DEVELOPMENT AND APPLICATION OF MULTIPLEXED IMMUNOASSAY AS DIAGNOSTIC TOOL IN ECOTOXICOLOGY.

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Hazard assessment of endocrine disruptors raises nowadays great concerns for human health as well as for the biotope balance. Great efforts have already been devoted to the development of biosensors for the study of the impact of endocrine disruptors on vertebrates. Recently, European scientific experts have highlighted the need to develop similar tools for the identification of these endocrine disruptors and for the assessment of their toxic effects and mode of action on key physiological functions on invertebrates. Biomarkers are recognized as relevant tools for diagnostic and hazard assessment of aquatic systems, and gammarus, a small crustacean, is a validated sentinel species for aquatic ecotoxicology in Europe.
The availability of direct and accurate methods for measuring biomarkers in invertebrates is thus essential, but the lack of multiplex assays for field monitoring remains a limitation. Indeed, biomarkers are quantified using indirect approaches such as biochemical assays and ELISA which are specific for one protein at a time. Even if the use of HPLC-MS technology is growing in this field, the cost of the apparatus and the needed level of technicity are high. Consequently, the use of protein biomarkers for field monitoring is currently expensive and very time-consuming impairing their larger use in ecotoxicology.
As a part of a large ecotoxicology project, our aim was to develop a multiplex assay for the measurement of biomarkers in Gammarus, based on the specific detection of proteotypic peptides and their quantification.

After identification of the appropriate proteotypic peptides from the target biomarkers and development of the corresponding antibodies, Bio-Plex® 200 with the xMAP® Technology was used to develop a competitive 4-plex assay on MagPlex® microspheres.
A systematic study was done on the procedure employed for bead coupling varying the length and the nature of the spacer as well as the composition of the buffer. Several types of secondary antibodies and phycoerythrin conjugates were also tested. Incubation time and temperature variations were also studied. Single assays were first developed and validated before multiplexing the assay.

The nature of the spacer as well as the treatment of the MagPlex® microspheres by saturating agents after coupling have a great impact on the sensibility of the assay. Appropriate choice of surfactant in the buffer minimized bead aggregation. The type of the secondary antibodies is less critical but the purity of the phycoerythrin conjugate is important.

The choice of the competitive format avoided the development of antibody pair for sandwich immunoassay and appeared to be very efficient. The LLOQ of the assay varied from 50 to 500 pg/mL. Each single assay and the 4-plex assay were successfully validated with respect to various performance criteria. Current experiments are underway on various environmental samples to fully explore the efficiency and limitations of the assay.