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Bericht über die Evaluierung des Nutzens von iNLABTEC Pipettenfilter

English translation of the report with above mentioned title

Report on the evaluation of the benefits of Inlabtec Pipette Filter Plugs

Dear Dr. Freydl

According to your assignment, we examined outside the accredited area whether the iNLABTEC pipette filter plugs provided by you are effective to prevent contaminations originating from a pipette.

Summary
The aim of this project was to check whether the iNLABTEC pipette filter plugs for 1 ml pipettes are an effective measure against contaminations. The available filters were introduced into two 1 ml air displacement pipettes and artificially contaminated with 5 to 50 µl of a Staphylococcus aureus suspension with a cell concentration of $5.4*10^7$ CFU / ml. Afterwards, it was investigated whether or not contaminations can be detected if sterile water was transferred onto culture media using the artificially contaminated 1ml pipettes, immediately after the contamination and one day later after leaving the pipette in a clean bench for drying the filter plug. Before pipetting sterile water samples onto culture media, the optical control of the filter plugs revealed no changes of the surfaces like drops or encrustations.
Although the artificial contamination level of the pipette filter plugs were at $1.9*10^6$ CFU / filter, respectively at $1.1*10^6$ CFU / filter, no contaminations of culture media has been observed after pipetting of sterile water with the contaminated pipettes. Therefore, the pipette filter plugs have completely retained the contamination suspension (Staphylococcus aureus) and are an effective measure to avoid contaminations in microbiological routine analysis.

Introduction
If liquids are not carefully pipetted splashes can occur which eventually deposit on the pipette and which can later sporadically contaminate subsequent samples. In molecular biology applications the use of filter tips are therefore standard, however, they are considerably more expensive than those tips without a filter. As an alternative to filter tips iNLABTEC AG developed soft filter inserts for 1 ml

air displacement pipette. The aim of this work was to test the usefulness and the retention capacity of these pipette filter plugs.

Methods

From a Staphylococcus aureus isolate a solution of $5.4*10^7$ CFU / mL was prepared as contamination suspension. For all microbial counts the horizontal method for quantitative determination of the aerobic mesophilic germs according to ISO 4833-1: 2013 was applied.

An iNLABTEC pipette filter plug was inserted into a first 1-channel Eppendorf Research plus pipette with a fixed volume of 1000 µl and the front of the filter plug inoculated with 5 µl of contamination solution. Immediately afterwards, the pipette was used to pipette 1 ml of sterile water into three Petri dishes which were processed and evaluated according to ISO 4833-1: 2013 (sterility control).

The same procedure was carried out after additional filter contamination with 5 µl (total contamination: 10 µl), 10 µl (total contamination: 20 µl) and 30 µl (total contamination: 50 µl).

A filter introduced into a second pipette was directly contaminated with 50 µl of contamination solution followed by pipetting of sterile water into Petri dishes for sterility controls.

To measure the viable cell counts present on the filter the contaminated filter plug of the first pipette was removed with a pair of sterile tweezers and extracted with 100 ml of buffered peptone water in a Bagmixer (Interscience) at stage 2 for 1 minute and subjected to microbial count according to ISO 4833-1: 2013.

The second pipette was stored overnight in a sterile bench for drying the contaminated filter. Next day 1 ml of sterile water was pipetted into three Petri dishes for sterility controls. Afterwards, the filter plug was removed and processed analogously to the first filter.

The colonies grown on sterility control plates were further differentiated by Gram staining and additional subculturing on rabbit plasma / fibrinogen agar plates (RPF, ThermoFisher Diagnostics). As phenotypical reference a colony from the dilution stage 10^{-5} of the Staphylococcus aureus contamination solution was streaked out on RPF plate and incubated in parallel for 2 days at 37 °C.

Results

Table 1 shows the cell concentration of the contamination suspension and the extracted filters. The filters were inoculated each with 50 µl contamination suspension of $5.4*10^7$ CFU / ml corresponding to $2.7*10^6$ CFU / filter. Therefore the recovery rate for filter 1 was 70 %, respectively 41 % for filter 2.

Table 1: Viable cell concentration

	Serial dilution							
Sample	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	CFU
Contamination Suspension	+++	+++	+++	+++	+++	53	6	$5.4*10^7$ / ml
Filter 1		+++	+++	187	24	1	0	$1.9*10^6$ / filter
Filter 2		+++	+++	100	15	2	0	$1.1*10^6$ / filter

Table 2 shows the results of the sterility controls using the artificially contaminated pipettes. On 16 plates no colony growth was detected. On 2 plates growth of one colony each was detected. The two colonies were Gram-negative cocci and developed on RPF medium after 24 hours on 37 °C only

small, respectively very small bright colonies. In contrast the subcultered reference colony of Staphylococcus aureus is Gram-positive and developed on RFP dark colonies with a typical coagulase phenotype. The two colonies of the sterility controls detected are therefore clearly not to be considered as originating from the artificially contaminated filters.

Table 2: Sterility controls

	CFU / plate		
Sample / sterility control	Plate 1	Plate 2	Plate 3
Filter 1; 5 µl	0	0	0
Filter 1; 10 µl	0	1	0
Filter 1; 20 µl	0	0	0
Filter 1; 50 µl	0	0	0
Filter 2; 50 µl	0	1	0
Filter 2; 50 µl (drying)	0	0	0

Conclusions

The total contamination level of the iNLABTEC filter plugs in these tests was by far greater than can be expected in practice even with the most careless pipetting practices. The contamination solution of 50 µl was adsorbed by the two filters within a short time so that no optical changes on the filter surfaces could be detected. The bacteriological recovery rates of 70 % and 41 %, respectively, showed that the bacteria are not inactivated or are only slowly inactivated, and that these are not significantly retained by the filters during extraction in buffered peptone water.

The occurrence of isolated colonies on agar plates after the incubation of sterile samples can be expected if pipetting is not carried out in a sterile environment. Gram staining and the atypical growth on RPF growth medium show that the two colonies isolated are not originating from the pipette filter plugs respectively from the contamination suspension.

The results of the sterile controls demonstrate that the tested iNLABTEC pipette filter plugs effectively prevent unintentional contaminations. It is nevertheless recommended to change the filters regularly, depending on the application, at shorter or longer intervals. It is to be expected that on the filter surface a coating or a crust can form by repeated splashes occurring over time, which could become a source of unwanted contaminations.

Best Regards

Dr. Christoph Müller
Sektionsleiter