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Studies of microbial recovery from stainless steel surfaces

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ABSTRACT

In microbiological environmental monitoring programs, swabs are widely used for monitoring of surfaces and operators contamination. Considering that cleaning methods are developed and validated to prevent the risk of producing contaminated products by confirming that the cleaning process is sufficient, it is important to establish method limits and select the proper cleaning techniques and detection methods. The present study comparatively evaluated the recovery capacity from samples surfaces precontaminated with *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231 using the swab and contact plates methods as well as the logarithmic reduction of microbial charge after the treatment of pre-inoculated stainless steel plates with chitosan solution 10mg/mL in 1M acetic acid.

Keywords: *stainless steel, microbial contamination, environmental monitoring methods.*

1. INTRODUCTION

In natural habitats, microorganisms are predominantly associated with solid surfaces and organized in communities known as biofilms [1,2,3,4,5]. Environmental monitoring should promptly identify potential sources of contamination in order to implement corrective actions before product contamination occurs. The monitoring program should include air, gases, operators, floors, walls and equipment surfaces, that came in contact with the product container and closures [6, 7, 8]. Environmental monitoring methods are not always able to recover microorganisms contaminating in the sampled area. In particular, low-level contamination can be particularly difficult to detect. Swabs are traditionally used for microbiological environmental sampling, despite their limited recovery capacity of contaminating microorganisms from different surfaces [9, 10, 11, 12, 13, 14]. In pharmaceutical industry many studies were developed concerning the recovery and release capacity from known microorganism's inocula of different swab materials (nylon floked, rayon, cellulose sponge-tipped). Swab sampling methods were developed for cleaning validation of residual active pharmaceutical ingredients in collected samples. For establishing cleaning methods is necessary to select a proper detection method of contaminants. One of the often overlooked details about monitoring the efficacy of surfaces disinfection using swab samples is that swabs could lead to errors associated with their construction.

Chitosan, poly [b-(1-4)-linked-2-amino-2-deoxy-D-glucose], is a non-toxic, hydrophilic, biocompatible, biodegradable and anti-bacterial product, obtained by partial deacetylation of chitin in alkaline conditions, a natural cationic polyaminosaccharide polymer[15]. Due to its

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biocompatibility and advantageous functional groups (amino and hydroxyl), chitosan is widely used in medicine (wound dressing material, drug delivery vehicle, candidate for tissue engineering) [16]. Yeast and moulds are the most sensitive groups, followed by Gram positive and Gram negative bacteria [17,18 19, 20]. The purpose of this paper was to evaluate the efficiency of the recovery of spiked microorganisms obtained from swab (inoculated and filtered) and contact plates, respectively. We evaluated the influence of chitosan solution 10 mg/mL in 1M acetic acid on different microorganism's viable cell counts from precontaminated stainless steel surfaces.

2. EXPERIMENTAL SECTION

The following materials were used to conduct the study: laminar flow hood, stainless steel plates (10 x 10 cm), glass spraying devices, sterile swab RediSwab® (*Biotrace International*), contact plates (55 cm), cellulose filter membrane 0,22 μm (*Millipore*), casein soy agar, Sabouraud dextrose agar, 10 mg/mL chitosan solution in 1M acetic acid. The test microorganisms were represented by reference strains (*Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus spizizenii* ATCC 6633, *Candida albicans* ATCC 10231). 24 stainless steel plates were sprayed each with 1mL microorganism suspension of different densities (10^4, 10^3, 10^2, 10 colony forming units(c.f.u.)/mL) from a distance of 15 cm, each of the six plates being spiked with the same density of microorganism suspension. After 5 minutes, 1 mL of 10mg/mL chitosan solution in 1M acetic acid was sprayed onto three stainless steel plates spiked with each inoculum. Eight stainless steel plates, two plates spiked with the same density of microbial inocula (one of this two plates sprayed with chitosan solution) have been swabbed and 1mL of swab solution was subcultured in agar media. Other eight plates processed in the same way have been swabbed and the entire solution of the swabs was filtered on a 0,22 μm membrane, further placed on the surface of the agar media. The last eight stainless steel plates, spiked as above, were collected with contact plates. Plates were incubated for 3-5 days, at 20-25° C on Saboraud dextrose agar (*Candida albicans)* and at 30-35° C for bacterial strains. After incubation the recovered colonies were counted by using this viable cell counts assay.

