

IonField Systems (IFS) is an advanced technology company dedicated to the manufacturing of plasma cleaning products that enable laboratories to protect against shortages, achieve better science, and reduce environmental waste.

The PureTIP Liquid Handler Companion (LHC) System by IFS utilizes a low temperature 'cold' atmospheric-pressure plasma process to destroy organic molecules residing on or inside pipet tips thereby enabling plastic tips to be repetitively reused and eliminating the need for solvent rinsing of all types of tips. On average, in 30 seconds, the PureTIP LHC produces dry, clean tips ready for immediate reuse.

Configured in the 96 and 384-well formats, PureTIP LHC easily integrates within widely used automated liquid handling platforms.

PureTIP LHC improves the reproducibility of results, shortens automation cycle times, reduces the number of lost runs, and eliminates environmental waste and liquid handling disposables. The result is a more effective and productive lab operation.



PureTIP LHC Components: PlasmaCharger Controller and Cleaning Station

Introduction

Although homogenous assays, which require minimal surface washing as compared to the more traditional sandwich-type (ELISA) procedures, have begun to dominate the field of high throughput screening, at least one liquid transfer operation of diverse material (compound or sample) is still required. Consequently, elimination of cross-contamination remains a concern in the microplate-based discovery laboratory. The demonstration of high-energy plasma as a safe and reliable alternative to the more typical 'tip washing' or 'tip replacement' techniques currently in use in the general laboratory revealed that the plasma treated pipetting devices were not only clean and dry, but, in fact, sterile. Here we discuss some of the early results leading us to this conclusion, and the potential utility of high-energy plasma to facilitate and accelerate the development and execution of bioassays involving bacterial and/or yeast cells.

Initial Studies

Cultures of *E. coli* (strain BL-21) were inoculated in LB broth and grown 24-36 hr. at 36.5 C to a density of approximately 1×10^8 /mL (OD 2.25). The harvested cells were used to prepare a 10-fold serial-diluted Master plate (1×10^9 - 1×10^2) cells/well, using an 8-channel EDP pipettor fitted with 20 μ L Rainin disposable tips. Duplicate 2 μ L aliquots were then transferred from the Master to the Test plate (cols 1 and 3). Similarly, duplicate 2 μ L aliquots were aspirated from the Master plate, then moved to the PureTIP LHC by IonField Systems and subjected to the cleansing protocol for 10- (col 2) or 20- (col 4) seconds, prior to being dispensed into 18 μ L fresh LB buffer. The plates were visually inspected after 24 hr. incubation at 36.5°C (**Figure 1**). Similar procedures were repeated with the yeast culture, *S. cerevisiae*, as shown in **Figure 2**.

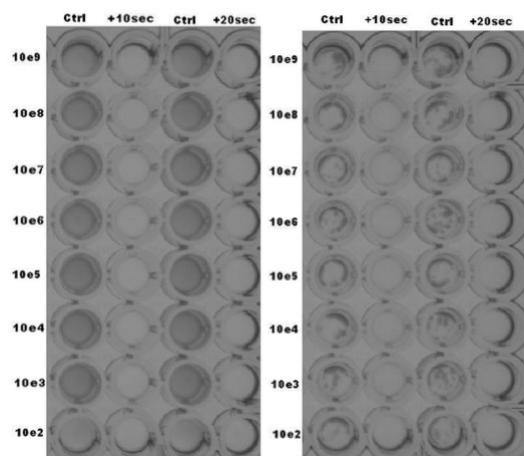
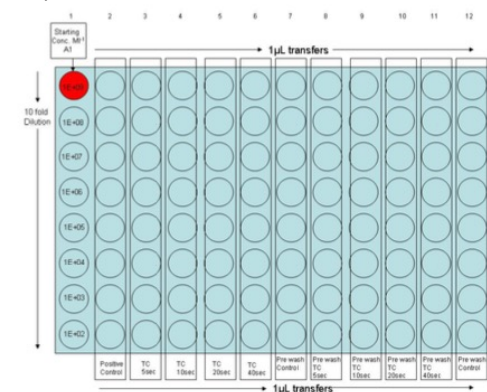


Figure 1: 1 μ L aliquots of serially diluted *E. coli* cultures, before or after TipCharger cleansing protocol. Cols 1 and 3 - Positive Control; Col 2 - following 10 second cleansing protocol; Col 4 - following 20 second cleansing protocol.

Figure 2: 1 μ L aliquots of serially diluted *S. cerevisiae* cultures, before or after TipCharger cleansing protocol. Cols 1 and 3 - Positive Control; Col 2 - following 10 second cleansing protocol; Col 4 - following 20 second cleansing protocol.

