A FLOW CYTOMETRY-BASED SYSTEM FOR DETECTION OF BACTERIAL CONTAMINATIONS IN CELL CULTURES

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The control of cell cultures for microbial contaminations is mandatory to set up reliable immunological assays and vaccine production procedures. Microbial contaminations derive from multiple sources and include bacteria, fungi (moulds and yeasts), mycoplasma and viruses. Bacterial contaminations are detected by visual inspection of cell cultures and/or bacteriological procedures. The sensitivity of these is often less than optimal, which may give rise to despicable delays in the detection of contaminated cultures and serious downstream losses. In particular, there is evidence that bacteriological media cannot provide suitable growth conditions for some slow-growing bacterial species and intracellular phases of bacteria such as staphylococci [1]. Moreover, several bacterial pathogens undergo mutations in their environment in order to survive and establish an infection. Many stressors are known to affect their size, growth, division and metabolism [2] and novel control procedures are badly needed. Flow cytometry has been used for a long time to detect bacteria [3], yeasts and fungi [4]. On the basis of these findings, we developed a flow cytometry-based detection procedure of bacterial contaminations of cultured cells and tissue culture media. The protocol is based on two dyes binding to nucleic acids of viable and dead bacterial cells, respectively. Our results showed that it is possible to discriminate the scatter and fluorescence profiles of bacteria from those of nucleoprotein particles released from necrotic and apoptotic cells (non-specific staining). Therefore, a bacterial contamination gate was defined on the basis of both forward scatter and fluorescence, and a threshold number of events in the gate was reckoned following examinations of several uncontaminated cell cultures of different origin, type (fibroblast, epithelial, mesenchymal) and species. Our procedure was shown to detect experimental bacterial contaminations within 4-5 hours of the inoculation. Most important, contaminated cell cultures of our diagnostic laboratory were revealed even before a positive bacteriological test. Each experimental contamination of cell cultures was carried out using one bacterial species at a time. Mycoplasma spp. was not investigated. Owing to the above, this novel and rapid test procedure has a considerable potential for routine applications and is conducive to more reliable, robust sterility controls of cell cultures and also immunological products like vaccines.

[1] Sendi P, Proctor RA. Staphylococcus aureus as an intracellular pathogen: the role of small colony variants. Trends Microbiol., 17(2):54-58, 2009. [2] Baatout et al. Physiological changes induced in bacteria following pH stress as a model for space research. Acta Astronautica, 60:451-459, 2007. [3] Winson MK, Davey HM. Flow cytometric analysis of microorganisms. Methods: Methods in Enzymology, 21:231-240, 2000. [4] Paau et al. Flow-microfluorometric analysis of Escherichia coli, Rhizobium meliloti, and Rhizobium japonicum at different stages of the growth cycle. Can J Microbiol., 23(9):1165-1169, 1977.