Milliflex® Quantum: A Fluorescence–based Platform for the Rapid Detection of Contaminants in Filterable Products

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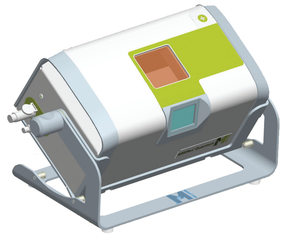
INTRODUCTION



Testing for microbial contamination in pharmaceutical production processes is necessary to monitor product quality. However, traditional microbiological methods are slow and require several days to obtain results. Therefore there is an increasing demand for rapid microbiological methods. Millipore's Milliflex Quantum system is a convenient and sensitive platform for the quantitative detection of contaminants in filterable samples. This rapid microbiological method is based on an universal enzymatic fluorescent staining of viable and culturable microorganisms. The fluorescent staining procedure is nondestructive, allowing downstream specific identification following a positive result. It was demonstrated that the Milliflex Quantum protocol is a fast and reliable alternative for the detection of microorganisms in water and complex matrices. The time to results were shorter than the time to results obtained with the compendial method.

PRINCIPLE OF DETECTION

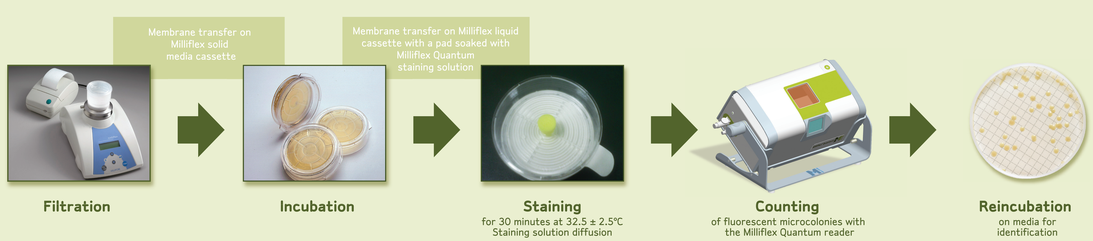
The principle of the fluorescence detection is based on an enzymatic reaction. The fluorogenic substrate used is a non–fluorescent viability marker that is cleaved by nonspecific intracellular enzymes resulting in a fluorescent product. Accumulation of fluorescence inside cells is an indicator of microbial metabolism activity and membrane integrity. The dye is diluted in a staining buffer allowing cell membrane permeabilization and thus dye introduction into cells.

MATERIAL AND METHODS

(A) Sterile water artificially contaminated with ATCC or environmental germs or **(B)** inprocess nonsterile water samples from pharmaceutical plants were filtered over mixed cellulose ester membranes using the Milliflex PLUS pump system. After incubation on Milliflex cassettes prefilled with Tryptic Soy Agar (TSA) or R2A agar at 32.5 ± 2.5 °C, or Sabouraud Dextrose Agar (SDA) at 22.5 ± 2.5 °C, membranes were transferred onto a cellulose pad soaked with the Milliflex Quantum staining solution and incubated for 30 minutes at 32.5 ± 2.5 °C. Fluorescent microcolonies were counted using the Milliflex Quantum reader (specific LED system). After detection, membranes were reincubated on media for traditional plate count (viability assay) and contaminants identification using the MicroSEQ® platform (Applied Biosystems). Compendial method was performed in parallel to control results concordance between both methods. Traditional plate count was done after a 7- day incubation. Fluorescent microcolonies counts and visual counts after reincubation of stained membranes were compared to results from compendial method.

(C) Chinese Hamster Ovary (CHO) cells culture from 4 to 6.10^6 cells/mL was spiked with *Bacillus cereus*, *Staphylococcus epidermidis* or *Propionibacterium acnes* and incubated at least for 16 hours at 37 °C. The culture was then filtered over mixed cellulose ester membranes and incubated on Milliflex cassettes prefilled with TSA at 37 °C. After the incubation period, membranes were stained following the procedure described above.

Milliflex Quantum Protocol Overview

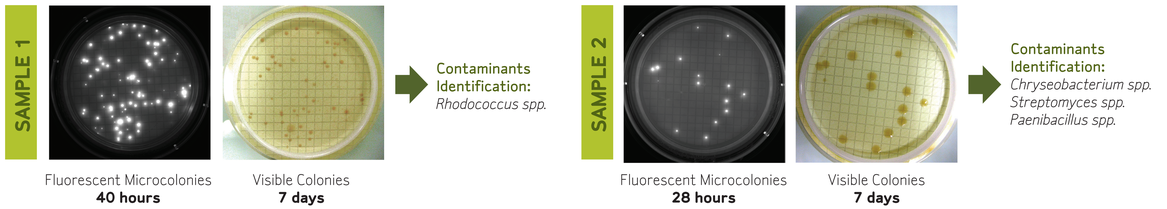


RESULTS

A Sterile Water Artificially Contaminated with ATCC or Environmental Germs

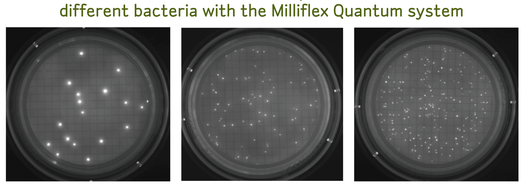
Microorganisms		Incubation Condition	Incubation time required for Milliflex Quantum detection
Escherichia coli	ATCC 8739	TSA 32.5 ± 2.5°C	8 hours
Pseudomonas aeruginosa	ATCC 9027		12 hours
Bacillus subtilis	ATCC 6633		8 hours
Staphylococcus aureus	ATCC 6538		12 hours
Candida albicans	ATCC 10231	SDA 22.5 ± 2.5°C	24 hours
Aspergillus brasiliensis	ATCC 16404		30 hours
Caulobacter spp.	Environmental strains	R2A 32.5 ± 2.5°C	30 hours
Micrococcus lylae			16 hours
Ralstonia spp.			24 hours

B Inprocess Nonsterile Water Samples from Pharmaceutical Plants



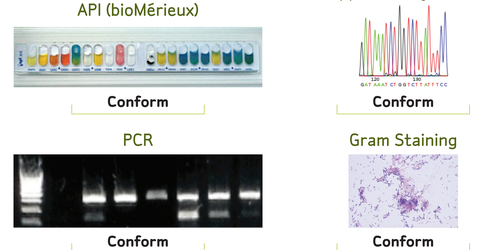
C Detection of Contaminants in Mammalian Cell Culture

Incubation time (on TSA at 37°C) required for the detection of different bacteria with the Milliflex Quantum system



B. cereus **8 hours** | *S. epidermidis* **9 hours** | *P. acnes* **48 hours**

D Identifications



CONCLUSION

Spiked or environmental germs were efficiently and rapidly detected with the Milliflex Quantum protocol with a 3 to 5 times reduction of the incubation time compared to the compendial method. As the method is nondestructive, each fluorescent microcolony detected will continue to grow to yield visible colonies allowing the identification of the contaminants using any available identification method.

The study demonstrated that the Milliflex Quantum platform could easily replace the traditional microbiological method with up to 5 days saved for slow growing strains. This method was also efficiently applied to the detection of contaminants in mammalian cell culture showing that the Milliflex Quantum system can be easily adapted to complex matrices. Detecting contamination early allows production to implement corrective action sooner.