Neutralizing Efficiency of Lecithin and Polysorbate 80 in ICR Contact Plates and Swabs for Surface Monitoring

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Introduction

Surface monitoring in cleanroom environments requires non-selective culture media which allow the detection of a broad range of microorganisms. At the same time these media should be supplemented with appropriate neutralizers to overcome or minimize antimicrobial activity at the sampling point as recommended in the ISO 14698.

In surface monitoring most often sanitizer or disinfectant residues may be a reason for false-negative results or limited detection of microorganisms.

There are several guidelines, such as pharmacopoeia (USP <61>) and ISO (ISO 11930 and ISO 18593), which list appropriate neutralizers towards various active ingredients of sanitizers or disinfectants (see table 1).

Active Ingredient	Recommended Neutralizer
Alcohol (such as IPA, ethanol)	polysorbate 80 or dilution
Aldehydes	sodium hydrogen sulfite, sodium thiosulfate, glycine, histidine
Sodium hypochlorite	sodium thiosulfate
Biguanides (such as chlorhexidine)	lecithin
Quaternary Ammonium Compounds (QAC)	polysorbate 80, lecithin
Phenolics	polysorbate 80, lecithin
Peracetic acid	buffer (e.g. phosphate buffer)
Hydrogen peroxide & VHP (non toxic degradation products)	pyruvate, catalase
Antibiotics, e.g. beta-lactam antibiotics	enzymes, e.g. beta-lactamases

Table 1 – Examples of antimicrobial agents and recommended neutralizers

The required neutralizer type as well as its concentration might be dependent on the amount of residues present on the sampling surface. The amount of antimicrobial residue by itself depends on the nature of disinfectant as well as on the workflow for disinfection. It can be assumed, that on a dried sanitized surface very low amounts of residues are present in case of oxidizing disinfectants, such as sodium hypochlorite or hydrogen peroxide as well as the volatile alcohols. Other active ingredients like aldehydes, biguanides, quaternary ammonium compounds as well as phenolics are expected to be more stable and are still present on the sanitized and dried surface.
To check the suitability of typical mixtures of neutralizers such as LT (lecithin and polysorbate 80) several commercially available disinfectants have been chosen for worst case neutralization efficiency tests.
The neutralization efficiencies are tested for the ICR Swab (ref 146529) and contact plates TSA Contact + LT – ICR+ (ref. 146552).

The following disinfectants/sanitizers are chosen as representatives for a broad spectrum of active ingredients:

Disinfectant Name (supplier)	Active ingredients according to supplier information	Used Abbreviation in this Poster
Actril® (Medivators Inc.)	1% hydrogen peroxide 5.2% acetic acid	H_2O_2
Aerodesin® 2000 (Lysoform Dr. Hans Rosemann GmbH)	30 to 35% Propan-1-ol 18 to 20% Ethanol 0.1% Glutaraldehyde	Aldehyde
Aniospray Quick (Laboratoires Anios)	50 to 100% Ethanol up to 2.5% QAC	QAC high
Cutasept® F (Bode Chemie GmbH)	50 to 70% Propan-2-ol 0.0025 to 0.025% QAC	QAC low
Klercide™ Sporicidal Active Chlorine (Ecolab Ltd)	0.5 to 1% Hypochlorite	Hypochlorite
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	Phenolic LpH
Vesphene® IIIst (Steris Corporation)	10-15% o-Benzyl-p-chlorophenol; 5-10% 2-Phenylphenol; 1-10% sulfonic acids, 1-5% phosphoric acid; 10% potassium hydroxide, 1-5% xylenesulfonate	Phenolic HpH

Table 2 – List of disinfectants for neutralization efficiency tests

Methods

Practical oriented Method used for ICR Swab Tests:
A stainless steel test surface of 5 x 5 cm is immersed in the disinfectant. After the surface has been allowed to dry for 3 hours the surface is swabbed. Subsequently, the growth medium (Soybean-Casein Digest Broth = SCDB with lecithin and polysorbate 80) is added to the swab which is inoculated with less than 30 CFU of the test strains. The swabs immersed in the broth medium are incubated and afterwards checked for turbidity. In case of Aniospray Quick, the stainless steel coupon was in addition also disinfected via a one-spray shot on the surface in addition to the above described immersion.

As a pre-test for determining the requirement of neutralizers the same test is performed with *S. aureus* ATCC® 6538)using Soybean-Casein Digest Broth (SCDB) without neutralizers instead of TSB + LT from the Swab reservoir.

Direct Plating Method used for Contact Plate testing:
25 µl of the disinfectant are spread on the agar surface. After 15-25 min, when disinfectant is absorbed into agar, a growth promotion test with selected microorganisms is performed. As 100 % control a contact plate from the same batch without disinfectant is used. This method is applied to contact plates only.

