A Laboratory Developed, 106 Mutation Cystic Fibrosis Carrier Screening Test Using the Agena MassARRAY®: Performance in the first 123,000 Tests

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Abstract

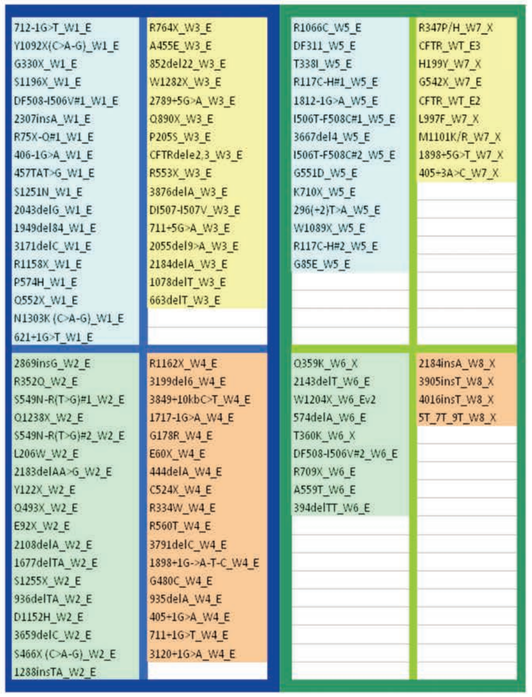
Introduction: In 2001 and 2004, the ACOG and ACMG recommended population-based carrier screening for cystic fibrosis (CF) using a panel of 23 mutations. Since that time, some have argued that expanded panels with additional mutations would be useful in some populations. We co-developed a CF screening test with Sequenom, Inc. that targets 106 mutations. Analytical verification of the assay has been presented (Farkas et al. JMD 2010;12:611-9). Here we report the data obtained from the first 123,172 test preformed at Mayo Clinic, and ask whether extended carrier screening panels can increase the sensitivity of CF carrier detection.

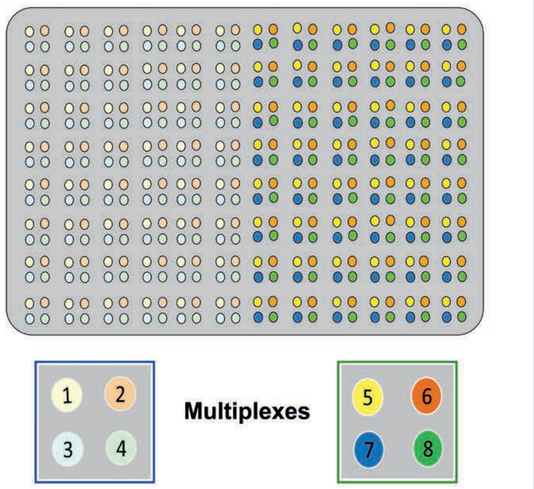
Methods: The MassARRAY® CF screening test was validated according to CLIA guidelines. The workflow consists of 8 multiplex PCR reactions, removal of dNTPs and primers with Agencourt® AMPure® XP resin (Beckman-Coulter), 8 multiplex single base extension (SBE) reactions, desalting with an ion exchange resin, spotting the extended SBE products onto a MassARRAY chip, and analysis by MALDI-TOF mass spectrometry. 48 samples and controls are run per batch in 384 well plates. All steps are automated, and the test requires 9 hours to complete. The method requires only general purpose laboratory reagents, thus the test is inexpensive at under $30 per reaction. Since validation, the MGL has tested 123,172 clinical samples.

Results: Validation of accuracy included analysis of a set of 43 samples that contained 60 different mutations and potential interferences (such as E75Q and 711+3A>G), 28 of these samples were compound heterozygotes. In addition, 594 samples were tested in parallel (15 runs) with the current Luminex 70+5 commercial assay, 25 of these samples had one mutation detected by the Luminex assay. For both sets of samples, there were no discordant results.

