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Application Note

Inactivation of Disinfectants using TSA based ICR Contact Plates with different Neutralizers

Abstract

This study summarizes the test results for three different disinfectants manufactured by Laboratoires Anios[1]. The neutralization efficiency of TSA contact plates with lecithin and Tween® 80 (LT)[2] or the Neutralizer A plate from the ICR portfolio range were tested in a "Direct Plating Test".

Introduction

For environmental monitoring of surfaces or personnel in cleanrooms[3], RABS and isolators, non-selective culture media with good growth promoting properties for a wide range of microorganisms are used.

The reasons for adding neutralizers to culture media are clearly explained by USP <1116>[4] and the FDA Aseptic Guide (2004)[5] which states: "Where appropriate, inactivating agents should be used to prevent inhibition of growth by cleanroom disinfectants or product residuals (e.g. antibiotics)", as well as in ISO 14698-1 where the following advice is given: "Appropriate additives shall be included to overcome, or minimize, the effects when residual antimicrobial activity at the sampling point is expected."

Antimicrobial activity may be represented by:

- Disinfectant or sanitizer residues on surfaces
- VHP residues after decontamination procedures
- Antibiotics in the production environment

To overcome antimicrobial activities and avoid false negative results, culture media are developed with appropriate neutralizers or enzymes to facilitate growth of microorganisms.

The ability of culture media to let these microorganisms grow depends on the activity of sanitizer residues on the surface. The sanitizer residues might be reactivated during the surface sampling process by humidification through the contact plates.

The load of residues, which is present on a dry surface after disinfection or sanitization, varies with the type of active agent. Ingredients like alcohol or hydrogen peroxide are significantly reduced due to evaporation or by chemical disproportionation to form non-toxic residues, whereas active substances such as quaternary ammonium compounds, aldehydes or biguanides leave stable residues after desiccation on the surface and may be reactivated.

Recommended neutralizers for several active ingredients are listed below (**Table 1**).

Table 1. Suitable neutralizer for different disinfectants

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.

In this study we evaluate the efficiency of two contact plates with a worst case “Direct Plating Test”. Thereby the disinfectants are spread onto the agar surface of the contact plates directly (25 µL are suitable for 55 mm plates).

After a standardized exposure time (15–20 min.) the treated plates and the control plates (without disinfectants) are inoculated with test strains. Test plates and control plates are from the same batch.

With regards to previously performed tests with quaternary ammonium compounds and polyhexamethylene biguanides, it could be concluded that 25 µL of a disinfectant per plate in the “Direct Plating Test” will give comparable results to the practical oriented test method (data not shown).

Material

Culture Media

In this study, the neutralizing efficiency of Neutralizer A Contact–ICR+ and Tryptic Soy Contact Agar (TSA) +LT – ICR+ were tested (**Table 2**).

Table 2. Tested contact plates for this study

Product	Article No.	Product Description
Neutralizer A Contact – ICR+	146697	Lockable contact plates for total viable count with Neutralizer A (mixture)[6]
Tryptic Soy Contact Agar +LT – ICR+	146552	Lockable contact plates for total viable count with lecithin and Tween® 80 (LT)[7]

Test Strains

Strains and incubation conditions used for examination of the recovery rate are listed in **Table 3**.

Table 3. List of tests strains (ATCC®)[8] and incubation conditions

Test Strain	Incubation Temperature [°C]	Incubation Time [days]
Bacillus subtilis (ATCC® 6633) (WDCM 00003)	30–35	≤ 3
Pseudomonas aeruginosa (ATCC® 9027) (WDCM 00026)		
Staphylococcus aureus (ATCC® 6538) (WDCM 00193)		
Staphylococcus epidermidis (ATCC® 14990) (WDCM 00132)		
Candida albicans (ATCC® 10231) (WDCM 00054)	20–25	≤ 5
Aspergillus brasiliensis (ATCC® 16404) (WDCM 00053)		

Disinfectants

The following disinfectants (**Table 4**) for examining neutralization efficiency of test plates were used