3. RESULTS SECTION

Recovery rates for Staplylococcus aureus ATCC 6538. The number of viable cell recovered from 4 log density suspension was 46.92% when using contact plates, 42.69% for filtered and 40.57% for inoculated swabs. Thus, the best recovery rates were obtained from 2 and 1 log density suspensions, i.e. 56.56% and 54.54%, respectively, using the filtered swab method (figure 1).

Recovery rates for Pseudomonas aeruginosa 9027. The number of viable cells recovered from 4 log density suspension was 41. 68 % using contact plates, 49.09% using filtrated swab and 44.08% using inoculated swab. The best recovery rate, i.e. 65.21% was obtained from 1 log density suspension, using the filtered swab method (figure 2).

Recovery rates for Bacillus spizizenii ATCC 6633. The number of viable cells recovered from 4 log density suspension was 32. 15 % using contact plates, 40. 88% using filtered swab and only 33.33% using the inoculated swab. The best recovery rates were obtained from 2 log density suspension, i.e. 85. 20% using the filtered swab method (figure 3).

Recovery rates for Candida albicans ATCC 10231. The number of viable cell recovered from 4 log density suspension was 51.48 % using contact plates, 58.66% using filtered swab and 52.47% using the inoculated swab. The best recovery rates were obtained from 2 and 1 log density suspensions, i.e. 86.27% and 126,92%, respectively, using the filtered swab method (figure 4).

The influence of chitosan solution 10mg/mL in 1M acetic acid on tested microorganism's viable cell counts.

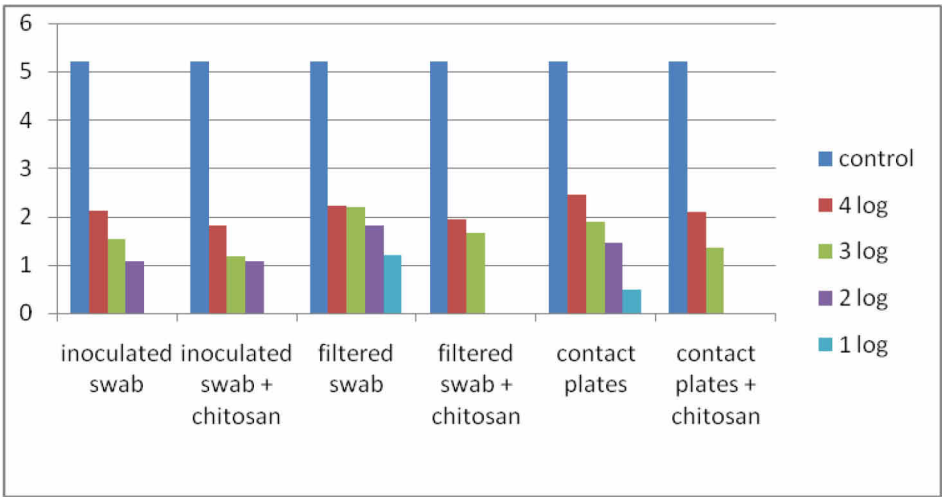

Figure 1: Number of viable cell counts (c.f.u./mL) of *Staphylococcus aureus* ATCC 6538 recovered from stainless steel surfaces

