Preliminary Studies of Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight for Detection of *CFTR* Variants

Agena® BIOSCIENCE

P673

Victoria M. Pratt Ph.D.[1], Andrew Bradford Ph.D.[1], Katarzyna Gawlik Ph.D.[1], Laura Mendez B.S.[1], Priscilla Hunt B.S.[1], Parastou Sadatmousavi M.S.[1], Vineet K. Dhiman Ph.D.[2], Dylan Hendricks M.S.[2], Gajendra Katara Ph.D.[3], Christine Banaszak M.S.[3], Matthias Jost Ph.D.[1], Glenn Sawyer B.S., M.B.A.[1]

[1]Agena Bioscience, San Diego CA | [2]Illinois Department of Public Health, Chicago IL | [3]Alverno Laboratories, Hammond IN

BACKGROUND

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is a targeted method to detect known genetic variants. Using MALDI-TOF, we initially developed an assay for 119 variants in the *CFTR* gene (Table 1). A preliminary assay was designed, tested for feasibility internally and sent to two volunteer laboratories for evaluation.

MATERIALS & METHODS

Assays for the targeted variants were designed using the online Agena Assay Design Suite (ADS, v2.0, San Diego CA). Primer sequences were aligned in silico against the GRCh38 reference genome. Primer–primer interactions within multiplex reactions were evaluated, and any primers exhibiting predicted interactions were redesigned and re-analyzed. This iterative process was repeated until no predicted interactions remained among primers within the same reaction well. A total of six wells were required to multiplex all variants of interest.

Genomic DNA is subjected to multiplex PCR amplification followed by shrimp alkaline phosphatase (SAP) to remove unincorporated dNTPs. In a subsequent reaction, probes that are adjacent to the variant of interest anneal to the PCR products and undergo single base extension. The detection platform uses matrix-assisted laser desorption/ionization time-of-flight mass spectrometry technology to determine the extension oligonucleotides mass and differentiate variants.

Reference materials were sourced from Coriell Cell Institute for Medical Research (Camden NJ) and Maine Molecular Quality Control, Inc. (Saco ME). DNA extracted from dried blood spots, buccal swabs, saliva, and whole blood was used for the studies. Feasibility studies were performed by multiple operators on samples using 2 different Agena MassARRAY Systems (San Diego CA) on different days, using both the 96- and 384-well format. The following parameters were guard-banded to determine optimum parameters and robustness: annealing temperature (58-62°C), PCR ramp rate (3-6°C/s), PCR reaction mix (0.5-1.5X), unincorporated extension primers (0.5-1.5X), $MgCl_2$ concentration (4.0±0.5mM) and recommended sample inputs (2-50ng).

Testing was performed by 2 external laboratories to determine accuracy and analytical sensitivity and specificity from orthogonally tested samples (e.g., next generation sequencing and MassARRAY). The external sites used DNA extracted from whole blood using a Roche MagNA Pure (Pleasanton CA) and dried blood spots using a laboratory-developed assay extraction method. Thermal cyclers used included: Applied BioSystems Incorporated (ABI) ProFlex, ABI Verti Pro, and ABI Verti (ThermoFisher, Waltham MA).

