

ORIGINAL ARTICLE

Clinical Laboratory Validation and Implementation of Quantitative, Real-Time PCR-based Detection of NPM1 Type A Mutation

Joelle Racchumi, Wayne Tam, Michael J. Kluk

Weill Cornell Medicine, Department of Pathology & Laboratory Medicine, New York, NY, USA

SUMMARY

Background: *NPM1* mutations have prognostic significance in acute myeloid leukemia (AML) and monitoring mutant *NPM1* levels during and after therapy has been described to predict relapse and survival. Despite the published significance of this molecular biomarker, routine monitoring for mutant *NPM1* levels has not been widely adopted in academic clinical laboratories. Therefore, our objective was to validate a quantitative, reverse transcription-PCR assay for the detection of *NPM1* Type A mutant transcripts for use in the clinical laboratory.

Methods: A quantitative, real-time, reverse-transcription PCR-based method for the detection of *NPM1* Type A mutant transcripts was validated for use in routine clinical practice. Results from this assay were compared to results from orthogonal methods, including next generation sequencing and digital droplet PCR.

Results: This real-time, reverse-transcription PCR-based method is sensitive (limit of detection: 0.0150% NCN and reproducible ($\leq 0.5 \log_{10}$ -fold variation)). We summarize the rigorous validation results and share observations that will help other clinical laboratories that may wish to implement this testing. We show the superior sensitivity of this assay compared to other assays (e.g., 45 gene Myeloid Next Generation Sequencing panel) and present a representative case which highlights the assay's utility in the pathologic assessment of cases with borderline morphologic or flow cytometric findings.

Conclusions: As molecular testing for residual disease in AML continues to expand, this sensitive and reproducible method will be an appropriate testing option for the detection of *NPM1* Type A mutant transcripts in clinical practice.

(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2020.200104)

Correspondence:

Michael J. Kluk, MD, PhD
Weill Cornell Medicine
New York-Presbyterian Hospital
Associate Professor of Clinical Pathology and
Laboratory Medicine
Department of Pathology and
Laboratory Medicine
Box 69
1300 York Ave. Office: K509
New York, NY, 10065
USA
Phone: +1 212-746-3972
Fax: +1 212-746-8173
Email: mik9095@med.cornell.edu

Manuscript accepted April 27, 2020

Supplementary Tables and Figures

Table 1. Analytic sensitivity. Cell line.

Sample; RNA dilution (% Pos. control RNA)	NPM Type A Mutant, number of copies	ABL, number of copies	% NCN, NPM1 Type A mutant/ABL	Mean, % NCN	SD
Cell lines dilution (% pos. control RNA)					
OCI-AML3_100%	449,078.875	74,524.29	602.594	603.331	1.042
OCI-AML3_100%	453,850.406	75,132.38	604.068		
OCI-AML3+MV411_10%	31,229.891	48,039.60	65.009	57.588	10.495
OCI-AML3+MV411_10%	26,652.297	53,127.45	50.167		
OCI-AML3+MV411_1%	3,235.177	46,716.25	6.925	6.738	0.264
OCI-AML3+MV411_1%	3,376.028	51,527.98	6.552		
OCI-AML3+MV411_10-1%	330.779	47,363.50	0.698	0.671	0.039
OCI-AML3+MV411_10-1%	322.527	50,119.39	0.644		
OCI-AML3+MV411_10-2%	32.126	56,495.57	0.057	0.071	0.020
OCI-AML3+MV411_10-2%	43.579	51,327.15	0.085		
OCI-AML3+MV411_10-3%	<u>3.231</u>	<u>54,812.52</u>	<u>0.006</u>	<u>0.006</u>	<u>0.000</u>
OCI-AML3+MV411_10-3%	<u>3.091</u>	<u>55,290.85</u>	<u>0.006</u>		
OCI-AML3+MV411_10-4%	1.817	68,993.86	0.003	0.002	0.002
OCI-AML3+MV411_10-4%	0.366	73,434.43	0.000		
OCI-AML3+MV411_10-5%	0.009	79,998.87	0.000	0.000	0.000
OCI-AML3+MV411_10-5%	0.000	74,978.66	0.000		
MV-4-11	0.001	70,750.01	0.000	0.000	0.000
MV-4-11	0.000	69,508.93	0.000		

Table 2. Probit analysis for Limit of Detection (LOD).

