EVALUATION OF THE BACTERICIDAL ACTIVITY OF THE DISINFECTANTS SepteFX 7D-11 AND SepteFX 6D-840 WHEN APPLIED TO THE MANNEQUIN USED IN CPR TRAINING

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A. Determination of the Ability of SepteFX 7D-11 and SepteFX 6D-840 to Disinfect the Synthetic Surfaces of the Mannequin used in CPR Training

Method Outline:

The method used was based on the Association of Official Analytical Chemists (AOAC) guidelines for the Efficacy Testing of Germicidal Sprays. From a 48 hour culture of *Staphylococcusaureus* (a common skin and nasal pathogen) containing a 5% bioburden (fetal calf serum - FCS), 10 µL was placed on the surface of 25cm squares of the synthetic mannequin material. This was done in ten replicates. The bacterial culture/bioload mixture was allowed to dry onto the square at 37^{o}C for 30 minutes.

SepteFX 7D-11 (Lot 923) was sprayed on the contaminated squares and allowed to remain in contact for 3 minutes. The excess disinfectant was drained from the squares which were then placed in nutrient broth and left for 30 minutes (1st Transfer). This would result in dilution or neutralization of the residual effect of the disinfectant which could have a bacteriostatic effect on the bacteria producing false negative results. The squares were then removed and placed in fresh nutrient broth (2nd Transfer). Both sets of media (1st and 2nd Transfers) were incubated at 35 - 37^{o}C for 48 hours.

The procedure was repeated for SepteFX 6D-840 (Lot 949) except that SepteFX 6D-840 was sprayed on the contaminated squares and allowed to remain in contact for 10 seconds. Positive and negative controls as required were set up

Results :

No growth was observed in any of the containers of nutrient broth containing the squares treated with SepteFX 7D-11 or SepteFX 6D-840. There was a 100% kill rate. The negative controls for sterility of media, fetal calf serum and sterilised squares of mannequin had no growth. The positive control showed growth indicating that the bacteria had been viable before being subjected to the disinfectants.

Conclusion :

Exposure of the contaminated surfaces to SepteFX 7D-11 and SepteFX 6D-840 resulted in the destruction of the bacteria even in the presence of a protein bioload (FCS) since no growth was observed. The effective contact time was also very short - 3 minutes and 10 seconds for SepteFX 7D-11 and SepteFX 6D-840 respectively. Therefore these agents are both very effective in disinfecting the surface of the mannequin.

B. Effect of SepteFX 7D-11 and SepteFX 6D-840 on elimination of *Staphylococcus aureus* from the nostrils of the mannequin

Method Outline :

The face of the mannequin was placed in an autoclavable plastic bag and sterilised. The experiment was performed in the laminar flow hood. In order to determine whether the nostril was sterile, a cotton-tipped swab was moistened in sterile nutrient broth, then used to wipe the inside surfaces of the nostril of the mannequin. Under aseptic conditions, the cotton tip of the swab was broken off and placed into a fresh tube of nutrient broth (negative control). A 10 µL volume of *S. aureus* /FCS mixture was placed in each nostril and left to dry for 30 minutes at 35 - 37^{o}C. A moistened cotton swab was then used to swab one of the inoculated nostril to indicate whether the bacteria were viable. The cotton tip of the swab was placed in nutrient broth (positive viability control).

SepteFX 7D-11 was sprayed into the inoculated nostril and left for 3 minutes after which a moistened swab was used to wipe the sprayed nostril thoroughly (Ist wipe). The cotton swab was placed in nutrient broth and left for 30 minutes to neutralize bacteriostatic action of residual disinfectant. A second moistened swab was used to wipe the same nostril, placed in nutrient broth and left for 30 minutes. After the elapsed time both swabs were removed from the nutrient broth, and their tips broken off into fresh tubes of nutrient broth. The following negative controls were also set up : uninoculated swab, uninoculated nutrient broth, uninoculated FCS. The test samples and controls were incubated at 35 - 37^{o}C for 48 hours.

The test was repeated for SepteFX 6D-840 with an exposure time of 10 seconds.