Table 1. *CFTR* 100+ Panel Content

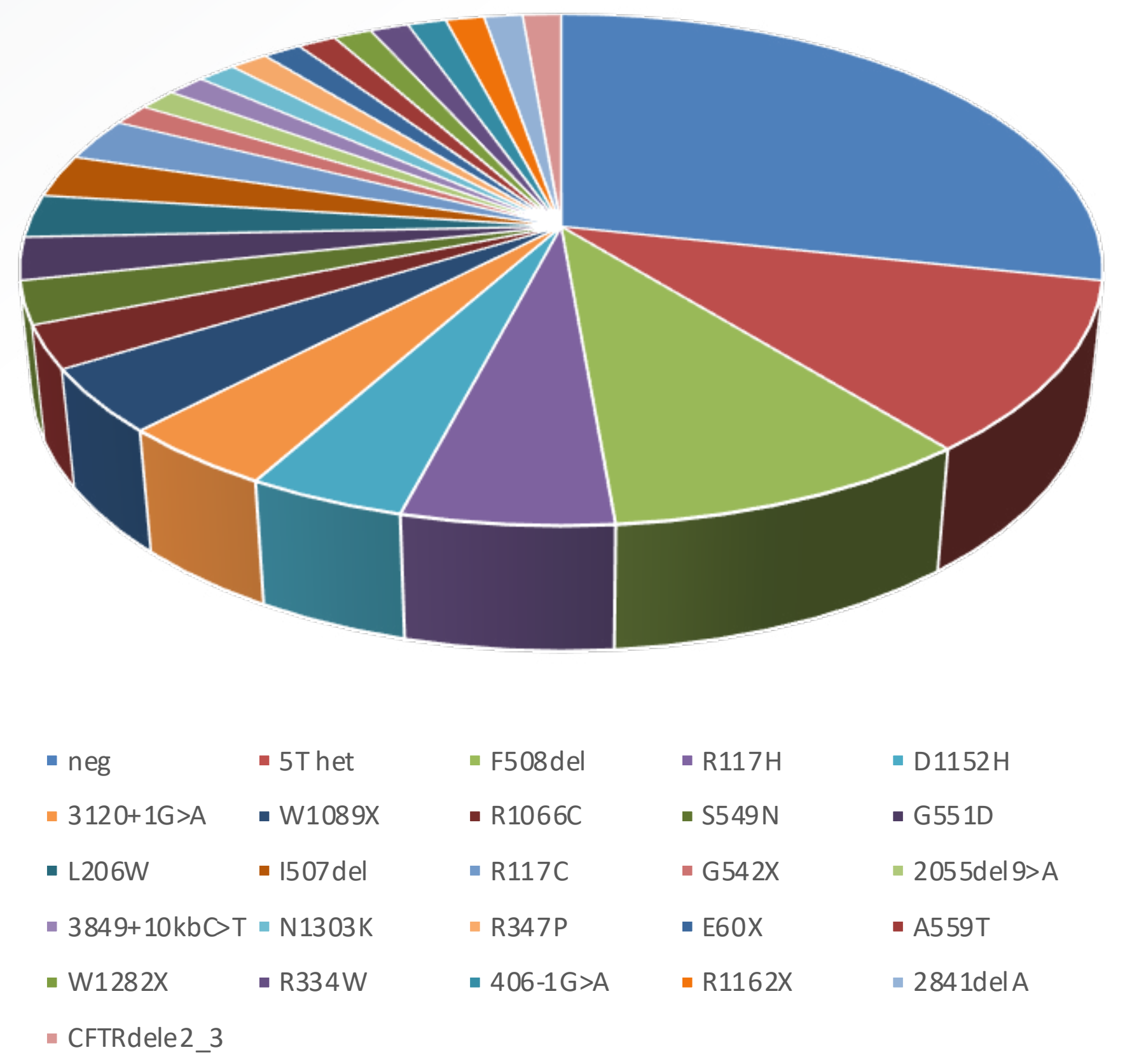
Legacy Name	rsID#	HGVS cDNA (NM 000492.4)	HGVS (GRCH38) (NC_000007.14)	ACMG Status
Q2X	rs397508740	c.4C>T	g.117480098C>T	Recommended
E60X	rs77284892	c.178G>T	g.117509047G>T	Recommended
P67L	rs368505753	c.200C>T	g.117509069C>T	Recommended
R75X	rs121908749	c.223C>T	g.117509092C>T	Recommended
G85E	rs75961395	c.254G>A	g.117509123G>A	Recommended
394delTT	rs121908769	c.262_263del	g.117509131_117509132del	Recommended
G91R	rs121908750	c.271G>A	g.117509140G>A	Recommended
406-1G->A	rs121908792	c.274-1G>A	g.117530898G>A	Recommended
Q98X	rs397508461	c.292C>T	g.117530917C>T	Recommended
Q98R	rs397508464	c.293A>G	g.117530918A>G	Recommended
444delA	rs121908801	c.313del	g.117530938del	Recommended
D110H	rs113993958	c.328G>C	g.117530953G>C	Recommended
R117C	rs77834169	c.349C>T	g.117530974C>T	Recommended
R117H	rs78655421	c.350G>A	g.117530975G>A	Recommended
621+1G->T	rs78756941	c.489+1G>T	g.117531115G>T	Recommended
F191V	rs141482808	c.571T>G	g.117534357T>G	Recommended
711+1G->T	rs77188391	c.579+1G>T	g.117534366G>T	Recommended
711+3A->G	rs397508761	c.579+3A>G	g.117534368A>G	Recommended
L206W	rs121908752	c.617T>G	g.117535285T>G	Recommended
L218X	rs397508777	c.653T>A	g.117535321T>A	Recommended
V232D	rs397508783	c.695T>A	g.117535363T>A	Recommended
935delA	rs121908772	c.803del	g.117536607del	Recommended
Q290X	rs397508808	c.868C>T	g.117536672C>T	Recommended
G330X	rs79031340	c.988G>T	g.117540218G>T	Recommended
R334W	rs121909011	c.1000C>T	g.117540230C>T	Recommended
T338I	rs77409459	c.1013C>T	g.117540243C>T	Recommended
1154insTC	rs387906360	c.1021_1022dup	g.117540251_117540252dup	Recommended
1161delC	rs121908774	c.1029del	g.117540259del	Recommended
R347H	rs77932196	c.1040G>A	g.117540270G>A	Recommended
R347P	rs77932196	c.1040G>C	g.117540270G>C	Recommended
R352Q	rs121908753	c.1055G>A	g.117540285G>A	Recommended
1288insTA	rs121908785	c.1155_1156dup	g.117542054_117542055dup	Recommended
1461ins4	rs397508189	c.1327_1330dup	g.117548758_117548761dup	Recommended
A455E	rs74551128	c.1364C>A	g.117548795C>A	Recommended
V456A	rs193922500	c.1367T>C	g.117548798T>C	Recommended
1504delG	rs397508196	c.1373del	g.117548804del	Recommended
1525-1G->A	rs397508200	c.1393-1G>A	g.117559463G>A	Recommended
S466X	rs121908805	c.1397C>A	g.117559468C>A	Recommended
L467P	rs139573311	c.1400T>C	g.117559471T>C	Recommended
I507del	rs121908745	c.1519_1521del	g.117559590_117559592del	Recommended