% NCN, NPM1 Type A Mutant/ABL	Samples	Detected
62	10	10
6.3	10	10
0.67	10	10
0.07	10	10
0.007	10	9
0.0007	10	5
0.00007	10	3
0.000007	10	0

Table 3. Real Time PCR vs. ddPCR.

Sample	Real Time PCR, % NCN, (NPM1 Type A Mutant/ABL)	dd PCR, % NCN, (NPM1 Type A Mutant/ABL)
P11_100%	355.5253	255.9524
P11_10% RNA dilution	51.9009	36.5452
P11_1% RNA dilution	5.6104	2.7967
P11_10-1% RNA dilution	0.8219	0.4374
P11_10-2% RNA dilution	0.0995	0.0593
P11_10-3% RNA dilution	0.0128	0.0085
Sample	Real Time PCR, % NCN, (NPM1 Type A Mutant/ABL)	dd PCR, % NCN, (NPM1 Type A Mutant/ABL)
P1_RNA dilution_run1	0.2765	0.3099
P1_RNA dilution_run2	0.2769	0.4058
P11_RNA dilution_run1	0.0961	0.0719
P11_RNA dilution_run2	0.106	0.0807
P16_RNA dilution_run1	0.0651	0.1411
P16_RNA dilution_run2	0.0879	0.1216
P_sample A_run1	0.043	0.027
P_sample A_run2	0.052	0.025

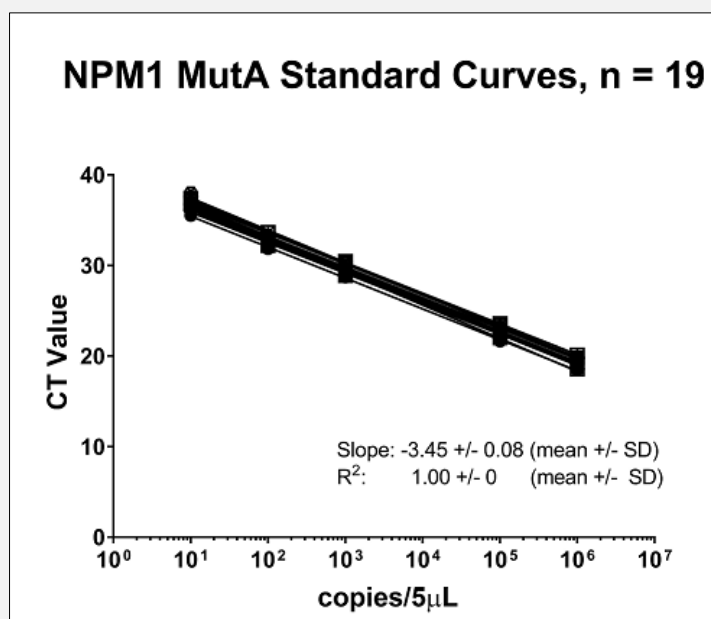


Figure 1.

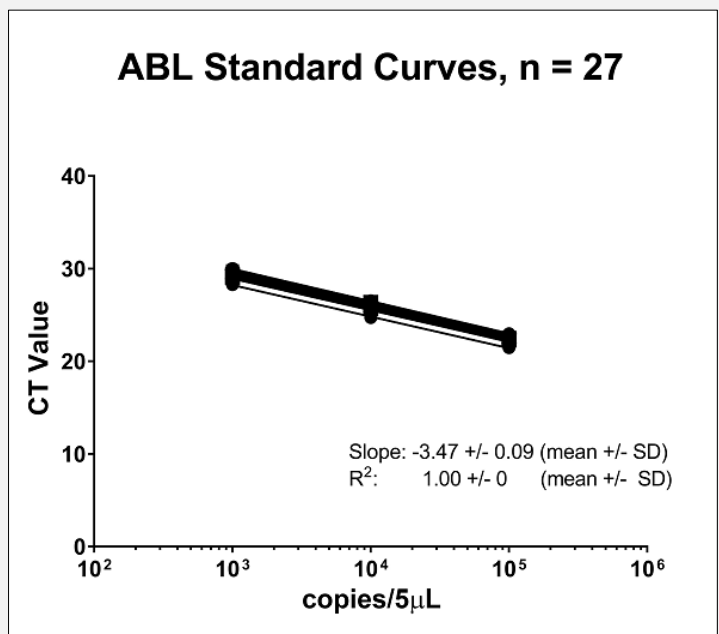


Figure 2.