Results and Discussion

According to the growth results summarized in figure 1 it can be concluded that oxidative disinfectants do not leave notable amounts of growth inhibiting residues on a surface after drying. *S. aureus* can grow in SCDB without any neutralizers when swabbing dried surfaces which are sanitized with hypochlorite, hydrogen peroxide or phenolics at low pH. Residues of aldehydes, quaternary ammonium compounds and phenolics at high pH values in the tested concentrations show growth inhibition of *S. aureus* at the standard incubation time. Even prolongation of the incubation up to 7 days does not improve the recovery rates.

All 6 test microorganisms were efficiently detected with the ICR Swab after swabbing a disinfected and dried surface using a broad range of disinfectants. There was one exception for the detection of low numbers of *S. aureus* after swabbing a surface which was previously immersed in a disinfectant with high concentration of quaternary ammonium compounds. This lowered detection rate of 4 positive out of 6 total samples could be increased to 6 (=100%) by applying the disinfectant via spraying instead of immersion of the surface into the disinfectant (data indicated in figure 2 – QAC high sprayed). This test results indicate that our chosen immersion test method is also a worst case method, because the supplier of disinfectant recommends to spray the disinfectant to the surface and furthermore to remove antimicrobial residues by additional wiping. We therefore conclude the neutralization efficiency of the ICR Swab is sufficient for all tested disinfectants when they are used in compliance with the supplier recommendation.

The neutralization efficiency of solid agar contact plates (TSA contact + LT –ICR+) also shows recovery rates of more than 70% for all tested disinfectants and microorganisms as shown in figure 3. From previous neutralization studies (not shown here) we concluded, that the chosen test method using an amount of 25 µl disinfectant per plate is comparable to the practical oriented test using the immersion method. Therefore we are confident, that the formulation of the chosen formulation of casein soybean digest agar supplemented with lecithin and polysorbate 80 shows excellent neutralization efficiency against disinfectants with active ingredients such as aldehydes, quaternary ammonium compounds, phenolics as well as hydrogen peroxide or hypochlorite in the tested concentration.

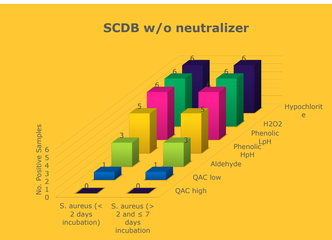

Figure 1 – Growth of *S. aureus* in alternative SCDB without neutralizers after swabbing a dry, sanitized surface using the ICR Swab (Number of positive results out of 6 samples)

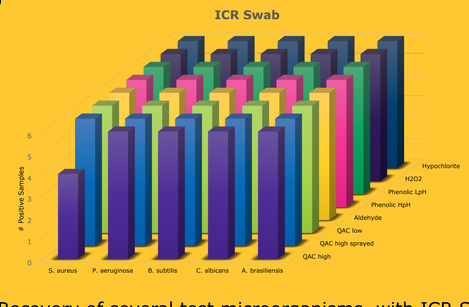

Figure 2 – Recovery of several test microorganisms with ICR Swab after swabbing disinfected surfaces (number of positive samples out of 6 samples tested)

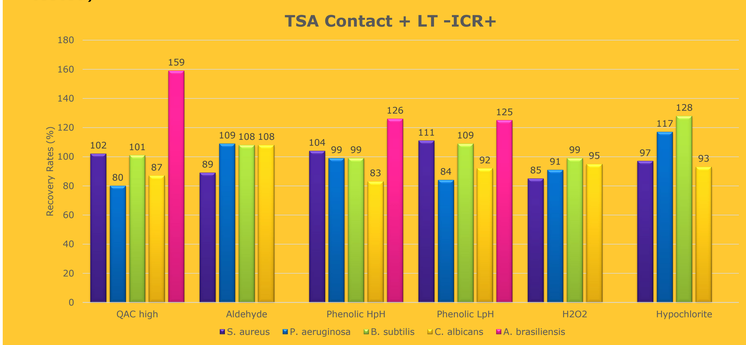

Figure 3 –Recovery Rates (%) on Contact plates TSA+LT contact – ICR+ of test microorganisms in the presence of 25 µl disinfectants (*A. brasiliensis* ATCC® 16404 was not tested for Aldehydes, H_2O_2 and Hypochlorite, as these test results are collected from separated test trials)

Summary

Overall it can be concluded, that all tested disinfectants can be efficiently neutralized with the SCDB medium of ICR Swabs as well with the TSA medium of ICR contact plates both supplemented with the neutralizers lecithin and polysorbate 80 (LT).

For disinfectants with high concentration of quaternary ammonium compounds, the cleaning and disinfection workflow should result a minimization of sanitizer residues. Procedures, recommended by the supplier of disinfectant, such as final wiping with sterile water of the sanitized surface should be followed to minimize the risk of chemical residues on the surface which facilitates the neutralization as well as the safety of manufacturing drug products.