F508del	rs113993960	c.1521_1523del	g.117559592_117559594del	Recommended
C524X	rs121908754	c.1572C>A	g.117559643C>A	Recommended
1716+1G->A	rs397508230	c.1584+1G>A	g.117559656G>A	Recommended
1717-1G->A	rs76713772	c.1585-1G>A	g.117587738G>A	Recommended
G542X	rs113993959	c.1624G>T	g.117587778G>T	Recommended
S549N	rs121908755	c.1646G>A	g.117587800G>A	Recommended
S549R	rs121909005	c.1645A>C	g.117587799A>C	Recommended
G551S	rs121909013	c.1651G>A	g.117587805G>A	Recommended
G551D	rs75527207	c.1652G>A	g.117587806G>A	Recommended
R553X	rs74597325	c.1657C>T	g.117587811C>T	Recommended
L558S	rs193922504	c.1673T>C	g.117587827T>C	Recommended
A559T	rs75549581	c.1675G>A	g.117587829G>A	Recommended
R560T	rs80055610	c.1679G>C	g.117587833G>C	Recommended
1811+1G->A	rs397508263	c.1679+1G>A	g.117587834G>A	Recommended
1811+1634A->G	rs397508266	c.1680-886A>G	g.117589467A>G	Recommended
R560S	rs397508267	c.1680A>C	g.117590353A>C	Recommended
A561E	rs121909047	c.1682C>A	g.117590355C>A	Recommended
1824delA	rs193922505	c.1692del	g.117590365del	Recommended
Y569D	rs397508276	c.1705T>G	g.117590378T>G	Recommended
E585X	rs397508296	c.1753G>T	g.117590426G>T	Recommended
1898+1G->A	rs121908748	c.1766+1G>A	g.117590440G>A	Recommended
1898+5G->T	rs121908796	c.1766+5G>T	g.117590444G>T	Recommended
A613T	rs201978662	c.1837G>A	g.117592004G>A	Recommended
G628R	rs397508316	c.1882G>A	g.117592049G>A	Recommended
2184insA	rs121908746	c.2052dup	g.117592219dup	Recommended
2184delA	rs121908746	c.2052del	g.117592219del	Recommended
R709X	rs121908760	c.2125C>T	g.117592292C>T	Recommended
2307insA	rs746418935	c.2175dup	g.117592342dup	Recommended
R764X	rs121908810	c.2290C>T	g.117592457C>T	Recommended
R785X	rs374946172	c.2353C>T	g.117592520C>T	Recommended
R792X	rs145449046	c.2374C>T	g.117592541C>T	Recommended
2622+1G->A	rs141158996	c.2490+1G>A	g.117592658G>A	Recommended
2789+5G->A	rs80224560	c.2657+5G>A	g.117602868G>A	Recommended
Q890X	rs79633941	c.2668C>T	g.117603542C>T	Recommended
Y913X	rs149790377	c.2739T>A	g.117603613T>A	Recommended
S945L	rs397508442	c.2834C>T	g.117603708C>T	Recommended
G970D	rs386134230	c.2909G>A	g.117606674G>A	Recommended
3120G->A	rs121908797	c.2988G>A	g.117606753G>A	Recommended
3120+1G->A	rs75096551	c.2988+1G>A	g.117606754G>A	Recommended
3199del6	rs121908767	c.3067_3072del	g.117610597_117610602del	Recommended
T1036N	rs397508498	c.3107C>A	g.117610637C>A	Recommended
3272-26A->G	rs76151804	c.3140-26A>G	g.117611555A>G	Recommended
R1066C	rs78194216	c.3196C>T	g.117611637C>T	Recommended