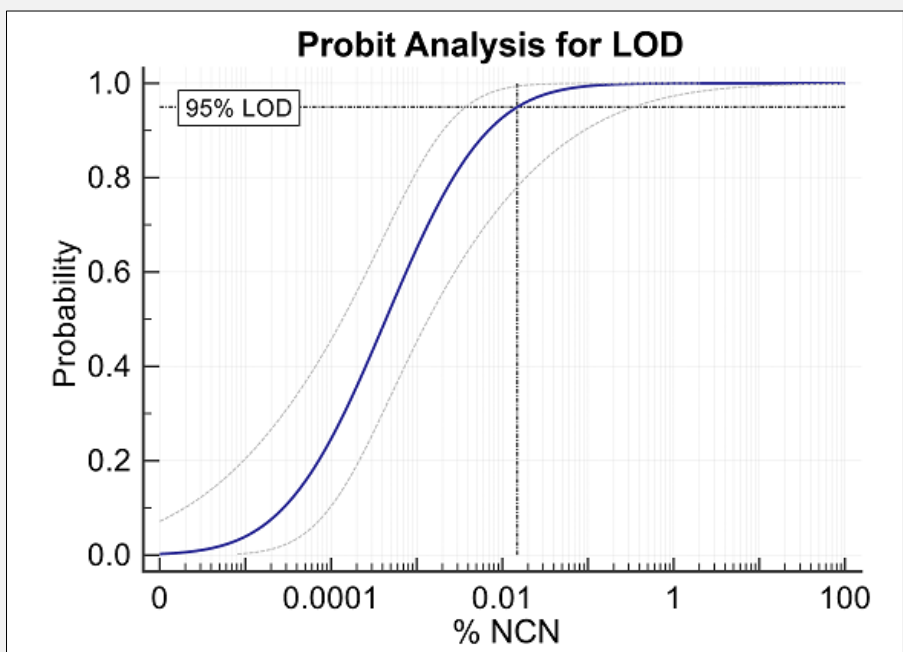


Figure 3.

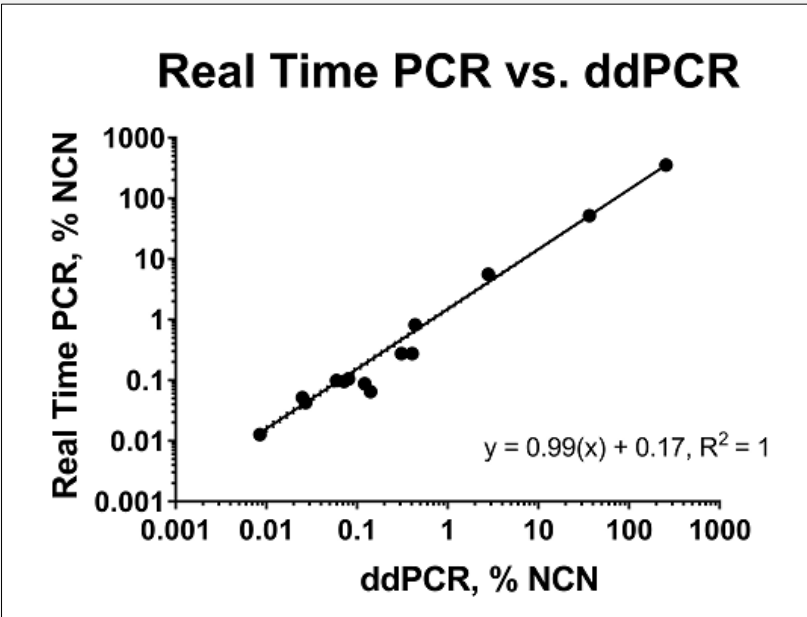


Figure 4.