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Application Note

Neutralization Efficacy of ICR Contact Plates against Phenolics and Quaternary Ammonium Compounds

Abstract

The disinfectant was applied to the agar, absorbed, and inoculated with microorganisms. An untreated reference plate without disinfectant was also inoculated and incubated in parallel.

Introduction

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

The reasons for adding neutralizers to culture media are clearly explained in USP <1116> and the FDA Aseptic Guide (2004) 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 ISO 14698-1 which points out: "Appropriate additives shall be included to overcome, or minimize, the effects when residual antimicrobial activity at the sampling point is expected." [3,4,5]

Antimicrobial activity may be represented by:

- Disinfectant or sanitizer residues on surfaces
- Vaporized hydrogen peroxide (VHP) residues after decontamination procedures
- Antibiotics in the production environment

Culture media are developed with appropriate neutralizers or enzymes to overcome antimicrobial properties and facilitate growth of microorganisms. The ability of culture media to let these microorganisms grow depends on the activity of sanitizer residues which might be reactivated during the surface sampling process by humidification through the contact plates.

The residue load, which is present on a dry surface after disinfection or sanitization, varies with the type of active agent. Active substances such as quaternary ammonium compounds and phenolics leave stable residues after desiccation on the surface and may be reactivated by the moisture of agar during sampling.

The recommended neutralizers against phenolics and quaternary ammonium compounds are Tween® 80 and lecithin. This study shows the neutralizer efficacy of three different formulations of ICR contact plates, which include both neutralizers in their mixtures.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Materials & Methods

We evaluated the efficacy of three contact plates with a worst case "Direct Plating Test". Thereby, the disinfectants were spread onto the agar surface of the contact plates directly (25 µL is suitable for 55 mm plates).

The treated and control plates (without disinfectants) were inoculated with test strains after a standardized 15-20 minute 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 1**).

Table 1: Contact plates tested in this study

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

* Tween® = polysorbate

Test Strains

Strains and incubation conditions used for examination of the recovery rate are listed in **table 2.**

Table 2: List of tests strains (ATCC®) 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)		
Candida albicans (ATCC® 10231)	20-25	≤ 5
Aspergillus brasiliensis (ATCC® 16404)		

Disinfectants

The following disinfectants (**table 3)** for examining neutralization efficacy of test plates were used.

Table 3: List of disinfectants tested for this report

Disinfectant	Supplier	Active Ingredients
ANIOSPRAY QUICK	Laboratoires Anios	50 to 100% Ethanol up to 2.5% QAC (Quaternary ammonium propionate)
Vesphene® IIIst*	STERIS Corporation	10-15% o-Benzyl-p-chlorophenol; 5-10% Potassium hydroxide; 5-10% 2-Phenylphenol; 1-10% Sulfonic acids, 1-5% Phosphoric acid; 1-5% sodium xylene sulfonate
LpH® IIIst**	STERIS Corporation	10-30% o-Benzyl-p-chlorophenol; 10-15% Isopropyl alcohol, 10-30% phosphoric acid; 5-10% Sodium 1-octanesulfonate; 5-10% 2-Phenylphenol; 1-5% Sodium xylene sulfonate; 1-5% Benzenesulfonic acid

*pH high 12.46; **pH low 0.25 (1% w/w dilution pH = 2.08)

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 10–100 CFU in the final inoculum.

Surface independent "Direct Plating Test"

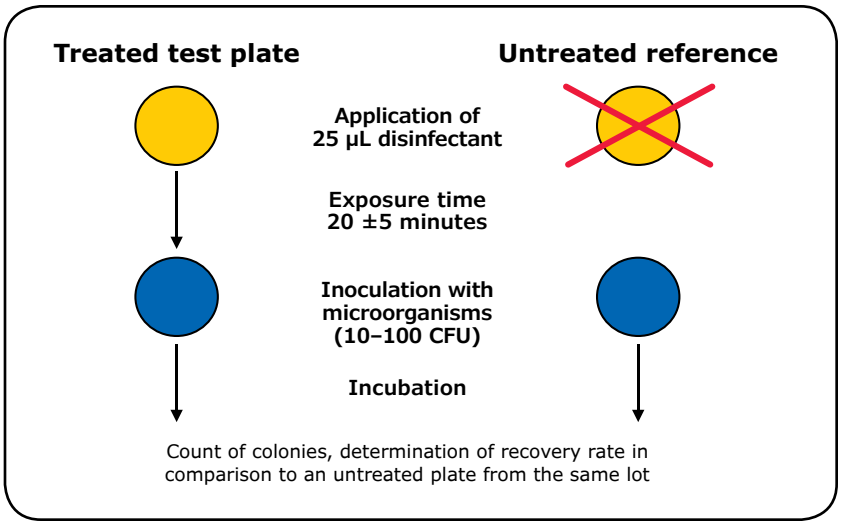
- For testing inactivation efficacy via "Direct Plating Test", the disinfectant was spread on the agar surface using a Drygalski Spatula (glass, 146 x 45 mm).
- After an exposure time of 20 ±5 minutes, the test plates were inoculated with 10–100 CFU of the recommended strain also using a Drygalski Spatula.
- Control plates from the same lot were inoculated and incubated in parallel, but without disinfectant.
- The plates were incubated as indicated in **table 2**.
- Each experimental condition was repeated five times and in two independent test trials