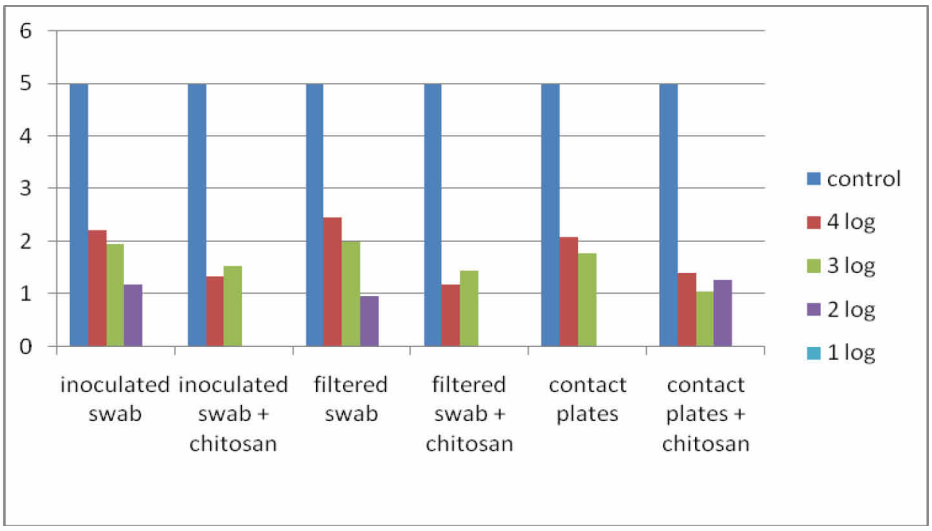

Figure 2: Number of viable cell counts (c.f.u./mL) of *Pseudomonas aeruginosa* ATCC 9027 recovered from stainless steel surfaces

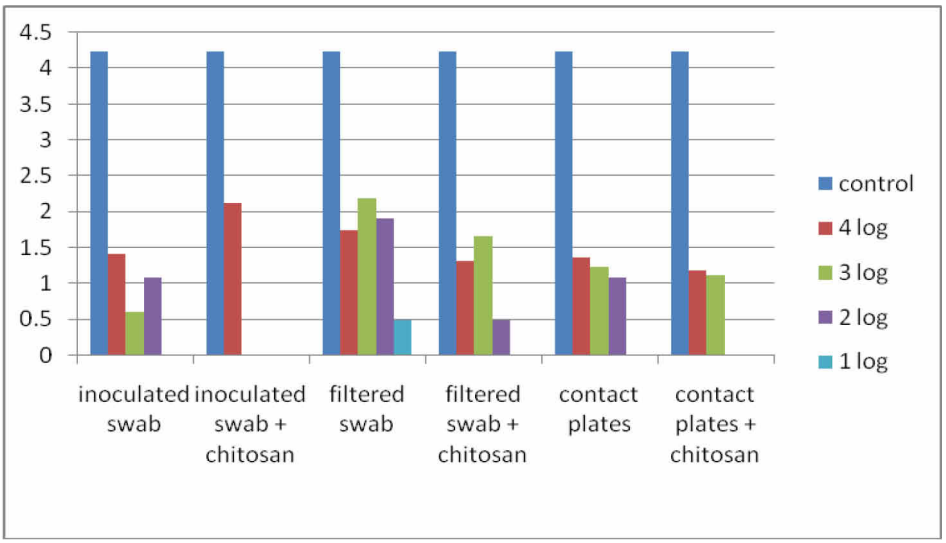

Figure 3: Number of viable cell counts (c.f.u./mL) of *Bacillus subtilis* ATCC *6633* recovered from stainless steel surfaces

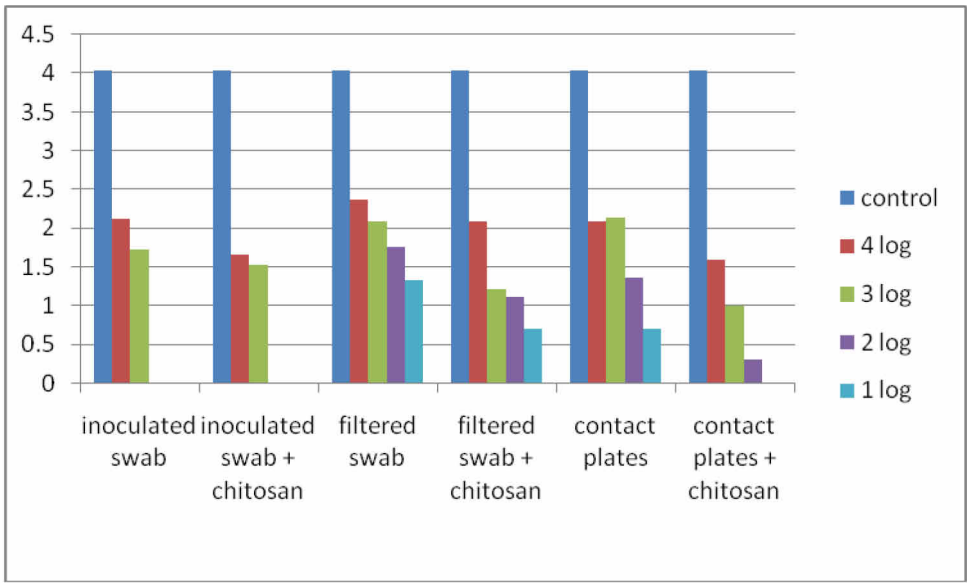

Figure 4: Number of viable cell counts (c.f.u./mL) of *Candida albicans* ATCC 10231recovered from stainless steel surfaces