Table 4. List of disinfectants tested for this report

Disinfectant	Supplier	Active Ingredients
Surfanios Premium IP sterile PAE[9]	Anios	• N-(3-Aminopropy)-N-Dodecylpropane-1,3-Diamine • Didecyldimethylammonium chloride • Propan-2-ol
Aniosurf ND Premium IP sterile PAE[10]	Anios	• Didecyldimethylammonium chloride • Propan-2-ol, • D-gluconic acid, compound with N,N''-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecane diamidine(2:1) Amines, N-C12-14-Alkyltrimethylenedi-
Anioxy Spore-Twin IP Sterile Concentre[11]	Anios	• Hydrogen peroxide • Acetic acid • Peracetic Acid

Method

Preparation of test strains

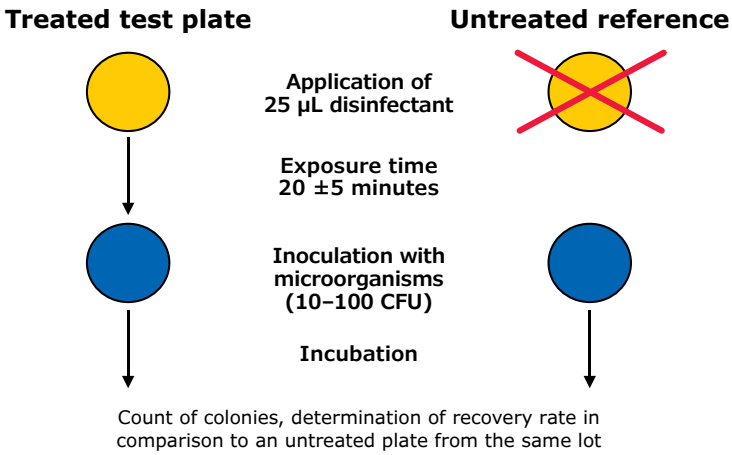
- Strains are recovered weekly from stock cultures on Columbia Blood Agar (bacteria) or Sabouraud Dextrose Agar (yeasts and molds)
- Sub-cultures are prepared as over-night cultures before testing.
- Dilutions are prepared in NaCl Peptone Buffer to achieve 10–100 CFU in the final inoculum.

Surface independent "Direct Plating Test"

- For testing inactivation efficiency via "Direct Plating Test", the disinfectant is spread on the agar surface using a Drigalski Spatula (glass, 146 x 45 mm).
- After an exposure time of 20 ±5 minutes, the test plates are inoculated with 10–100 CFU of the recommended strain also using a Drigalski Spatula.
- Control plates from the same lot are inoculated and incubated in parallel, but without disinfectant.
- The plates are incubated as indicated in **Table 2**.
- Each experimental condition is repeated five times.
- The neutralization of disinfectants for the test plates is defined as sufficient if the recovery on test plates with 25 µL of disinfectant is 50–200%, compared to the control plates (without disinfectant).

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

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

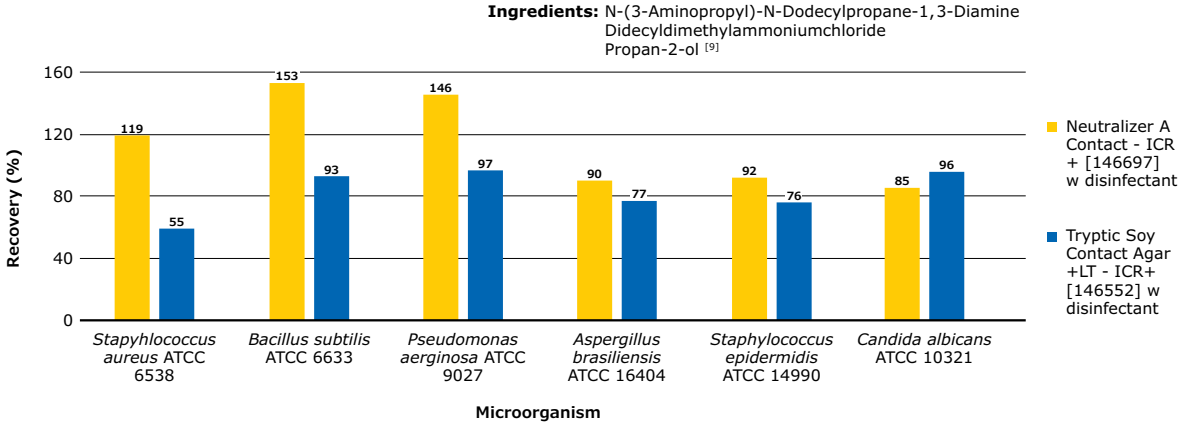


Results

The capacity of the used contact plates to neutralize the active ingredients of several disinfectants is tested by “Direct Plating Test”.

