Development and clinical validation of a novel diagnostic test for Mucin1 kidney disease (MKD)

Brendan Blumenstiel[1] ,Matthew DeFelice[1], Ozge Birsoy[1,2], Qing Yu[1], Zach Leber[1], Niall Lennon[1]

[1] **The Broad Institute of Harvard and MIT,** [2] **Partners Healthcare Laboratory for Molecular Medicine**

Abstract

Mucin -1 Kidney Disease (MKD), also known as medullary cystic kidney disease type 1, is a rare autosomal dominant inherited kidney disease which leads to progressive loss of kidney function and end stage renal disease. Progression of the disease most often requires dialysis or kidney transplantation in the later decades of life. Previous reporting has linked this progressive tubulo-interstitial disease to a difficult to detect mutation markedly underrepresented in massively parallel sequencing. The mutation is identified as a single base insertion in a single copy of a 60bp repeat unit comprising a long (~1.5-5 kb), GC-rich (~85%) VNTR sequence in a coding region of the MUC1 gene. This single base cytosine duplication extends a 7C homopolymer to an 8C homopolymer, creating a frameshift which introduces an early stop codon and is expected to result in a truncated protein (Kirby *et al*. 2013).
The inherent difficulty in detecting this specific mutation using standard commercial methods required the development of a novel assay for MKD diagnosis in a clinical setting. Here we describe the development, clinical validation and implementation of a mass-spectrometry based CLIA assay for accurately diagnosing patients with MKD. Validation of the MKD diagnostic assay tested across 48 samples in six replicate runs demonstrated a conclusive call rate of 95.5% and accuracy of 100%.

Mutation Description

- Single base cytosine insertion extends 7C homopolymer to 8C within 60bp VNTR
- Causes frameshift introducing an early stop codon
- +C insertion destroys MwoI restriction site enabling enrichment by digestion

Muc1 VNTR Sequence

ACCAGGCCGGCCCCGGGCTCCACCG**CCCCCCC**AGCCCACGGTGTCACCTCGGCCCCGGAC

Muc1 VNTR +C Insertion

ACCAGGCCGGCCCCGGGCTCCACCG**CCCCCCCC**AGCCCACGGTGTCACCTCGGCCCCGGAC

Reading Frame Shift

...ACCG**CCCCCCC**AGCCCACGGT...
ThrAlaProProAlaHisGly

...ACCG**CCCCCCCC**AGCCCACGGT...
ThrAlaProPro**Ser**ProArg

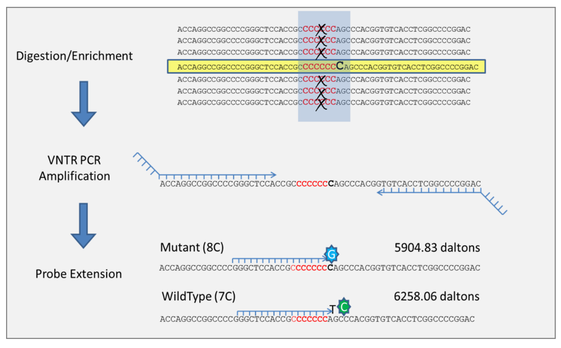
MwoI Restriction Endonuclease

5'...GCNNNNNNNGC...3'
3'...CGNNNNNNNGC...5'

MwoI Digest

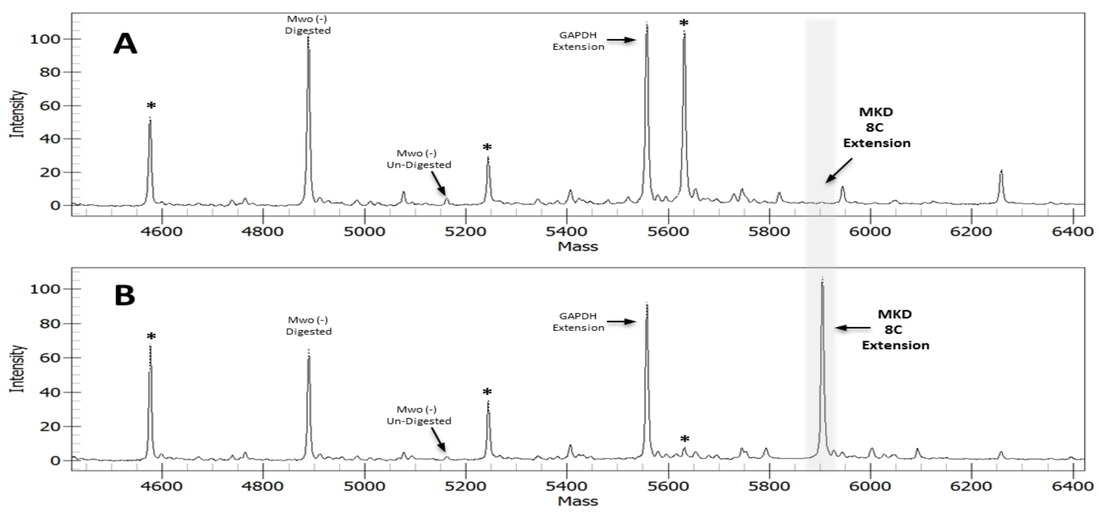
Assay Workflow

- Restriction digest gDNA with MwoI enriches for 8C insertion over wildtype background
- Tailed PCR amplifies insertion containing repeat copies
- Probe extension
- Analyte separation/ detection by MALDI-TOF MS



