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Evaluation of the INNO-LiPA *CFTR*iage test principle

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Background/Objectives

Nearly 2000 cystic fibrosis (CF)-associated mutations have been reported across the cystic fibrosis transmembrane regulator (*CFTR*) gene, some though with a very low frequency in the general population, which makes the detection of cystic fibrosis complex. Efficient and accurate population screening for cystic fibrosis requires an assay that can simultaneously detect multiple mutant and wild-type sequences of the *CFTR* gene. Contrary to cumbersome and more expensive sequencing-based approaches, reverse-hybridization assays, such as line probe assays (LiPA), are straightforward and easy to perform, not requiring expensive instrumentation. The LiPA assay is particularly advantageous since it is fast and simple to perform. Moreover, this assay is amenable to automation (Auto-LiPA, Fujirebio Europe, Gent, Belgium or TENDIGO, Fujirebio Europe, Gent, Belgium) and thus particularly suitable for clinical settings where multiple samples can be processed simultaneously. The LiPA assays are limited in the number of oligonucleotides that can be immobilized on a carrier and hence on the mutations that can simultaneously be analyzed. To ensure sufficient coverage of the *CFTR* mutations, in line with the newest guidelines and regulations, multiple LiPA assays (#5) need to be performed to complete the genetic analysis, which is time-consuming, requires more reagents, resulting in a higher cost.

As result, there is a need for efficient and simple methods that allow the simultaneous analysis of multiple *CFTR* mutations. More particularly, an improvement of the current reverse-hybridization-based approach, by increasing the number of *CFTR* mutations that can be analyzed without increasing the number of assays to be performed.

Methods

The INNO-LiPA *CFTR*iage is the solution to the above-mentioned problems. The INNO-LiPA *CFTR*iage is used for the simultaneous analysis of 88 *CFTR* mutations based on reverse-hybridization. To do so, an amplification product of the *CFTR* gene is hybridized with pools of oligonucleotide probes immobilized on the INNO-LiPA *CFTR*iage strip. Only when necessary, a second hybridization reaction on the relevant INNO-LiPA *CFTR* strip follows. In this case, the same amplification product of the INNO-LiPA *CFTR*iage can be reused to test the individual oligonucleotides probes that are comprised in the positive pool(s) from the INNO-LiPA *CFTR*iage strip. Thus, the positive pool(s) from the INNO-LiPA *CFTR*iage indicate(s) which reverse-hybridization assay(s) with individual oligonucleotide probes need(s) to be performed in the second hybridization reaction to complete the genetic analysis of *CFTR* mutations (Figure 1). Advantageously, this methodology allows to reduce the number of INNO-LiPA *CFTR* assays with individual oligonucleotide probes that need to be performed to complete the genetic analysis to a maximum of 2 additional strips.

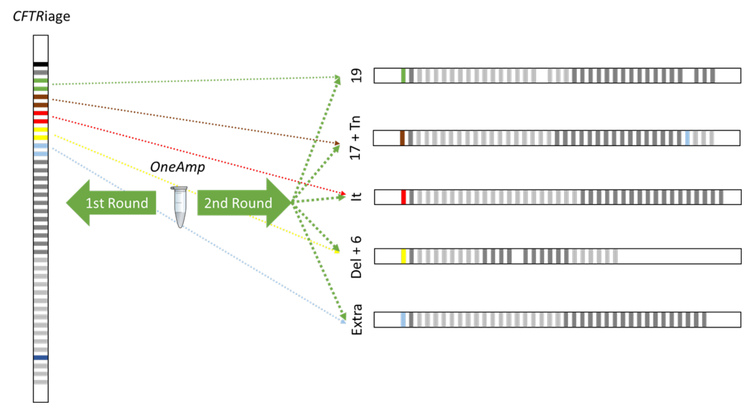

***Figure 1**: CFTRiage test approach: The (possibly) positive pools in the first hybridization (INNO-LiPA CFTRiage) indicate which reverse-hybridization assays with individual oligonucleotide probes need to be performed in the second hybridization reaction to complete the genetic analysis of CFTR mutations.*

To further reduce the number of INNO-LiPA *CFTR* assays, the INNO-LiPA *CFTR*iage strip (Figure 2) also contains, in addition to the pooled mutation lines, a series of 12 individual mutation lines and corresponding wild type probes of the most frequent *CFTR* mutations (Table 1). By this, the complete analysis of 88 mutations can be achieved in more than 90 to 95% of the samples after this first hybridization assay.

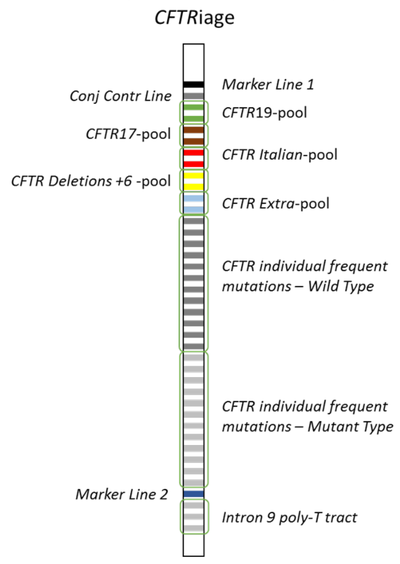

***Figure 2**: CFTRiage strip lay-out*

***Table 1**: List of the individual mutation lines on the CFTRiage strip with the respective allele frequency according to the CFTR2 variants list (March 2019)*

CFTR mutation	Frequency
F508del	69,74%
G542X	2,54%
G551D	2,10%
R117H	1,31%
N1303K	1,58%
W1282X	1,22%
621+1G->T	0,93%
1717-1G->A	0,86%
2789+5G->A	0,72%
A4455E	0,35%
*CFTR*dele2,3 (21kb)	0,29%
L927P	0,02%

Results

The INNO-LiPA *CFTR* portfolio exists of 5 different typing kits each designed for a particular set of individual *CFTR* mutations (INNO-LiPA *CFTR* 19 (#18); INNO-LiPA *CFTR* 17+Tn Update (#17+Tn); INNO-LiPA *CFTR* Italian Regional (#21); INNO-LiPA *CFTR* Deletions+6 (#14); INNO-LiPA *CFTR* Extra (#18)). To check for all these mutations, 5 amplification and hybridization assays have to be performed. Knowing that the goal for *CFTR* testing is to find 2 compounds heterozygous *CFTR* mutations in affected patients, the introduction of the INNO-LiPA *CFTR*iage reduces the complete mutation analysis to maximum 3 hybridization assays using 1 amplification. Because the most frequent mutations are also present on this INNO-LiPA *CFTR*iage strip this number of hybridization assays will be reduced to only 1 in 90 to 95% of the cases.

CONCLUSION

The INNO-LiPA *CFTR*iage is a simple method that allows the simultaneous analysis of multiple *CFTR* mutations in one run despite the availability of limited probe positions and this by pooling relevant mutation sequences on one probe location on the INNO-LiPA *CFTR*iage strip. Thanks to this together with the availability of the most frequent *CFTR* mutations as individual lines on the INNO-LiPA *CFTR*iage strip the *CFTR* analysis of 88 *CFTR* mutations can be reduced from 5 assays to 1 assay (in 90% to 95% of the cases) and with a maximum of 3 hybridization assays to obtain the same result.