Multicenter Validation Study of a Novel StripAssay for Cystic Fibrosis

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Introduction

Cystic fibrosis (CF), with an incidence of approximately 1 in 3000 live births in Caucasians, is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene. To date, more than 1500 CFTR mutations have been described, the majority being very rare or private. Worldwide, the most frequent mutation F508del accounts for 30-72% of CF chromosomes depending upon ethnicity. Overall there is great heterogeneity in the remaining pathogenic mutations, as type and distribution vary substantially between populations.

Methods

We have developed a reverse-hybridization assay for the rapid and simultaneous analysis of 34 CFTR mutations, as well as the IVS8 polyT (5T/7T/9T) variants. In the first validation phase 117 pretyped DNA samples from different CF centers across Europe were used to validate the CF StripAssay.

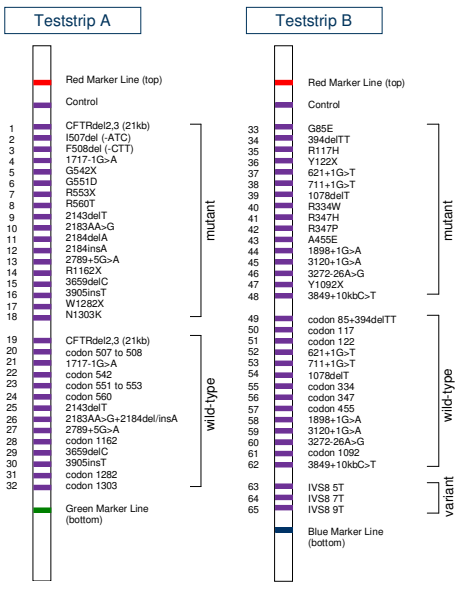
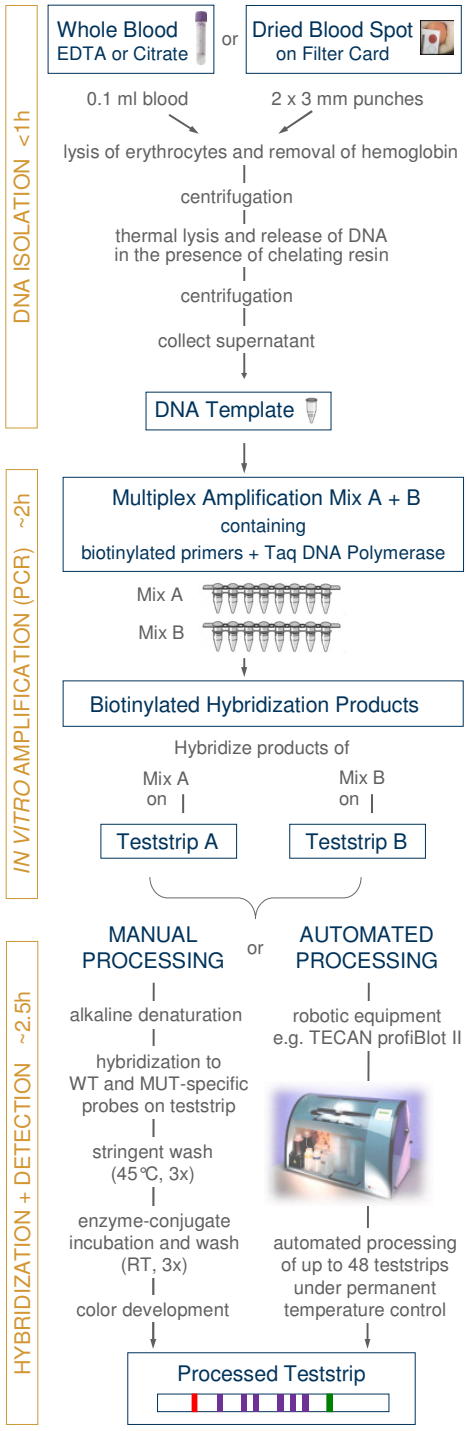


Figure 1. Schematic Teststrips of CF StripAssay.

Assay Procedure



Results

Mutation	SA	IH	Mutation	SA	IH
F508del	58	58	A455E	2	2
G551D	7	7	3272-26A>G	2	2
621+1G>T	6	6	Y1092X	2	2
G542X	6	6	R1162X	2	2
3849+10kbC>T	6	6	711+1G>T	1	1
2183AA>G	6	6	1078delT	1	1
2184insA	5	5	R560T	1	1
R334W	5	5	3120+1G>A	1	1
R553X	5	5	3905insT	-	-
3659delC	5	5	*R117C*	-	2
N1303K	5	5	*711+3A>G*	-	2
CFTRdel2,3(21kb)	4	4	*S549L*	-	2
394delTT	4	4	*1525-1G>A*	-	1
R347P	4	4	*I506L*	-	1
1717-1G>A	4	4	*1677delTA*	-	1
2184delA	4	4	*R553Q*	-	1
2789+5G>A	4	4	*1811+1G>C*	-	1
W1282X	4	4	*1898+1G>C*	-	1
R117H	3	3	*Del ex11 - part ex12*	-	1
G85E	3	3	*Del part ex12 – 14A*	-	1
1898+1G>A	3	3	*D1152H*	-	1
2143delT	3	3	*3600+2insT*	-	1
I507del	2	2	*R1158X*	-	1
Y122X	2	2	*R1162L*	-	1
R347H	2	2	*G1349D*	-	1
Wild-type				62	43
Total				**234**	**234**

Table 2. CF alleles identified with CF StripAssay (SA) and In-House (IH) methods in 117 DNA samples. Mutations in *italics* are not present on teststrips.

Genotyping results from 111 out of 117 (95%) pretyped samples were confirmed by the CF StripAssay. Discrepant results were all due to probes not present on teststrips, and in one case due to deletional mutations that were not detectable by the StripAssay.

Summary

- **CONVENIENCE:** ready-to-use reagents and prefabricated teststrips; inexpensive equipment (thermocycler, waterbath, shaker); reliable results.
- **SAMPLE SIZE:** requires small amounts of samples (10-50 ng DNA), which is of particular importance for prenatal diagnosis and newborn screening.
- **EFFICIENCY:** accessible to automation using robotic equipment and interpretation software (StripAssay Evaluator).

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