CYP2D6 NEW GENOTYPING METHODS

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Background :

Cytochrome P450 2D6 is a highly polymorphic and well-characterised drug-metabolizing enzyme. It is responsible for the biotransformation of about 20% of all currently prescribed drugs. More than 80 alleles have been described and CYP2D6 genotype is related to CYP2D6 activity. Several techniques have been developed to genotype CYP2D6 such as Multiplex PCR, RealTime PCR, SNapshot, ... but none of them include the analysis of the most relevant alleles in a single assay. Furthermore, the presence of highly homologous pseudogenes and the possibility to get gene duplications or deletions make genotyping of CYP2D6 even more challenging.

Luminex xTAG® technology is a new easy genotyping method that allows the simultaneous detection of most relevant alleles found in different ethnicities as well as deletions and duplications. Multiplex Ligation-dependent Probe Amplification® (MLPA) is quite recent approach allowing a semi-quantitative detection of gene copy number (potentially interresting for deletions and duplications).

The aim of the present study was on one hand to compare the Luminex multiplex genotyping technique with commonly used TaqMan® assays and on the other hand to compare the Luminex assay with MLPA.

Methods :

97 DNA from Brazilian women with breast cancer treated with tamoxifen were screened for CYP2D6 polymorphisms. DNA was extracted by a Salting Out method and by an automated extraction method (Magna Pure Compact® (*Roche Diagnostics*)). DNA was analyzed by the Luminex xTAG® CYP2D6 Kit v3 (*Luminex Corporation*) and for all patients, 7 selected SNPs were compared with the TaqMan® assays (TaqMan® Drug Metabolism Genotyping Assays (*Applied Biosystems*)). All the samples were screened for duplications and deletion with Luminex® assay and SALSA® MLPA® kit P128 Cytochrome P450 (MRC Holland).

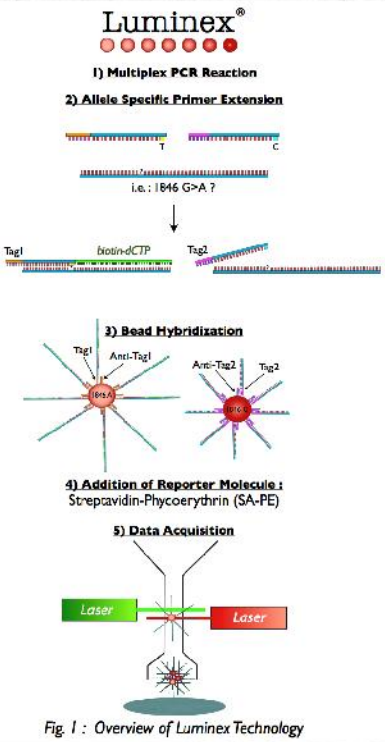

Fig. 1 : Overview of Luminex Technology

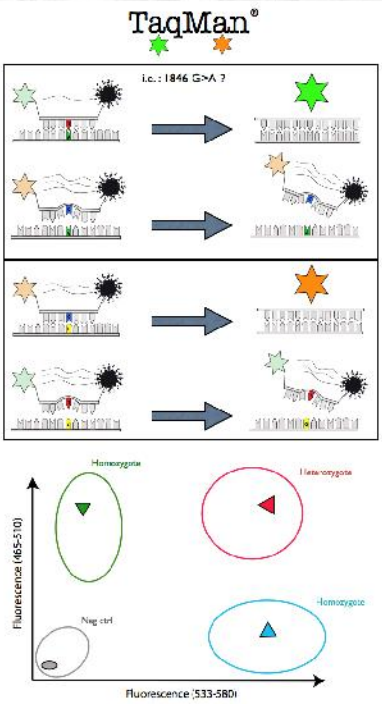

Fig. 2 : TaqMan detection (FAM-VIC)

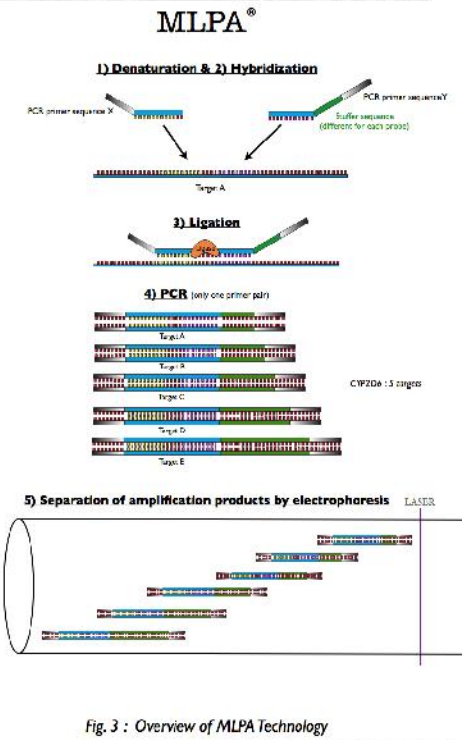

Fig. 3 : Overview of MLPA Technology

Results :

