Early detection of microbial contaminants in cell-based products using the Milliflex® Rapid System combined with a selective lysis solution

MilliporeSigma

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Introduction

Cell-based processes are essential in the biotechnology industry for producing complex molecules such as recombinant proteins and monoclonal antibodies. To ensure maximum patient safety and industrial productivity, these cell culture batches and downstream processes must be rigorously monitored for microbial contamination. Rapid microbial detection is critical, allowing manufacturers to respond quickly in the event of contamination, thereby improving process control and safeguarding product quality.

The Milliflex® Rapid System 2.0 offers an advanced, automated solution for the rapid detection and quantification of viable contaminants—including bacteria, yeasts, and molds—in filterable samples. Using adenosine triphosphate (ATP) bioluminescence technology, the system delivers faster total viable count results than traditional methods like membrane filtration and pour plates. It is designed for both rapid sterility and bioburden testing.

For mammalian cell cultures, where the detection of microbial ATP can be complicated by the presence of interfering mammalian ATP, a method based on a selective mammalian cell lysis solution was developed to eliminate the ATP background, enabling accurate colony-forming unit (CFU) counts. In that study, samples of mammalian cells at low and high cell density were artificially contaminated with microorganisms and prepared to get a rapid total count with the Milliflex® Rapid System 2.0.

Methods

Chinese hamster ovary CHO-S cells were grown up to 6 10^6 cells/mL for low density and up to 1 10^7 cells/mL for high density. CHO-S cells were inoculated with *Pseudomonas paraeruginosa* WDCM 00026, *Bacilllus spizizenii* WDCM 00003, *Staphylococcus aureus* WDCM 00032, *Clostridium sporogenes* NCTC 12935, *Cutibacterium acnes DSM 1897, Candida albicans* WDCM 00054.
Each inoculated CHO-S cell sample followed protocols described in the table 1 according to cell density to remove interfering ATP background.
Filtered samples were then incubated onto Rapid Sterility agar cassettes (RSTM) before Milliflex® Rapid System 2.0 read out. Inoculation controls were done in parallel and read with Milliflex® Rapid System 2.0.
Performance of the detection was assessed for each sample by calculating the recovery in % versus the control. Each test was done in 5 replicates.

Table 1

Steps	Low Cell Density Protocol (10^6 cells/mL)	High Cell Density Protocol (10^7 cells/mL)
1. Sample Preparation	Mix mammalian cells inoculated with around 50 CFUs of microorganism with lysis solution (4 mL per 1 mL of cells) and 5 units apyrase.	Split the inoculated sample into multiple tubes (max 2 mL per tube). Add 9 mL lysis solution and 5 units apyrase per 1 mL of cells.
2. Vortexing	Vortex for 4 seconds.	Vortex each tube for 4 seconds.
3. Incubation	Incubate at RT for 15 minutes.	Incubate at RT for 15 minutes. Briefly vortex before filtration.
4. Filtration Process	Pre-wet with 10 mL Fluid D. Pour 20 mL NaCl peptone/PBS, vortex tube briefly, pour mix into the funnel, complete to 100 mL with NaCl peptone/PBS, and filter. Rinse membrane with 3 x 100 mL Fluid D, then with a final 100 mL NaCl peptone/PBS.	Pre-wet membrane with 10 mL Fluid D. Add 20 mL NaCl peptone/PBS and sample content, bringing volume to 100 mL. Use multiple membranes if necessary. Filter max 3 tubes (~6 mL) per membrane. Rinse membrane with 3 x 100 mL Fluid D, then with a final 100 mL NaCl peptone/PBS or 400 mL NaCl peptone/PBS.
5. Membrane Incubation	Incubate membrane in conditions suitable for microorganism detection.	Incubate membrane in conditions suitable for microorganism detection.

Milliflex® Rapid detection tower

Results & Discussion

The table 2 shows the Milliflex® Rapid results for the detection of microorganisms inoculated in low cell density samples

Table 2

CHO-S Sample	Milliflex® Rapid read out	Time to result (h)	Recovery %
no lysis		NA	NA
B. spizizenii + lysis		8	70
P. paraeruginosa + lysis		16	78
S. aureus + lysis		16	89
C. sporogenes + lysis		20	86
C. acnes + lysis		72	71
C. albicans + lysis		24	81

The table 3 shows the Milliflex® Rapid results for the detection of microorganisms inoculated in high cell density samples

