

## A feasibility study for the optimization of Biesse Bioscreen as benchless detector for microplastics in liquid media

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Plastics is an essential commodity of modern society. Although not yet defined as a persistent, bioaccumulative or toxic pollutant as such, concerns regarding the observed and predicted negative effects of plastic materials on the environment, with specific regard to plastic littering, have been raised by the scientific community (1). Globally increasing production, continued and uncontrolled release and plastic deterioration into smaller fractions urges the understanding of ecosystem tolerance and effects of plastic and microplastics on organisms. Microplastics are not a specific kind of plastic but rather any type of plastic fragment that is less than five millimeters in length (MC, defined as <5mm). Two classifications of microplastics currently exist. Primary microplastics are any plastic fragments or particles that are already 5 mm in size or less before entering into the environment. These include microfibers from clothing, microbeads, and plastic pellets (2,3,4). Secondary microplastics are microplastics that are created from the deterioration of larger plastic products once they enter the environment through natural weathering processes. Such sources of secondary microplastics include water and soda bottles, fishing nets, and plastic bags (5). Both types are recognized to persist in the environment at high levels, particularly in aquatic and marine ecosystems. Additionally, plastics degrade slowly, often over hundreds if not thousands of years. This increases the probability of microplastics being ingested and incorporated into, and accumulated in, the bodies and tissues of many organisms (6). The entire cycle and movement of microplastics in the environment is not yet known, but several lines of evidence suggest that microplastics pollution impacts both marine and freshwater ecosystems. Many studies have investigated the presence of microplastics in both natural ecosystems or other systems such as effluents from wastewater treatment plants. However, there is still a pressing need for adequate and reproducible microplastics detection methods and it is within this framework that we are working.

We recently evaluated the instrument Bioscreen as a diagnostic tool for the detection of bacteria in urinculture (7). Bioscreen is an instrument developed by ASI (Milan, Italy) based on a technology patented in collaboration with the University of Urbana-Champaign, IL USA (patent number: US7,973,2944 B2; Jul. 5, 2011). The Bioscreen method allows measuring the concentration of fluorescent particles in a liquid medium (8, 9, 10, 11, 12, 13). The instrument is equipped with a rotating and translating sample holder. Bioscreen uses pattern recognition data analysis technique for measuring the concentrations and for characterizing fluorescent particles on the basis of size, shape, diffusion constant and/or composition.

In the attempt to apply this instrument to the detection of plastics we first selected a fluorescent probe able to bind plastic samples. We chose Nile red, a fluorescent dye that binds the surface of plastics but not most natural materials. We mainly focused on two synthetic plastic samples: PVC fragments of 250  $\mu\text{m}$  and HDPE fragments of 150  $\mu\text{m}$ . These two kinds of plastics were chosen as representative respectively of high density and low density plastics (PVC density= 1.393  $\text{g}/\text{cm}^3$  and HDPE density= 0.94  $\text{g}/\text{cm}^3$ ). As Bioscreen analyzes liquid samples, we also selected specific solvents for each type of plastic to keep the particles in suspension (i.e. avoiding them to float or to precipitate). Further we performed several experiments in function of the kind of analyzed plastics and its concentration. After the effectiveness of Bioscreen in detecting plastics was verified, we carried out some measurements on real samples. In particular, we analyzed samples of wastewater at the inlet or outlet of specific units of a municipal wastewater treatment plant. The samples were also analyzed by FTIR measurements. In comparing the different measurements, we found that Bioscreen was able to detect the presence of plastic in the same samples for which the FTIR did. These first results demonstrate that Bioscreen is a potentially useful tool for detecting microplastics in liquid media.

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