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REVIEW

USE OF FLOW CYTOMETRY FOR THE EVALUATION OF DISINFECTANT EFFECTIVENESS

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Summary

Flow cytometric analysis is used to evaluate various micro-organisms' susceptibility to antibacterial agents and disinfectants. This paper describes a study where *Candida albicans* was used to assess the method against three disinfectants. The disinfectants examined were: 70% - ethanol and two 70%- ethanol based commercial hand disinfectants with smoothing and cleaning additives. To detect the presence of damaged microbial cells EtBr was used. With the method, a 1 minute sample incubation time showed 36.25% ± 1.45 and 35.0% ±2,0 dead cells present as a result of commercial disinfectants-1 and -2, incubation; and 86.5% ±2.8 after the incubation with 70%- ethanol. With a 3 - minute incubation time, the corresponding results were 43.25% ±3.95; 43.5% ±2.6; 86.5% ±4.3. These were significant in multi-group comparison. With a 5-minute incubation time, *C. albicans* samples showed an antiseptic activity against the chemicals under investigation of: 85.5% ±2.7; 84.5% ±2.4 and 91.3% ±3.57 respectively. Here there was no significant difference.
Key words: flow cytometry, microorganism susceptibility evaluation, disinfectant, yeast, hand sanitisation

Résumé

L'utilisation de la cytométrie de flux pour évaluer l'efficacité désinfection

L'analyse par cytométrie de flux est utilisée pour évaluer la sensibilité de divers micro-organismes à des agents antibactériens et des désinfectants. Cet article décrit une étude où *Candida albicans* a été utilisé pour évaluer l´éfficacité de trois désinfectants. Les désinfectants examinés étaient les suivants: 70% - éthanol et deux 70% à base d'éthanol, désinfectants commerciaux pour les mains avec des additifs de lissage et de nettoyage. EtBr a été utilisé pour détecter la présence de cellules microbiennes endommagées. Avec ce procédé, après un temps d'incubation de 1 minute, il a été montré que 36,25% ± 1,45% et que 35,0 ± 2,0 de cellules mortes présentes avec les désinfectants commerciaux -1 et -2; et une valeur de 86,5% ± 2,8 après l'incubation avec 70% - éthanol. Avec un temps d'incubation de 3 minutes, les résultats correspondants étaient 43,25% ± 3,95; 43,5% ± 2,6; 86,5% ± 4,3. Ceux-ci étaient significatifs en comparaison multi-groupe. Avec un temps d'incubation de 5 minutes, les échantillons *C. albicans* ont montré une activité antiseptique contre les produits chimiques sous enquête de: 85,5% ± 2,7; 84,5% ± 2,4 et 91,3 ± 3,57%, respectivement. Ici, il n'y avait pas de différence significative.
Mots clés: cytométrie de flux, l'évaluation de microorganismes de sensibilité, désinfectant, la levure, la désinfection des mains

Introduction

Flow cytometry can be used as a rapid assay to assess the effect of different antimicrobial agents. One important type of agent to assess are disinfectant products designed as hand sanitizers. Various commercial 70%- ethanol or - isopropyl alcohol based hand disinfectant solvents theoretically have different antiseptic dynamics different to medical ethyl alcohol, at the same concentration. Moreover, when using such products a short exposure time is less effective.

Flow cytometry methods have been used in clinical

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and sanitary microbiology fields since the 1990s, and the opportunities offered by the methods are well demonstrated [1,2]. Moreover, the use of flow cytometry to assess the functional characteristics of causative agents has been discussed in scientific publications. Cytometry is gradually becoming a more common means to evaluate viability, metabolic activity and antibiotic-susceptibility; although there have been insufficient standardized clinically significant tests performed to date [3-5]. The standard methods of diagnostic of antibiotic-susceptibility are labour intensive and time-consuming; sometimes such tests take up to 24 hours to isolate a pure strain and 72 hours in total to complete the analysis. Such timescales account for significant delays with the administration of the appropriate antibacterial therapy. To use technologies, that decrease time and labour consumption, is both economically rational and would contribute to the effectiveness of antibiotic therapy.

The existing express methods of antibiotic response diagnostic (including microscopy, Raman spectrometry, MALDI-TOF spectrometry, resonance mass-spectrometry, fluid scope-technology, isothermal microcalorimetry, magnetic beadrotation, nano-pore technology) are costly to run and require expensive specific equipment [6]. Furthermore, they are not always advantageous in the respect of time and labour consumption, particularly when compared to cytometric methods of microorganism detection. In terms of advantages, flow cytometry will, firstly, enable a faster process and one that is less costly; secondly, the method will determine the dynamic characteristics of antimicrobial agent and pathogen interaction.