Neutralization of Surfanios Premium IP sterile PAE

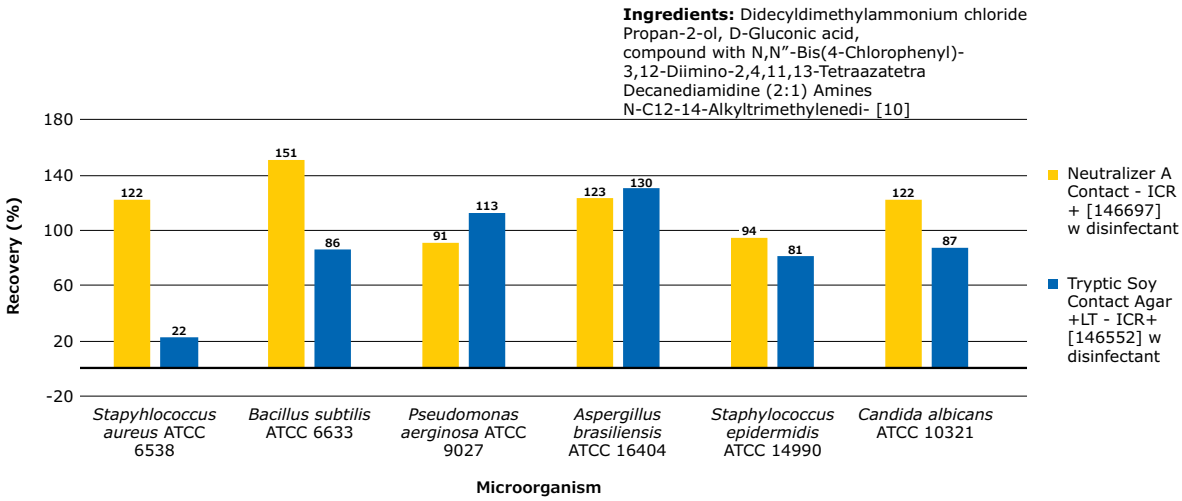
Figure 2. Recovery of test strains on Neutralizer A Contact – ICR+ plates (146697) and Tryptic Soy Contact Agar +LT – ICR+ (146552) in presence of 25 µL Surfanios Premium IP sterile PAE



Test results show that all tested microorganisms can be recovered in the presence of 25 µL Surfanios Premium IP sterile PAE (**Figure 2**), indicating the efficient neutralization of active residues of this disinfectant by included neutralizers.

Neutralization of Aniosurf ND Premium IP sterile PAE

Figure 3. Recovery of test strains on Neutralizer A Contact – ICR+ plates (146697) and Tryptic Soy Contact Agar +LT – ICR+ (146552) in presence of 25 µL Aniosurf ND Premium IP sterile PAE

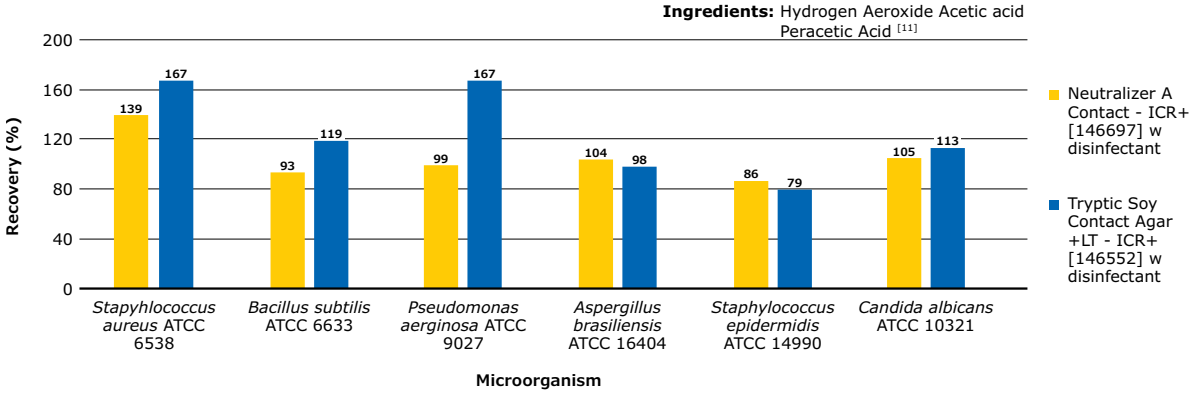


Test results show that all tested microorganism can be recovered in the presence of 25 µL Aniosurf ND Premium IP sterile PAE (**Figure 3**).

