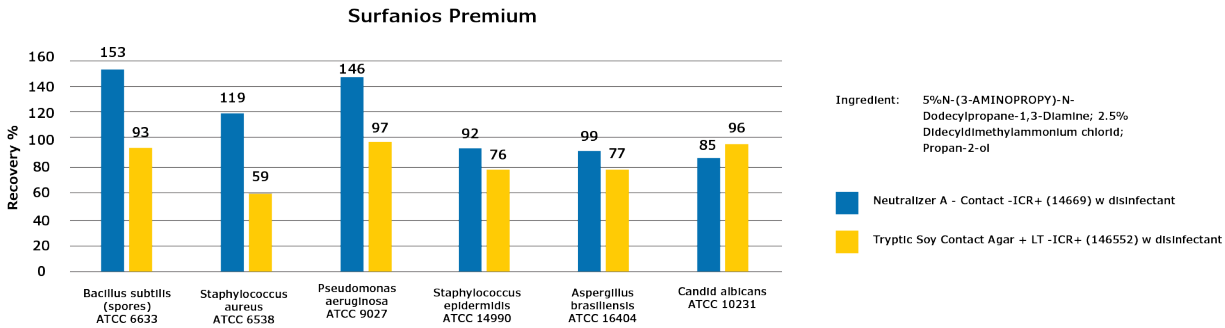
Figure 1: Workflow of the surface independent "Direct Plating Test"



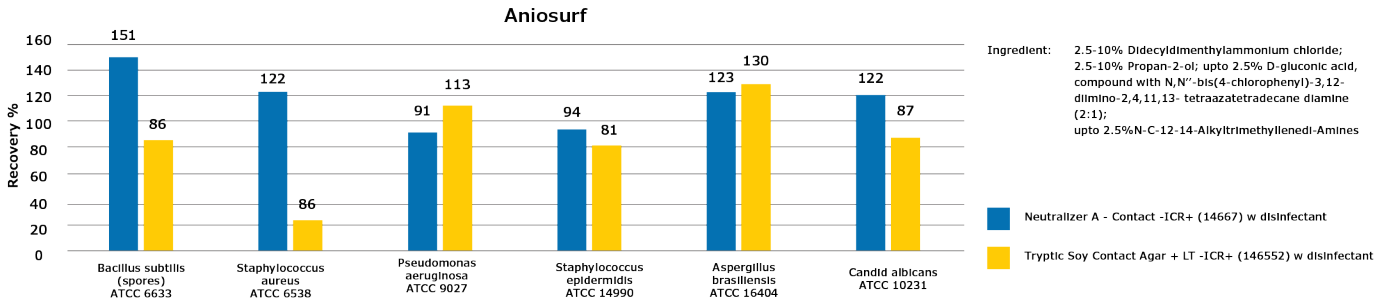
Results

Average recovery rates of the tests are shown in **Figures 2 to 5**. The recovery rates are given as percentages of the results of untreated plates from the same lot.