Table 1: Number of viable cell counts (% log) of *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus spizizenii* ATCC 6633 and *Candida albicans* ATCC 10231 recovered from stainless stel surfaces

	Inoculated swab							
	-chitosan(log)				+chitosan(log)			
	4	**3**	**2**	**1**	**4**	**3**	**2**	**1**
S.aureus	**40.57**	**36.42**	**33.75**	**0**	**34.80**	**43.09**	**33.75**	**0**
P. aeruginosa	**44.08**	**48.62**	**39.46**	**0**	**26.45**	**38.34**	**0**	**0**
B. subtilis	**33.33**	**18.5**	**48.43**	**0**	**50.11**	**0**	**0**	**0**
C. albicans	**52.47**	**56.57**	**0**	**0**	**41.08**	**50.32**	**0**	**0**

	Filtered swab							
	-chitosan(log)				+chitosan(log)			
	4	**3**	**2**	**1**	**4**	**3**	**2**	**1**
S.aureus	**42.69**	**52.14**	**56.56**	**54.54**	**37.30**	**39.28**	**0**	**0**
P. aeruginosa	**49.09**	**49.87**	**65.21**	**0**	**23.64**	**35.83**	**0**	**0**
B. subtilis	**40.88**	**36.84**	**85.20**	**39.02**	**30.73**	**51.08**	**21.52**	**0**
C. albicans	**58.66**	**68.75**	**86.27**	**126.92**	**51.73**	**37.15**	**54.41**	**67.30**

	Contact plates							
	-chitosan(log)				+ chitosan(log)			
	4	**3**	**2**	**1**	**4**	**3**	**2**	**1**
S.aureus	**46.92**	**44.76**	**45.62**	**21.81**	**40.38**	**32.38**	**0**	**0**
P. aeruginosa	**41.68**	**44.11**	**0**	**0**	**27.65**	**26.06**	**42.14**	**0**
B. subtilis	**32.15**	**38.08**	**48.43**	**0**	**27.89**	**34.36**	**0**	**0**
C. albicans	**51.48**	**70.39**	**66.66**	**67.30**	**39.10**	**32.89**	**14.70**	**0**

*Colored cells are indicating the log densities for which an important decrease of viable cell counts 9>10%) was observed.

Chitosan solution reduces viable cell counts obtained by the filtered swab method, with 56.56% and 54.54%, when using 2 log and 1 log bacterial density inocula of *Staphylococcus aureus* ATCC 6538 and with 65.21% when using 2 log bacterial density inoculum of *Pseudomonas aeruginosa* ATCC

9027. *Bacillus spizizenii* ATCC 6633 recovery rates obtained with filtered swab were reduced with 63.68% on 2 log suspension density. The most important decrease in viable cell counts were obtained for *Candida albicans* ATCC 10231 using 1 log microbial density inoculum, quantified by the contact plates method (67.3%) and filtered swab method (59.62%).

4. CONCLUSIONS

This study evaluated the comparative recoveries of spiked microorganisms obtained using the swab method (inoculated and filtered) and respectively, contact plates method. The best recovery rates were obtained for filtered swab and contact plates method on *Candida albicans* ATCC 10231, using 1 log density suspension and for *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231, when using 2 log density suspensions. Chitosan solution 10 mg/mL in 1M acetic acid reduced the viable cell counts of *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 9027, the most significant decrease of mcirobial recovery rate being obtained when using filtered swab (65.21%).