The aim of the study was to use cytometric methods to evaluate the disinfectant activity of selected substances. Hand disinfectants, commonly used in healthcare settings, were used as sample substances. Hand sanitisers play a key role infection control for healthcare staff. For personnel safety reasons, these agents are typically alcohol based [7].

Material and Methods

Following the purpose of the study, has been used as material and methods, the below mentioned.

Test microorganism

The strain used was derived from the ATCC culture collection. A yeast, residential to human skin, was selected: *Candida albicans* CBS8837.

Before the use, the yeast cultures were recovered in an optimal fluid medium and grown till the late-log growth stage. Sterile physiological solution, filtered through 0.2 μm, was used as the vehicle solution. The final concentration obtained was $1x10^6$/mL. The final value was limited by the design of the cytometer. A x10 dilution series was completed, in triplicate.

Flow cytometry

A PartecCyFlow cytometer was used for the analysis. Logarithmic scale 3 was used for the population identification and background gating. To evaluate the positively stained population, logarithmic scale 4 was used. Physiological solution, unstained growth and the stain solution without microbial cells, were used to provide an overall blank for background gating.

The flow cytometry fluorescent dyes for staining viable and dead cells were Ethidium bromide (EtBr) and C21H20BrN3 intercalating agent with the molecular mass of 394.33 g/mol. The water solution used was 5 mcg/ml at the rate of 5 mcl per 100 mkl of the growth. The incubation time was 5 minutes; a 488 nm laser was used for FL4 detection. The stained population was considered dead.

Disinfecting agents

Hand disinfectants were decided as the sample agents. The solutions are alcohol-based and are used for fast skin disinfection. A 70% ethanol solution was used as a reference solution and AHD 2000 disinfectants, Lysoform Dr. Hans, based on 70% Ethanol; and HandSanitazer (Tork), with 65% ethanol and 5% isopropyl alcohol were chosen as the test agents.

Results

To evaluate the disinfectant ability of the agents *Candida albicans* was used as the challenge culture, while hand disinfectants AHD 2000, Lysoform Dr. Hans, based on 70% ethanol and Hand Sanitazer (Tork), with 65% ethanol and 5% isopropyl were used as the agents. The incubation time comprised of: 1 minute, 3 minutes and 5 minutes. The results are presented in table 1.

For statistical analysis, Discriminate Function Analysis was used to find the mean, error of mean, the normality of distribution and the 95% - confidence range.

The reference ethanol solution showed high disinfection effect independent of the incubation time. Both hand

Table 1 - The Results of Disinfection Effect of Alcohol-containing Hand Disinfectants

Exposure Time	AHD 2000 (CI ±95% PI+, %)	Hand Sanitazer (CI ±95% PI+, %)	Ethanol 70% (CI ±95% PI+, %)
1 minute	30.81 % - 34.89% *	31.43% - 34.57% *	80.81% - 90.11%
3 minutes	34.82% - 47.18%*	38.81% - 42.79%*	78.48% - 88.72%
5 minutes	82.43% - 89.17%	79.69% -86.27%	85.91% - 91.41%

* - confidence range between the groups (p level < 0,05)

disinfectants revealed a considerable inhibition of the disinfecting effect and showed a significant difference in the effectiveness compared with pure ethanol during 1 and 3-minute exposure periods; however, no significant difference was found between the two sample solutions. At five minutes, there was no significant difference between the three agents.

Conclusions

The results of the study indicated that the given method can be used as a rapid antibacterial effect assay; moreover, one important advantage of cytofluorometry is that it does not only allow an evaluation of the final result; it also provides a clear indication of the dynamics of the antimicrobial effect. The dynamics of the antiseptic effect of ethanol and that of alcohol-based hand disinfectants' significantly differs at a similar ethanol concentration. This is possibly due to the skin-smoothing additives contained in the latter. However, at the exposure time of 5 minutes, neither of the solutions involved showed a significant difference and antimicrobial effectiveness was demonstrated.

The time of disinfectant effect development is one of the most important characteristics of an agent and is hard to reveal using classical bacteriological methods. Whether, in practice, staff who apply hand disinfectants are able to wait for 5 minutes for the antimicrobial effect to occur is a matter of conjecture.

The total time required for the analysis presented comprised 30 minutes after the pure strain isolation was prepared. This indicates that flow cytometry has the potential to be a rapid and effective test method. In relation to the products examined, other microorganisms can be considered to assess the different disinfectant products more fully; the purpose here was to present an effective test method.

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