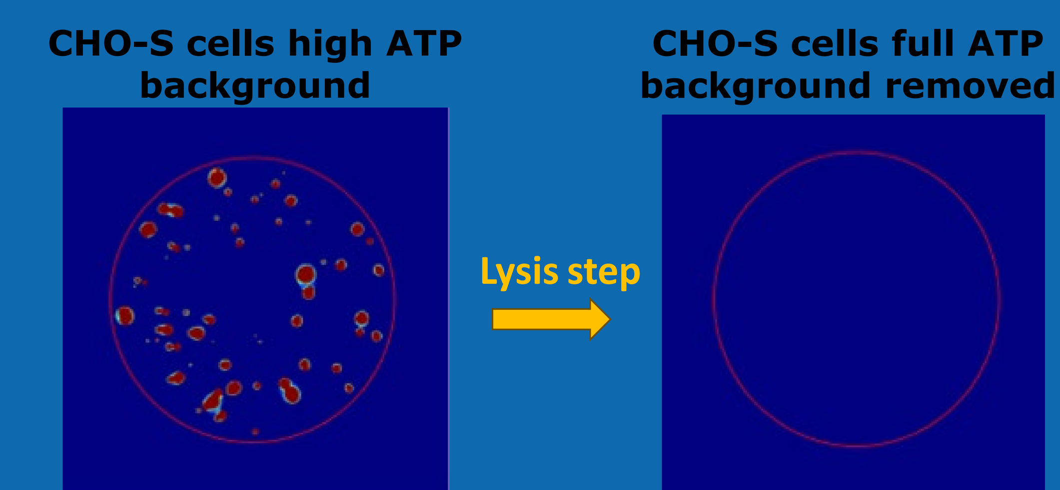
Table 3

CHO-S Sample	Milliflex® Rapid read out	Time to result (h)	Recovery %
no lysis		NA	NA
B. spizizenii + lysis		8	97
P. paraeruginosa + lysis		16	106
S. aureus + lysis		16	72
C. sporogenes + lysis		20	91
C. acnes + lysis		72	76
C. albicans + lysis		24	77

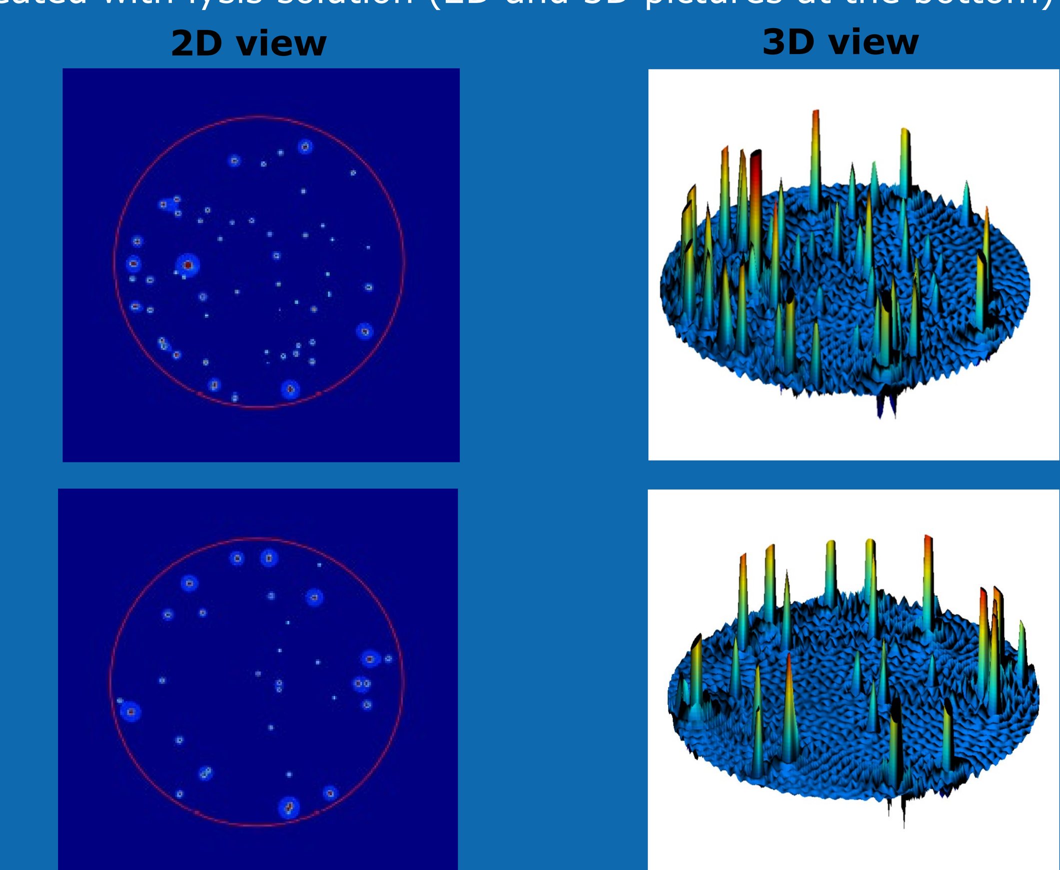
Results presented in table 2 and 3 show a proper detection of all microorganisms inoculated in CHO-S samples when selective lysis solution is applied according to the developed protocols. Recoveries are ranging from 70 to 106 %.

Milliflex® Rapid pictures

- Example of ATP background removal with the selective lysis solution on CHO-S sample

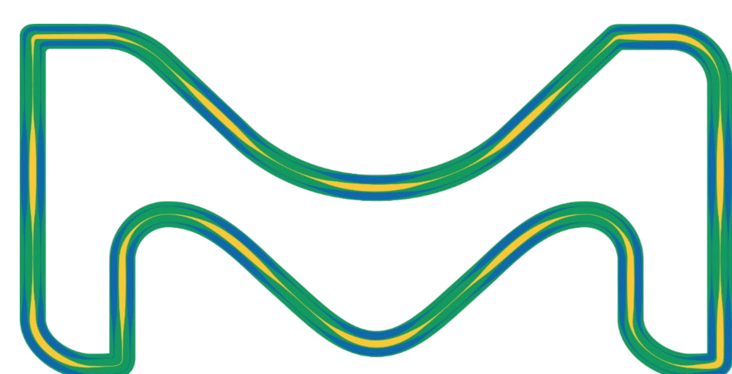


- Example of *C acnes* detection in the absence of CHO-S cells (2D and 3D pictures in the top) and in the presence of CHO-S cells treated with lysis solution (2D and 3D pictures at the bottom)



Conclusion

The selective mammalian cell lysis solution together with the developed sample preparation method enable the monitoring and the rapid detection of contamination in cell-based products with the Milliflex® Rapid System 2.0



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