5. REFERENCES

[1] Landini P., Cross-talk mechanisms in biofilm formation and responses to environmental and physiological stress in Escherichia coli, *Research in Microbiology*, 160, 259, **2009**.
[2] Saviuc C., Grumezescu A., Chifiriuc M.C., Bleotu C., Stanciu G., Hristu R., Mihaiescu D., Lazăr V., In vitro methods for the study of microbial biofilms, *Biointerface Research in Applied Chemistry*, 1, 31-35, **2011**.
[3] Holban A., Lazăr V., Inter-kingdom cross-talk: the example of prokaryotes - eukaryotes communication, *Biointerface Research in Applied Chemistry*, 1,95-110, **2011**.
[4] Panus E., Chifiriuc M.C. , Banu O., Mitache M., Bleotu C., Rosoiu N., Lazăr V, Comparative study of resistance and virulence markers in Escherichia coli strains isolated from hospital surfaces, clinical specimens and drinking/ marine waters, *Biointerface Research in Applied Chemistry*, 1, 24-30, **2011**.
[5] Saviuc C., Grumezescu A.M., Holban A., Chifiriuc C., Mihaiescu D., Lazar V., Hybrid nanostructurated material for biomedical applications, *Biointerface Research in Applied Chemistry*, 1, 64-71, **2011**.
[6] U.S. Food and Drug Administration Guidances for Industry-sterile Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice, September **2004**.
[7] European Commission Guide to Good Manufacturing Practice, Revision to Annex1, May **2003**.
[8] Borchert D., GMP Manual, Good Manufacturing Practice and implementation, Maas&Peither GMP Publishing, 1st edition, **2010**.
[9] Ritter N.M., Advant SJ, Hennessey J, et al. What is test method qualification? Proceedings of the WCBP CMC strategy forum, 24 July 2003. *BioProcess Int.*, 2, 8, 32-46, **2004**.
[10] Kanarek A.D., Method validation guidelines. BioPharm Int Web site. September 15, 2005. Available at: http://biopharminternational.findpharma.com/ biopharm/article/articleDetail.jsp? id=185606&pageID=4. Accessed December 13, **2009**.
[11] Boudreau S.P., McElvain J.S., Martin L.D., Method validation by phase of development: an acceptable analytical practice. *Pharmceutical Technoology*, 28, 54-66, **2004**.
[12] United States Food and Drug Administration. Guide to Inspections Validation of Cleaning Processes. FDA. Available at: http://www.fda.gov/ora/Inspect_ ref/igs/valid.html. Accessed May 6, **2009**.
[13] LeBlanc D.A., Establishing scientifically justified acceptance criteria for cleaning validation of finished drug products. *Pharmaceutical Technology*, 19, 5, 136-148, **1998**.
[14] Gugliotta T., Smiths Detection – Scientific: Ion Mobility Spectrometry (IMS) for Cleaning Validation. Institute of Validation Technology Cleaning Validation and Critical Cleaning Processes Conference; July 24-27, Chicago, **2007**.
[15] Saviuc C., Grumezescu A.M., Oprea E., Radulescu V., Dascalu L., Chifiriuc M.C., Bucur M., Banu O. , Lazar V., Antifungal activity of some vegetal extracts on Candida biofilms developed on inert substratum, *Biointerface Research in Applied Chemistry*, 1, 15-23, **2011**.
[16] Grumezescu A.M., Saviuc C., Holban A., Hristu R., Croitoru C., Stanciu G., Chifiriuc C., Mihaiescu D., Balaure P., Lazar V, Magnetic chitosan for drug targeting and *in vitro* drug delivery response, *Biointerface Research in Applied Chemistry*, 1, 160-165, **2011**.

[17] Grumezescu A.M., Mihaiescu D.E., Tamaș D., Hybrid materials for drug delivery of rifampicin: evaluation of release profile, *Biointerface Research in Applied Chemistry*, 1, 229-235, **2011**.
[18] Forsyth R.J., Van Nostrand V., Using visible residue limits for introducing new compounds into a pharmaceutical research facility, *Pharmaceutical Technology*, 29, 4, 134-140, **2005**.
[19] Siegerman H., Hollands W., Strauss M., Optimum swabbing techniques for cleaning validation. How to succeed in the search for nothing: effective swabbing techniques for cleaning validation, Volume 1, **2006**.
[20] Verghese G. Selection of cleaning agents and parameters for cGMP processes. Paper presented at: Interphex Conference; March 17-19, Philadelphia, **1998**.