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%, compared to the control plates (without disinfectant).

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 below.

ISO 11930 describes the interpretation of results and conclusion on neutralizer efficacy 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%..."

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



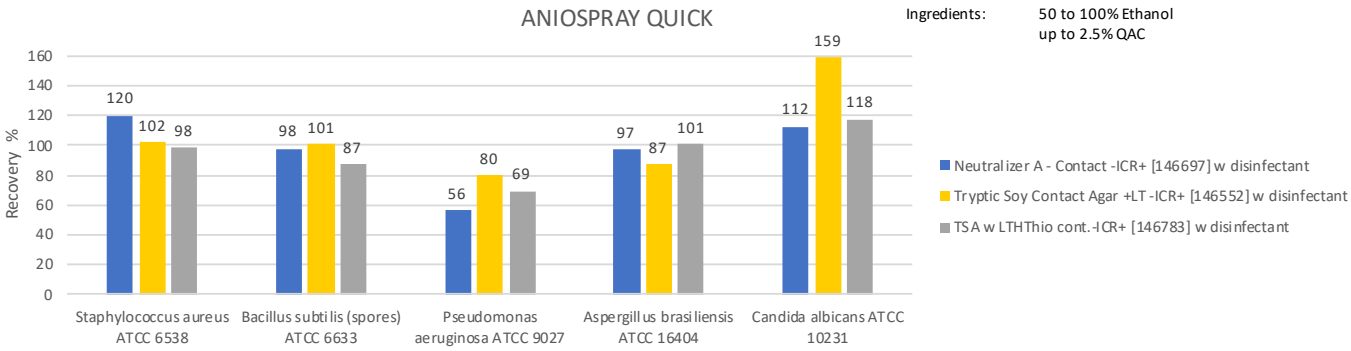
Results

Average recovery rates of the 2 independent test trials are shown in figures 3-5. The recovery rates were calculated in comparison to an untreated plate from the same lot.

Neutralization of ANIOSPRAY QUICK

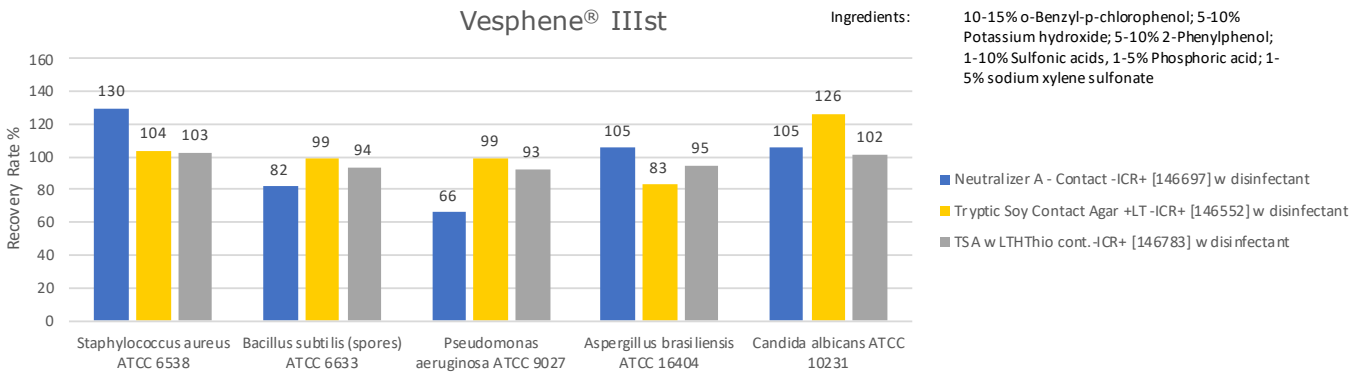
The results in **figure 3** demonstrate that all tested microorganisms could be recovered in the presence of 25 µL ANIOSPRAY QUICK indicating effective neutralization of active residues.