Results:

No bacteria were recovered from the nostrils of the mannequin after they were treated with either SepteFX 7D-11 for 3 minutes or SepteFX 6D-840 for 10 seconds. This was evidenced by the fact that no growth occurred in the recovery medium - nutrient broth. The positive controls all had growth and the negative controls did not grow.

Conclusion :

SepteFX 7D-11 and SepteFX 6D-840 were able to eliminate *S. aureus* that had been used to contaminate the nostrils of the mannequin. The presence of a protein bioburden, did not affect the efficacy of the disinfectants in killing the bacteria since no growth was observed. This, therefore, is an indication that these disinfectants should still be effective in the presence of other protein-containing materials such as blood or saliva. In some instances, proteins might increase the ability of the bacteria to resist a disinfecting agent by surrounding them with a protective layer that the disinfectant cannot penetrate; or the protein themselves could also inactivate the disinfectant. Furthermore the spray method of applying the disinfectants appears to be extremely effective in disinfecting areas such as crevices and corners that might be diifficult to access by other modes of disinfectant application such as wiping.

C. The Effect of the Disinfectants SepteFX 7D-11 and SepteFX 6D-840 on the Rubber Material used to make the Mannequin.

Method Outline :

Twenty five centimetre squares of the rubber material were prepared and placed in 4 groups that were treated differently. The squares were soaked in water, SepteFX 7D-

11, or SepteFX 6D-840 and dried over a two week period, or left untreated.

Group 1 - squares were left at room temperature 22-25^{o}C;

Group 2 - squares were soaked in deionized water and dried at 37^{o}C

Group 3 - squares were soaked in SepteFX 7D-11 and dried at 37^{o}C

Group 4 - squares were soaked in SepteFX 6D-840 and dried at 37^{o}C

The protocol was as follows : pieces were alternately soaked for approximately 8 hours, then dried overnight . This was repeated for ten cycles including one extended cycle each of soaking and one of drying that lasted for 3 days.

The squares were checked each day and at the end of two weeks for changes in appearance, flexibility and whether there was a build-up of residues.

Result:

There were no obvious changes in the appearance of the squares that were treated with water, SepteFX 7D-11 or SepteFX 6D-840 and those that were left untreated. The colour remained the same , the flexibility was similar, and there was no cracking or discoloration. The only noticeable difference was with the squares that were treated with SepteFX 6D-840. There was a very slight film build - up which was not present in the other sample groups.

The material was also analysed to determine whether there would be a residual amount of disinfectant absorbed onto the mannequin after it was treated with SepteFX 7D-11 and SepteFX 6D-840. The squares of material were sprayed with SepteFX 7D-11 then left for 3 minutes and with SepteFX 6D-840 then left for one minute. They were wiped to remove excess disinfectant then immersed in deionized water to permit any absorbed disinfectant to diffuse into the water. Samples of the water were then analysed by HPLC for the presence of the active ingredients - ethanol and chlorhexidine - from the disinfectant. This would be an indicator of the presence of the disinfectant left on the mannequin.

No ethanol or chlorhexidine was detected in the samples.

Conclusion :

The disinfectants SepteFX 7D-11 and SepteFX 6D-840 were not detrimental to the material used in the make-up of the mannequin. Although there was a slight film left after treatment with SepteFX 6D-840, this was not easily noticed. Moreover, the conditions under which the experiment was carried out were extreme. Normally, the mannequin would not be stored at 37oC, but at ambient room temperature 20-25oC; neither would it be subjected to such long contact times with the disinfectants. The time of exposure for SepteFX 7D-11 and SepteFX 6D-840 to be effective is only three minutes and ten seconds respectively. Therefore, it is unlikely that any cumulative effect of the film deposit due to SepteFX 6D-840 on the mannequin would be noticeable under normal use and conditions.

In addition, it appears that once the samples are disinfected, there would be little or no adverse effect due to residues of the disinfecting agents remaining on the surface of the mannequin since none of the active agents were detected

SUMMARY

SepteFX 7D-11 and SepteFX 6D-840 are effective and safe disinfectants that can be used to disinfect the mannequins used in CPR classes. They are effective in a short period of time, even in the presence of a protein bioload and are especially useful in areas that might be difficult to access by other disinfectants. They are not detrimental to the mannequin since they do not destroy or damage the material that the mannequin is made of and are not absorbed onto it in any appreciable amount.

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