MALDI-TOF MS Analyte Detection

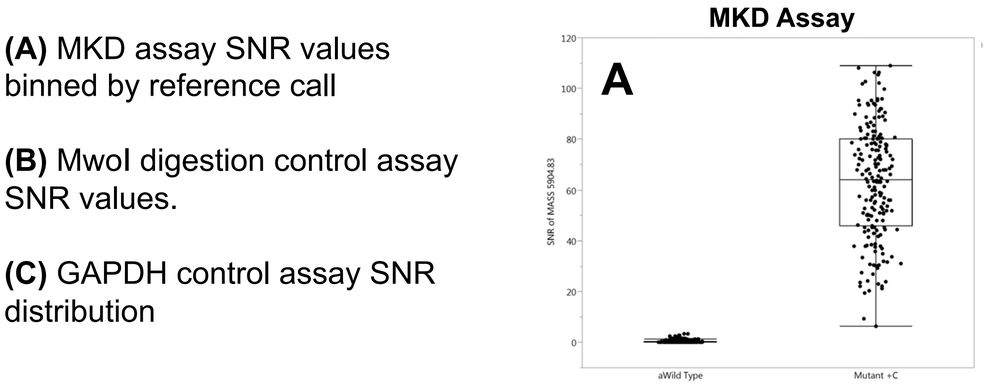
Accurate detection of the +C insertion is performed by assessing the extension analytes of three multiplexed probe extension assays using MALD-TOF MS. GAPDH control, MwoI digestion control and the MKD assay. Presence of the causal 8C allele determined by a strong peak at 5904.83 daltons. Passing controls and a lack of a peak at 5904.83 da indicates the lack of the insertion. **(A)** Spectra from W/T control sample. **(B)** Spectra from MKD affected patient sample



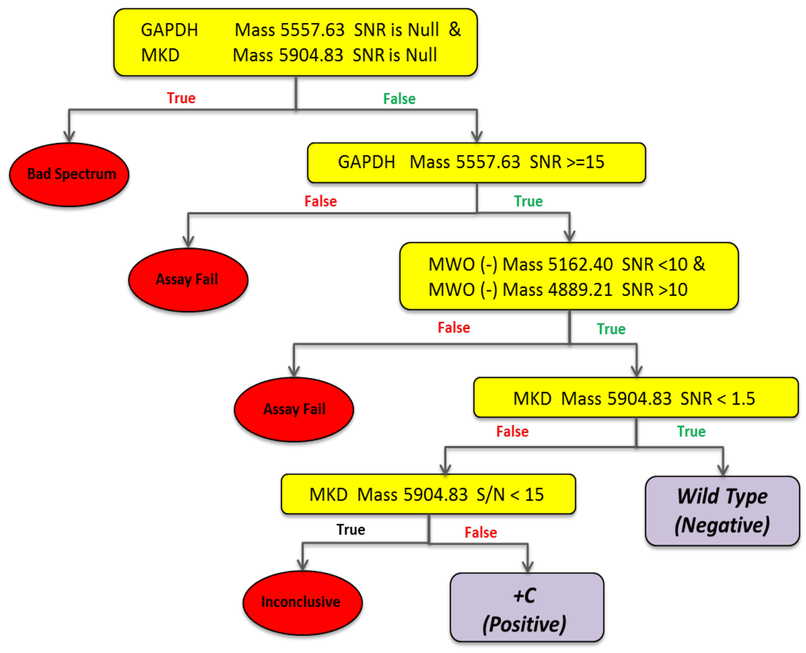
Assay Performance and Intensities

Signal to noise ratios (SNR) for multiplexed assays for 384 individual tests.

- **(A)** MKD assay SNR values binned by reference call
- **(B)** MwoI digestion control assay SNR values.
- **(C)** GAPDH control assay SNR distribution



MKD Diagnostic Assay Calling Flowchart



Validation Results

- 24 HapMap wildtype controls and 24 known +C affected samples were tested by two personnel across a total of 6 replicate runs.
- Validation sample set run using 20ng and 100ng input.
- Total conclusive call rate = 95.5%
- Accuracy = 100%

Validation set	Inconclusive %	Assay Fail %	Analytical sensitivity %	Analytical specificity %	Accuracy %
Plate #1, Rep 1	2	0	100	100	100
Plate #1, Rep 2	0	0	100	100	100
Plate #2, Rep 1	0	2	100	100	100
Plate #2, Rep 2	0	2	100	100	100
Plate #3, Rep 1	15	2	100	100	100
Plate #3, Rep 2	4	0	100	100	100
Average	**3.5**	**1**	**100**	**100**	**100**
Plate #1, 20ng input	2	0	100	100	100
Plate #1, 100ng input	0	0	100	100	100

Acknowledgements

Slim Initiative in Genomic Medicine for the Americas