**Doctoral Thesis (by Mgr Jindra Vrzalova) – Receiver Report**

**Multiplex xMAP immunoanalysis and examples of its application**

Biomarkers are referred to analytes in biological samples, or any measurement that predicted a disease state or response to a specific drug therapy. Developments in genomics and proteomics have renewed the interest in biomarkers as useful clinical tools. Biomarkers constitute a rational approach, which, at optimal level reflect the biology of the disease. Therefore it is a challenge to identify relevant biomarkers to implement them for critical clinical decisions.

Multiple immunoassays allow users to measure concentrations of a panel of factors simultaneously in a single assay. The Multiplex xMAP immunoanalysis is a test platform that carries out multiplex immunoassays in a manner similar to conventional sandwich ELISAs. The need for evaluation and validation of assays to be applied in clinical routine is mandatory, in particular to stratify patients, and this is also valid for xMAP applications.

The presented Thesis is focusing on a very interesting and important protein marker field - measuring panel biomarkers in the clinical situation with Luminex xMAP technology platform in oncological diagnostics. The scientific study of the protein marker xMAP immunoanalysis panels in this Doctoral Thesis is of high quality, and add new information how to apply xMAP technology in a proper way in oncological diagnostics. Strengths and weaknesses for Luminex xMAP technology platform have been examined in detail and is well demonstrated in the Doctoral Thesis.

- **Aim of the study** is well answered in the Thesis
- **Introduction** is quite acceptable dealing with presenting various immunoassays including proteomics. However, I lack a more detailed presentation of Ciphergen/Vermillion marker panel assays. In connection with Mass spectrometry for detection in proteomics SELDI is discussed but nothing about clinical applications in oncology. We see a number of SELDI applications in the literature in oncology eg ovarian cancer (Triage test).
- **Methods** - the basic presentation of xMAP technology is well reported as well as a number of alternative high-value diagnostic tests.
- **Clinical applications of xMAP immunoanalysis** reported in the literature is discussed in the Thesis (Protein expression profiling, Immunodiagnostics, Genetic diseases and Genomic research) and make a platform for the present investigation of xMAP immunoanalysis in oncology
- **Experimental part – preanalytical investigations** are very important demonstrating the differences in xMAP signal between selected body fluids, the sensitivity of various markers and long term stability – new technical characteristics generated for xMAP. The number of replicates, assayed are relatively limited indicating some weaknesses in calculated median and average levels ie differences in body fluids. However, this analytical approach demonstrates the performance characteristics of xMAP technology in particular for some inflammatory markers and growths factors. Furthermore, this put also a focus on the requirement of an Internal Quality Control as well as an External Quality Control Programme.
Experimental part – clinical part ovarian cancer; a very small patient cohort (pathological as well as benign) studied which markedly limits a more conclusive judgement regarding the clinical utility of the applied xMAP panel. The clinical definition of benign disease should be more precise for that patient cohort. It is well demonstrated in the Thesis that the xMAP panel for ovarian cancer is not acceptable in comparison with single markers. Table 14 should be revised – the magnitude for some markers are not correct written, sensitivity should be presented with one decimal as well as CV (%) and cut off - in particular due to the small number of patients.

Experimental part – clinical part metastatic process; the outcome of xMAP human bone panel appears similar to the individual bone biomarkers. However, the patient material is very small which definitely reduces the overall conclusion regarding clinical capacity of xMA technology. An extensive statistical evaluation were performed on the biomarker test data even if the generated marker data is based upon a very small patient cohort. If we are critical we could perhaps say that the small number of observations cannot be overlooked. A comprehensive discussion of bone markers and their potential applications in clinical routine are performed. Some educational parts (basic information) are included in the discussion of data in this section and these parts may be put in introduction of “metastatic process” in order to make the discussion more focused on scientific comparison. It is quite obvious from presented results that data generated with xMAP human bone panel appears to come out very good.

Experimental part – clinical part prostate cancer; this section is very well presented in Thesis with a balanced presentation of generated data in relation to reported data in literature. In contrast to earlier clinical sections there are a definitely higher number of patients included for analysis, but not sufficient for a proper statistical treatment of subdivided groups. The number of PIN-patients are extremely low to be considered in a statistical evaluation and the outcome may only be a tendency.

Conclusions – most important findings generated with xMAP protein panels in this Thesis is satisfactory summarized. Consequently, the aim of the experimental part of Thesis is fulfilled. Strengths of xMAP technology (small sample volumes, many analytes assayed in parallel, xMAP results correlate well with many traditional methods) as well weaknesses of xMAP technology (selection of body fluids, limitations in composition of existing xMAP panels, not sufficient sensitivity, too large differences in analyte concentrations) are discussed.

References – the written references in the reference list do not follow International rules for publications. Perhaps you follow a local rule otherwise the style should be accordingly: Authors, title, journal in short, volume, pages and year.

I will also make the author observant on some general and small points that may be considered in the final version of the Thesis;

- It is pointed out in Conclusions that it is pilot projects in the experimental part where xMAP technology has been applied but this might also be more focused in the different clinical sections ie data generated must be checked in larger and relevant patient groups
- The small and condensed introduction to each clinical application section is good but some parts in Discussion in particular “metastatic process” should be moved to introduction
- Linguistic corrections should be performed throughout Thesis (grammar, a checking of spelling, selection of words etc) if it is an intention to publish the material in English. The language is quite OK but there are some errors that may be corrected.
- In the Analytical Section (4.1.1) you have Results interpretation before Results.
- References should be given consequent in the text throughout the Thesis.

Stockholm, Sweden

March 18, 2009

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