Additional Studies

In a second series of experiments, an 8-channel EDP pipettor with 20 μ L Rainin tips was used to prepare *E. coli* and yeast cultures (as above) in microplates. Serial dilutions containing 1×10^9 - 1×10^2 cells/mL were located in col 1. To control the experiment, 1 μ L aliquots were transferred from col 1 directly into cols 1 and 12 of the Test plate using V&P Scientific 1 μ L slotted pins. Test samples (cols 3-6) were prepared by transferring 1 μ L aliquots from col 1, then subjecting the loaded tips to the cleansing protocol for 5-, 10-, 20-, or 40-seconds prior to dispensing into 20 μ L/well fresh LB broth. The effect of pre-rinsing the pipet tips prior to cleansing was evaluated in cols 7-11. Test samples (1 μ L) were transferred from, then returned to col 1, and the tips were rinsed in distilled water prior to the 5-, 10-, 20-, or 40-second cleansing protocol, then dispensed into fresh LB broth. Plates were incubated 24 hr. at 36.5°C and evaluated by visual



Continuing Studies

More recently, these studies have been extended to include septum-piercing needles, designed to support the propagation of cells in bioreactor environments. In these processes, septum-piercing needles are used to add media, inoculate cells, and add reagents for pH control, as well as to collect samples for in-process evaluation. For these experiments, Teflon® coated stainless steel probes (Sias AG, Switzerland) or 27.5-gauge septum-piercing needles (BD, Franklin Lakes, NJ) were mounted on a Xantus® Robotic Liquid Handling System (Sias AG, Switzerland) fitted within an AirFiltronix hood with HEPA filtration. *E. coli* (strain BL-21) was inoculated and incubated in LB broth at 36.5°C for 24 hr., to an OD of 2.5. Transfer needles were contaminated in this broth, then immersed in fresh bacterial growth medium stored in sterile 96-well plates. Positive control samples were immersed and evaluated for growth by visual inspection after 72 hr. incubation at 36.5°C. Negative control samples were exposed in the hood for 30 min, then evaluated for contamination after 72 hr. incubation at 36.5°C. See Figure 3 and Figure 4 for test results.

Plasma Time Experiment

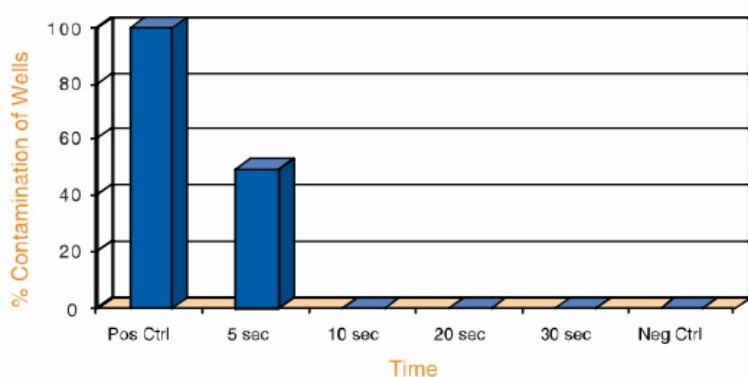


Figure 3: Plasma Time Experiment: Dip needle into *E. Coli* at a concentration of 10^9 cells/mL, clean for X seconds and dip into bacterial growth media. Incubate for 72 hours.

Plasma Depth Experiment

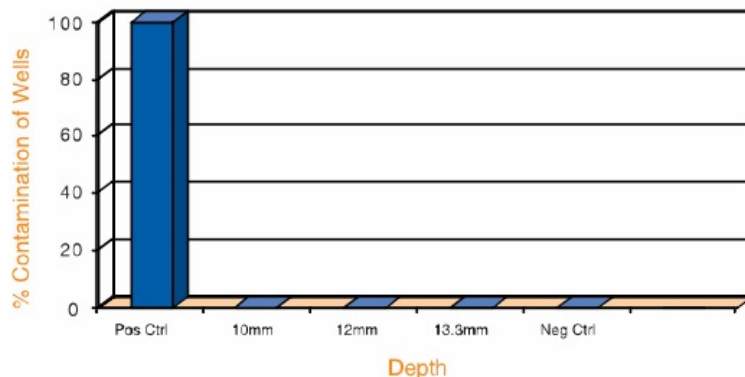


Figure 4: Plasma Depth Experiment: Dip needle X mm into *E. Coli* at a concentration of 10^9 cells/mL, clean for 20 seconds and dip X mm into bacterial growth media. Incubate for 72 hours.

Conclusions

Plasma based cleaning has been used throughout the manufacturing sector for several decades, primarily using reduced pressure and special gas mixtures. IonField Systems has invented a practical means of delivering the benefits and efficacy of plasma technology to the biopharmaceutical laboratory using normal room air, at atmospheric pressure and room temperature, expending about as much power as a light bulb. The PureTIP LHC System has been shown to effectively cleanse metal cannula, plastic pipet tips, and pin tools – here we present evidence that these liquid delivery tools are not only cleaned, but sterilized.

The initial experiments demonstrate effective elimination of up to 2×10^6 viable bacteria or yeast cells in $2 \mu\text{L}$ volumes after a 10–20 second exposure to the plasma field. Additional studies showed that the effect could be visualized with plasma field exposures as small as 5 seconds, and that 100% sterilization could be obtained within 40 seconds. Further, by incorporating a single rinse cycle (i.e., delivering the test sample into a diluent), 100% sterilization can be obtained within 20 seconds for bacterial or yeast cultures.