Neutralization of Surfanios Premium IP sterile PAE

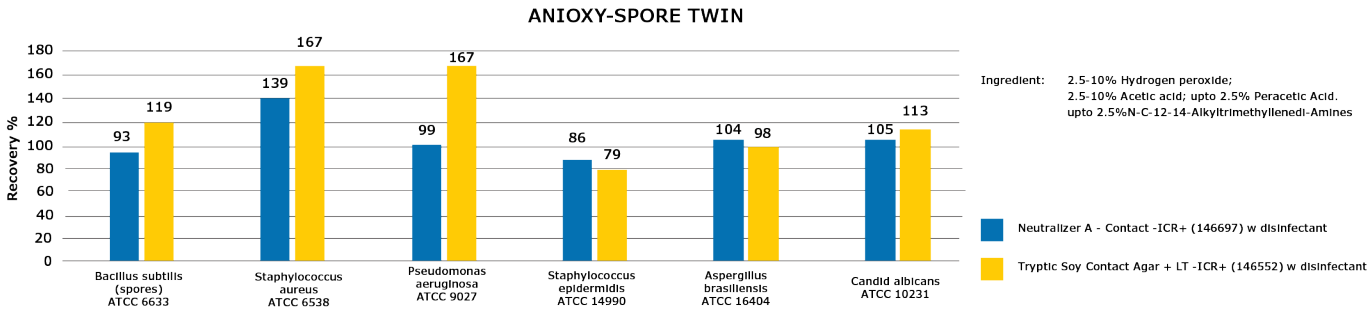


Neutralization of Aniosurf ND Premium IP sterile PAE



Neutralization of Anioxy Spore-Twin IP sterile concentrate

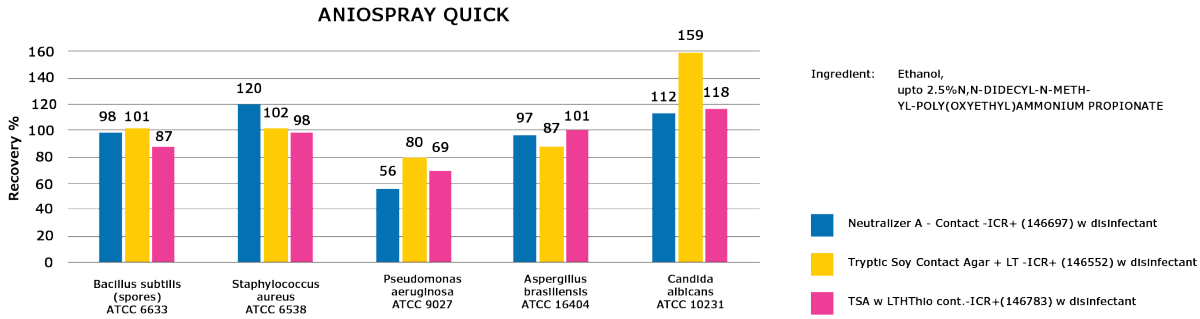
Figure 4. Recovery of test strains on Neutralizer A – ICR+ plates (146697) and Tryptic Soy Contact Agar +LT – ICR+ (146552) in the presence of 25 µL Anioxy Spore-Twin IP Sterile Concentrate



The test results show that all tested microorganism strains could be recovered in the presence of 25 µL Anioxy Spore-Twin IP sterile concentrate, indicating the efficient neutralization of active residues of this disinfectant by the contained neutralizers **(Figure 4)**.

Neutralization of ANIOSPRAY QUICK

Figure 5. Recovery of test strains on Neutralizer A Contact – ICR+ plates (146697), TSA +LT – ICR+ (146552) and TSA w LTHThio cont.-ICR+ (146783) in the presence of 25 µL ANIOSPRAY QUICK



The results in **Figure 5** show that all tested microorganism strains could be recovered in the presence of 25 µL ANIOSPRAY QUICK, indicating the effective neutralization of active residues

Conclusions and Discussions

This study summarizes the results of the neutralization efficiency of three TSA media including either lecithin and Tween® 80 or lecithin, Tween®80, histidine and thiosulfate, or the Neutralizer A mixture as inactivating agents. The neutralization efficiency was defined as sufficient when the counts reached at least 50% of those on control plates without disinfectants. The “Direct Plating Test” method was used for all plates.

The four tested disinfectants Surfanios Premium IP sterile PAE, Aniosurf ND Premium IP sterile PAE, Anioxy Spore-Twin IP and ANIOSPRAY QUICK can all be sufficiently neutralized by Neutralizer A Contact – ICR+, whereas TSA +LT – Contact – ICR+ plates are suitable to inactivate only ANIOXY SPORE TWIN, Surfanios Premium and ANIOSPRAY QUICK at the recommended working dilutions, but not Aniosurf ND Premium at the tested concentration (*S.aureus* recovery rate below 50%). Similarly, the earlier publication of Hedderich and Klees had revealed lower detection rates for gram-positive strains like *Staphylococcus aureus* on such media when using combinations of chlorine-containing compounds with quaternary ammonium compounds.[9]

In addition, TSA w LTHThio contact.-ICR+ (146783) plates were used for tests with ANIOSSPRAY QUICK. These were shown to inactivate the active ingredients of the disinfectant.

Literature

1. Laboratoires Anios ; Pave du moulin; 59260 Lille-Hellemmes France
   Laboratoires Anios (11.05.2015) Safety Data Sheet (Regulation (EC) n° 1907/2006 – Reach) Surfanios Premium IP Sterile Concentre – 2590000 Version 6.1
   Laboratoires Anios (12.03.2015) Safety Data Sheet (Regulation (EC) n° 1907/2006 – Reach) Aniosurf ND PremiuM – 2436000 Version 4.2
   Laboratoires Anios (26.05.2015) Safety Data Sheet (Regulation (EC) n° 1907/2006 – Reach) Anioxy-Spore Twin IP Sterile Concentre – 2343000 Version 2.1
   Laboratoires Anios, Version 2.2 (27-12-2016) Safety Data Sheet ((EC) n° 1907/2006 - REACH); Aniospray Quick NPC
2. FDA Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice; United States Pharmacopoeia 40 NF 35: <1116> Microbiological Control and Monitoring of Aseptic Processing Environments
3. ISO 14698-1(2003): Cleanrooms and associated controlled environments - Biocontamination control - Part 1: General principles and methods
4. EN 17141 (2020) European standard Cleanrooms and associated controlled environments - Biocontamination control
5. For more information regarding neutralizers for several active ingredients see also USP: <61> and <1227>; EP: 2.6.12, and ISO 18593
6. Tween is a registered trademark of Croda International PLC, UK
7. ATCC is a registered trademark of American Type Culture Collection (ATCC); 10801 University Boulevard Manassas, VA 20110 USA
8. ISO 11930 (2019): Cosmetics -- Microbiology -- Evaluation of the antimicrobial protection of a cosmetic product
9. Hedderich R. and Klees A.-G. (2012): Neutralization of Disinfectants by Culture Media Used in Environmental Monitoring; Environmental Monitoring – A Comprehensive Handbook, Volume 6; 159–180

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11/2020