Figure 3: Recovery of test strains on Neutralizer A Contact – ICR+ plates (146697), TSA +LT – ICR+ (146552) and TSA w LTHThio cont.-ICR+ (146783) in presence of 25 µL ANIOSPRAY QUICK



Neutralization of Vesphene® III st Phenolic Disinfectant

Figure 4: Recovery of test strains on Neutralizer A Contact – ICR+ plates (146697), TSA +LT – ICR+ (146552) and TSA w LTHThio cont.-ICR+ (146783) in presence of 25 µL Vesphene® IIIst

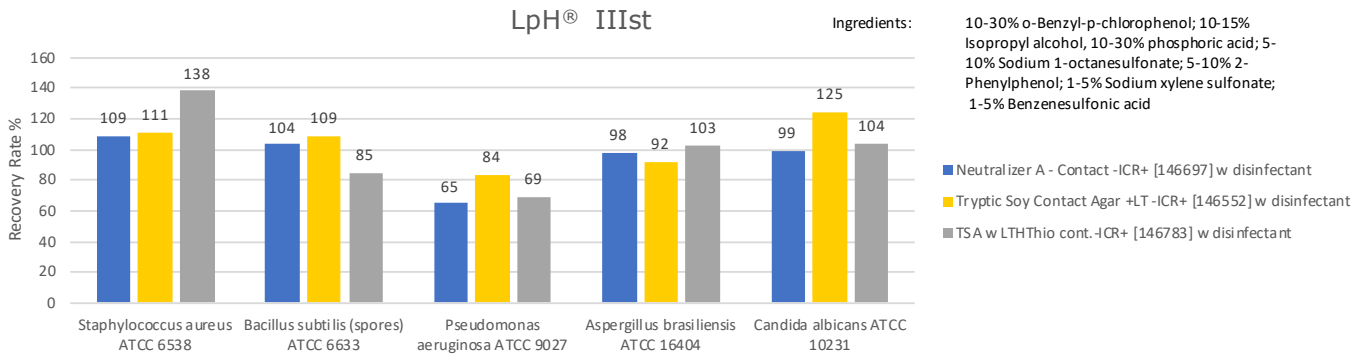


In the presence of Vesphene® IIIst all microorganisms can be detected with recovery rates over 50%.

Neutralization of LpH® III st Phenolic Disinfectant

LpH III was successfully neutralized by all three contact plate formulations.

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 presence of 25 µL LpH® IIIst



Conclusion and Discussion

Two disinfectants containing phenol (Vesphene III and LpH III) in addition to one disinfectant containing a quaternary ammonium (Anios Spray Quick) were tested for neutralization efficacy with three different contact agar plates. The average recovery rate demonstrated the efficacy of neutralization when TSA contact plates including either lecithin and Tween® (Cat. no. 146552), Neutralizer A mixture (146697) or with lecithin, Tween®, histidine and thiosulfate (146783) as inactivating agents were used.

In the "Direct Plating Test", the recovery rates for each microorganism were over 50%. The neutralization efficacy of the three contact agar plates (Neutralizer A Contact – ICR+ (146697), Tryptic Soy Contact Agar +LT – ICR+ (146552) and TSA w LTHThio cont.-ICR+ (146783)) was therefore effective at inactivating all 3 disinfectants.

Literature

(1) Laboratoires Anios; Pavé du moulin; 59260 Lille-Hellemmes France

Laboratoires Anios, Version 2.2 (27-12-2016) SAFETY DATA SHEET ((EC) n° 1907/2006 - REACH); ANIOSPRAY QUICK NPC

(2) STERIS Corporation; 7501 Page Avenue; St. Louis, MO 63133 USA

STERIS Corporation, 08/28/2018 Version 1.0; SAFETY DATA SHEET (Acc. to Federal Register/ Vol. 77 No. 58/ Monday, March 26, 2012/ Rules and Regulations); Vesephene® IIIst Phenolic Disinfectant

STERIS Corporation, 07/12/2018 Version 1.0; SAFETY DATA SHEET (Acc. to Federal Register/ Vol. 77 No. 58/ Monday, March 26, 2012/ Rules and Regulations); LpH® IIIst Phenolic Disinfectant

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

(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) ISO 11930 (2019): Cosmetics -- Microbiology -- Evaluation of the antimicrobial protection of a cosmetic product

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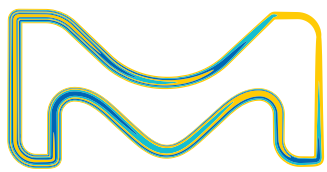

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