Similar results were obtained for both extraction methods indicating that automated techniques are also suitable for genotyping methods including long fragment PCR analysis as for CYP2D6. Ten commercial controls (*Coriell*) were tested by Luminex® and TaqMan® assays and gave successful results. Among the 679 SNPs analysed by both methods 673 gave concordant results (99,1%). There were a perfect match between the results of Luminex and MLPA for the detection of deletion and duplications. According to the well-known Brazilian ethnic intermixing, we found a wide range of different CYP2D6 genotypes using the Luminex® assay.

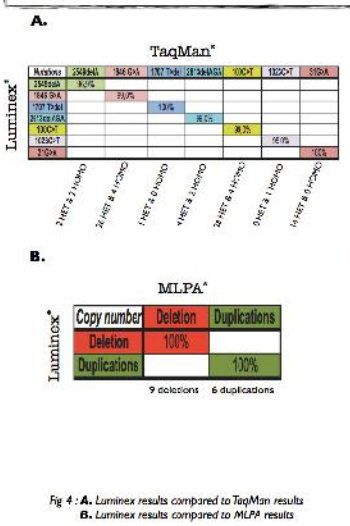

*Fig 4 : **A.** Luminex results compared to TaqMan results*
__B.__ Luminex results compared to MLPA results

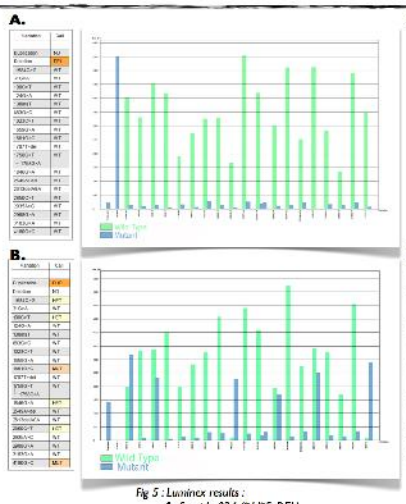

Fig 5 : Luminex results :
*__A.__ Sample 024 (*1/*5, DEL)*
*__B.__ Sample 006 (*2/*4, DUP)*

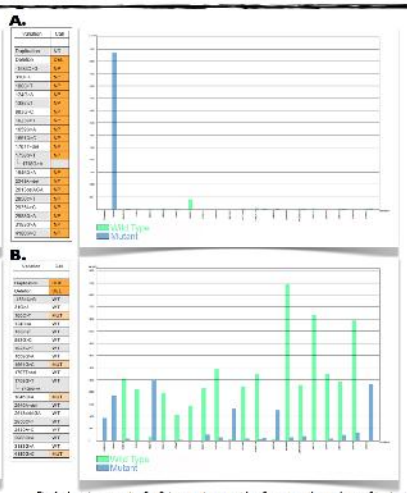

Fig 6 : Luminex results for 2 interesting samples from an other cohort of patients.
*__A.__ *5/*5 (DEL, DEL)*
*__B.__ *4/*5 (DEL, DUP)*

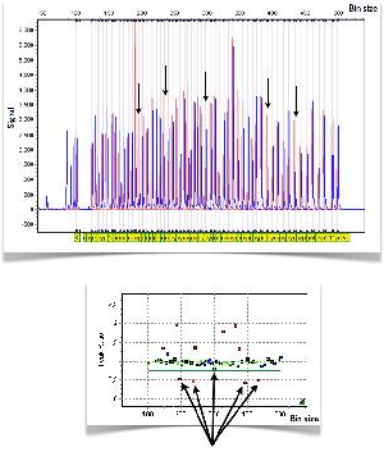

*Fig 7 : MLPA results for sample 024 (*1/*5, DEL).*

Conclusion :

Luminex xTAG® CYP2D6 kit v3 provides a powerful and easy handling technique allowing the determination of 19 SNPs on a single assay, corresponding to 15 different alleles. It has to be considered as an interesting alternative to the AmpliChip® CYP450 Test (*Roche Diagnostics*) for CYP2D6 genotyping of patients from different ethnicities. In comparison with other existing techniques, it allows a wider screening including CYP2D6 deletion (*5) and duplications analyses. MLPA is a powerful tool for the detection of deletion and duplications for the CYP2D6 gene.

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