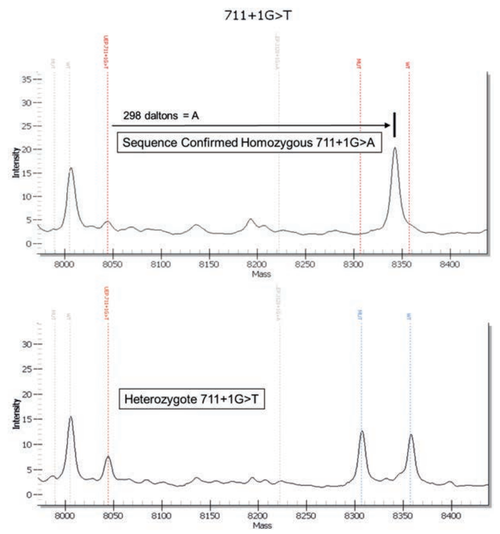
Discussion: Of the clinical samples tested, 3,153 were for a possible or known diagnosis of CF, pancreatitis, or male infertility; the remainder had indications for testing of routine carrier screening, a family history of CF, a partner that was a known CF carrier, or no indication given. Of the samples tested, 472 had two mutations (226 homozygotes and 246 compound heterozygotes). Of these, there were 203 deltaF508 homozygotes, and 184 cases with one deltaF508 with another mutation. There were 4,647 cases with one CF mutation identified. Of the 123,172 total cases tested, this represents a 3.8% carrier frequency, which is concordant with literature estimates. These numbers do not include 498 carriers of one copy of the R117H variant with a 5T allele. These results are reported, but not as CF carriers – rather carriers of a mild variant not typically associated with classical CF.

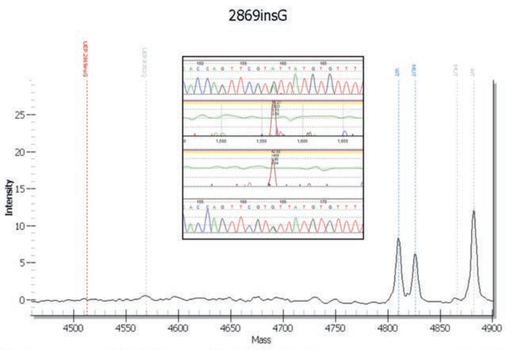
Methods





Example Interesting Cases



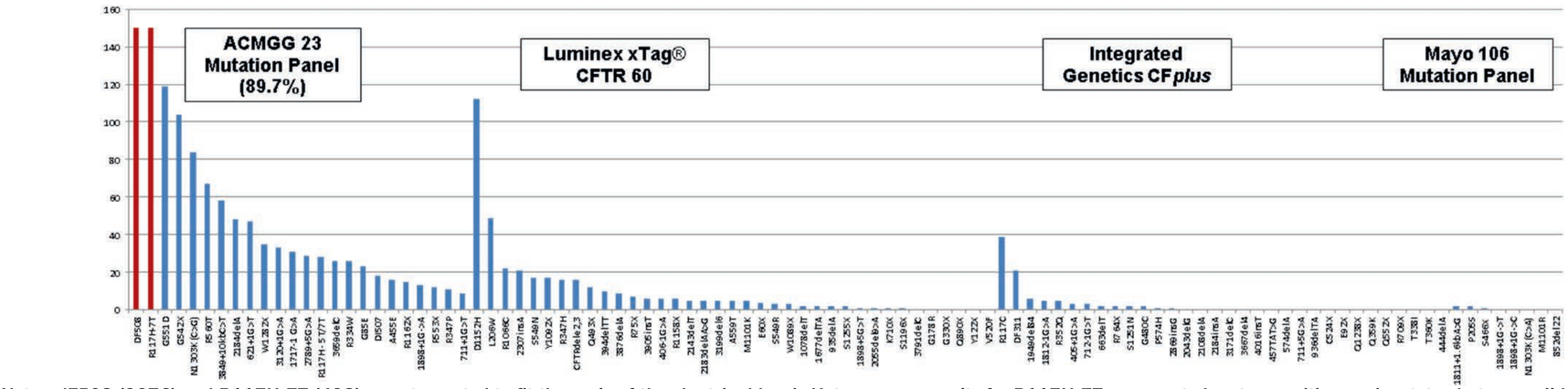


Probe	UEP.2869insG	CCAGCACCAGTTCGT	4512.90
Analyte	WT	CCAGCACCAGTTCGTA	4810.20
Analyte	MUT	CCAGCACCAGTTCGTG	4826.20

Note: The 2869A>G point mutation was detected during follow-up sequencing for the 2869insG alteration. This marker will remain on the follow-up sequencing list.

Results

Distribution of mutations identified compared to the ACMGG recommended 23 mutation panel and two commercially available extended panels.



Note: dF508 (3276) and R117H-7T (498) were truncated to fit the scale of the chart (red bars). Heterozygous results for R117H-7T are reported, not as positive carrier status but as a mild variant.

Discussion

We collaborated with Sequenom (now Agena Biosciences) to develop a novel, 106 mutation assay designed for high throughput, cystic fibrosis carrier screening using the Mass Array® MALDI-TOF platform. The method has proven to be robust, user friendly, and compatible with a high degree of automation. We have used it to process over 120,000 samples. The observed carrier rate of 3.8% is in agreement with published values.

We confirm the value of extended panel testing for CF carrier screening, with slightly over 10% of the positives being outside of the ACMGG 23 mutation panel.