The recovery rates for *Staphylococcus aureus* are weak on TSA with LT, whereas the neutralizer A mixture shows excellent inactivation properties. The test results confirm, that for high concentrated quaternary ammonium compounds combined with chlorhexidine digluconate the standard neutralizers lectithin and Tween® 80 are not sufficient. For those compounds there is a need for additional neutralizers represented in the Neutralizer A mixture, as previously reported by Hedderich and Klees[12].

Neutralization of Anioxy Spore-Twin IP sterile concentre

Figure 4. Recovery of test strains on Neutralizer A – ICR+ plates (146697) and Tryptic Soy Contact Agar +LT – ICR+ (146552) in presence of 25 µL Anioxy Spore-Twin IP Sterile Concentre (dilution according to manufacturer's instruction)



Test results show that all tested microorganism can be recovered in the presence of 25 µL Anioxy Spore-Twin IP sterile Concentre indicating the efficient neutralization of active residues of this disinfectant by the included neutralizers (**Figure 4**).

Discussion and Conclusion

This study summarizes the results of the neutralization efficiency tests with TSA contact plates including either lecithin and Tween® 80, or the Neutralizer A mixture as inactivating agents. The microorganisms' recovery rate, defined as ≥ 50% in "Direct Plating Test", the neutralization efficiency is sufficient for inactivating the disinfectant.

The three tested disinfectants (Surfanios Premium IP sterile PAE, Aniosurf ND Premium IP sterile PAE, Anioxy Spore-Twin IP sterile concentre) can be sufficiently neutralized by Neutralizer A Contact – ICR+, whereas TSA + LT – Contact – ICR+ plates are suitable to inactivate the recommended working dilutions of Anioxy spore Twin and Surfanios, but is not efficient to inactivate Aniosurf ND in the tested concentration.

References and further reading information

1. Laboratoires Anios
   Pavé du moulin
   59260 Lille-Hellemmes
   France

2. Tween is a registered trademark of Croda International PLC, UK

3. Cleanrooms and associated controlled environments –
   Biocontamination control – Part 1:
   General principles and methods (ISO 14698-1:2003)

4. United States Pharmacopoeia 40 NF 35
   <1116> Microbiological Control and Monitoring of Aseptic Processing Environments

5. FDA Guidance for Industry (2004):
   Sterile Drug Products Produced by Aseptic Processing –
   Current Good Manufacturing Practice.

6. Neutralizer A Contact – ICR+
   Merck KGaA, Darmstadt, Germany, 2016
   Technical Data Sheet
   Neutralizer A – Contact – ICRplus
   Ordering number: 1.46697.0020 / 1.46697.0200

7. Tryptic Soy Contact Agar + LT ICR+
   Merck KGaA, Darmstadt, Germany, 2016
   Technical Data Sheet
   Tryptic Soy Contact Agar + LT – ICRplus
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8. ATCC® is a registered trademark of American Type Culture Collection (ATCC)
   10801 University Boulevard
   Manassas, VA 20110 USA

9. LABORATOIRES ANIOS, 11.05.2015
   SAFETY DATA SHEET (REGULATION (EC) n° 1907/2006 – REACH)
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   Version 6.1

10. LABORATOIRES ANIOS, 12.03.2015
    SAFETY DATA SHEET (REGULATION (EC) n° 1907/2006 – REACH)
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11. LABORATOIRES ANIOS, 26.05.2015
    SAFETY DATA SHEET (REGULATION (EC) n° 1907/2006 – REACH)
    ANIOXY-SPORE TWIN IP STERILE CONCENTRE – 2343000
    Version 2.1

12. Hedderich R. and Klees A.-G. (2012):
    Neutralization of Disinfectants by Culture Media Used in Environmental Monitoring;
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