R1066H	rs121909019	c.3197G>A	g.117611638G>A	Recommended
W1089X	rs78802634	c.3266G>A	g.117611707G>A	Recommended
W1098C	rs397508533	c.3294G>C	g.117611735G>C	Recommended
S1118F	rs146521846	c.3353C>T	g.117611794C>T	Recommended
R1158X	rs79850223	c.3472C>T	g.117627525C>T	Recommended
R1162X	rs74767530	c.3484C>T	g.117627537C>T	Recommended
3659delC	rs78984783	c.3528del	g.117627581del	Recommended
W1204X	rs121908765	c.3611G>A	g.117627664G>A	Recommended
3791delC	rs121908811	c.3659del	g.117627712del	Recommended
3849+5G->A	rs193922520	c.3717+5G>A	g.117627775G>A	Recommended
3849+10kbC->T	rs75039782	c.3718-2477C>T	g.117639961C>T	Recommended
3876delA	rs121908784	c.3744del	g.117642464del	Recommended
S1255X	rs76649725	c.3764C>A	g.117642484C>A	Recommended
3940delG	rs763843966	c.3808del	g.117642528del	Recommended
W1282X	rs77010898	c.3845G>A	g.117642565G>A	Recommended
4016insT	rs121908808	c.3889dup	g.117652857dup	Recommended
N1303K	rs80034486	c.3909C>G	g.117652877C>G	Recommended
CFTRdele2,3	structural variant	c.(53+1_54-1)_(273+1_274-1)del	N/A	Additional
E92X	rs121908751	c.274G>T	g.117530899G>T	Additional
G178R	rs80282562	c.532G>A	g.117534318G>A	Additional
1078delT	rs75528968	c.948del	g.117540178del	Additional
I336K	rs397508139	c.1007T>A	g.117540237T>A	Additional
Q493X	rs77101217	c.1477C>T	g.117559548C>T	Additional
poly T (5T)	rs1805177	c.1210-7_1210-6del	g.117548634_117548635del	Additional
poly T (9T)	rs1805177	c.1210-7_1210-6dup	g.117548634_117548635dup	Additional
I506V	rs1800091	c.1516A>G	g.117559587A>G	Additional
I507V	rs1801178	c.1519A>G	g.117559590A>G	Additional
F508C	rs74571530	c.1523T>G	g.117559594T>G	Additional
1677delTA	rs121908776	c.1545_1546del	g.117559616_117559617del	Additional
V520F	rs77646904	c.1558G>T	g.117559629G>T	Additional
1811+1G->C	rs397508263	c.1679+1G>C	g.117587834G>C	Additional
G628R_G>C	rs397508316	c.1882G>C	g.117592049G>C	Additional
2055del9->A	rs121908779	c.1923_1931delinsA	g.117592090_117592098delinsA	Additional
2143delT	rs121908812	c.2012del	g.117592179del	Additional
2183AA->G	rs121908799	c.2051_2052delinsG	g.117592218_117592219delinsG	Additional
K710X	rs75115087	c.2128A>T	g.117592295A>T	Additional
E725K	rs199791061	c.2173G>A	g.117592340G>A	Additional
Y1092X_C>A	rs121908761	c.3276C>A	g.117611717C>A	Additional
Y1092X_C>G	rs121908761	c.3276C>G	g.117611717C>G	Additional
M1101K	rs36210737	c.3302T>A	g.117611743T>A	Additional
D1152H	rs75541969	c.3454G>C	g.117614699G>C	Additional
3905insT	rs121908789	c.3773dup	g.117642493dup	Additional

RESULTS

The assay performance during guard-banding was optimal at recommended performance parameters, with suboptimal performance for DNA concentration at 2ng and low $MgCl_2$ concentration, especially for 394delTT.

The external laboratory results (68 samples) were 100% concordant with previously tested results, for 100% accuracy. Three samples failed testing. Fifty-two chromosomes where the reference was positive tested positive and 78 chromosomes where the reference was negative tested negative. Figure 1 includes the variants included in the orthogonal testing. The analytical sensitivity is 100% (95% CI; 93.1-100%) and the analytical specificity is 100% (95% CI; 95.3-100%).

Figure 1. Variants included in orthogonal testing



ACKNOWLEDGEMENTS

The entire Agena Team: Aaron Florece, Aleksey Nakorchevsky, Amber Lane, Glenn Wise, Maggie Lowe, Heath Metzler, Nichole Rupp, Sharron Ohgi, Shelley Spisak, Wayne Ge, Darryl Irwin, Paul Yates.

DISCLOSURES

VMP, AB, KG, LM, PH, PS, MJ, and GS are employees of Agena Bioscience.

CONCLUSIONS

MALDI-TOF is a targeted method to detect known variants. It has mid-range multiplexing capabilities, allowing the detection of approximately 25 variants/well. As such, we developed a targeted assay to detect over 100 *CFTR* variants. The assay was designed to detect the 100 variants as recommended by ACMG, plus additional variants that were recommended by a voice of the customer survey.

Overall, using MALDI-TOF to detect *CFTR* variants performed well in initial studies. One of the limitations of this study is that not all the variants in the assay could be evaluated in clinical samples or reference materials. Currently there are no commercially available reference materials to verify the accuracy of performance for all the variants in the assay. While a commercial control from Maine Molecular Quality Control, Inc. and Coriell DNA samples from the CDC's-GeT-RM studies were used, synthetic oligos had to be created for many of the variants.

Orthogonal studies performed in the external laboratories were 100% concordant with the previous results. A total of 68 DNA samples were tested. The most common variant in the data set, excluding 5T, was as expected F508del (n=7), followed by R117H (n=4). Note that the samples included in the orthogonal testing were not random but were chosen to include as many variants as possible.

Initial customer feedback indicated that the assay performed well using DNA extracted from a variety of sample types. Customers also suggested adding three variants (I506V, I507V, and F508C) that might interfere with targeted testing, to ensure accurate genotyping. These variants are not pathogenic but will ensure that the assay can accurately detect F508del and I507del when present.

Additional studies are underway both internally and externally to improve and further delineate assay performance.

REFERENCE

JL Deignan, AR Gregg, WW Grody, MH Guo, H Kearney, KG Monaghan, KS Raraigh, J Taylor, CJ Zepeda-Mendoza, C Ziats. Updated recommendations for *CFTR* carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG), Genetics in Medicine, 2023 25(8):1098-3600.