



P2.118: Development and Performance Evaluation of a Real-Time PCR Assay for EGFR Mutations to Guide Targeted Therapy in NSCLC

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INTRODUCTION

CLINICAL CHALLENGE



~85%

lung cancer cases in Indonesia are NSCLC.¹



34-67%

Indonesian NSCLC patients harbor EGFR mutations.^{1,2}



National Health Insurance-reimbursed TKI requires accessible EGFR testing.

SOLUTION



Leveraging expanded real-time PCR infrastructures post COVID-19.

DIAGO EGFR Mutation Detection Kit was developed as an open-system assay for broad implementation across molecular laboratories.

25 EGFR mutations

detected in **3 reactions/sample**

DISCUSSION & CONCLUSION

Actionable Take Away Message

- The real-time PCR assay covers 25 EGFR mutations with 100% sensitivity & 96.23% specificity compared with Sanger sequencing.
- Direct Ct-based analysis and open-system compatibility enable broad implementation across molecular laboratories.

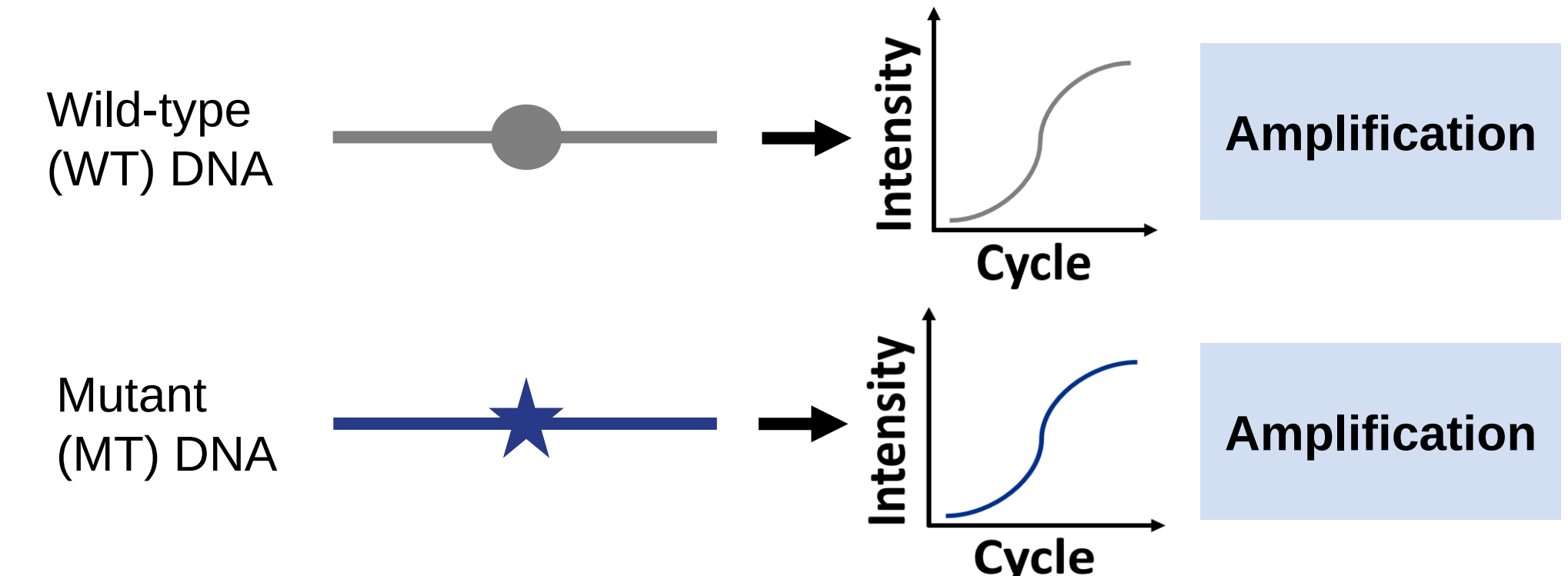
Future Direction for Research

- Evaluate circulating tumor DNA-based testing.
- Assess impact on treatment selection and patient outcomes.

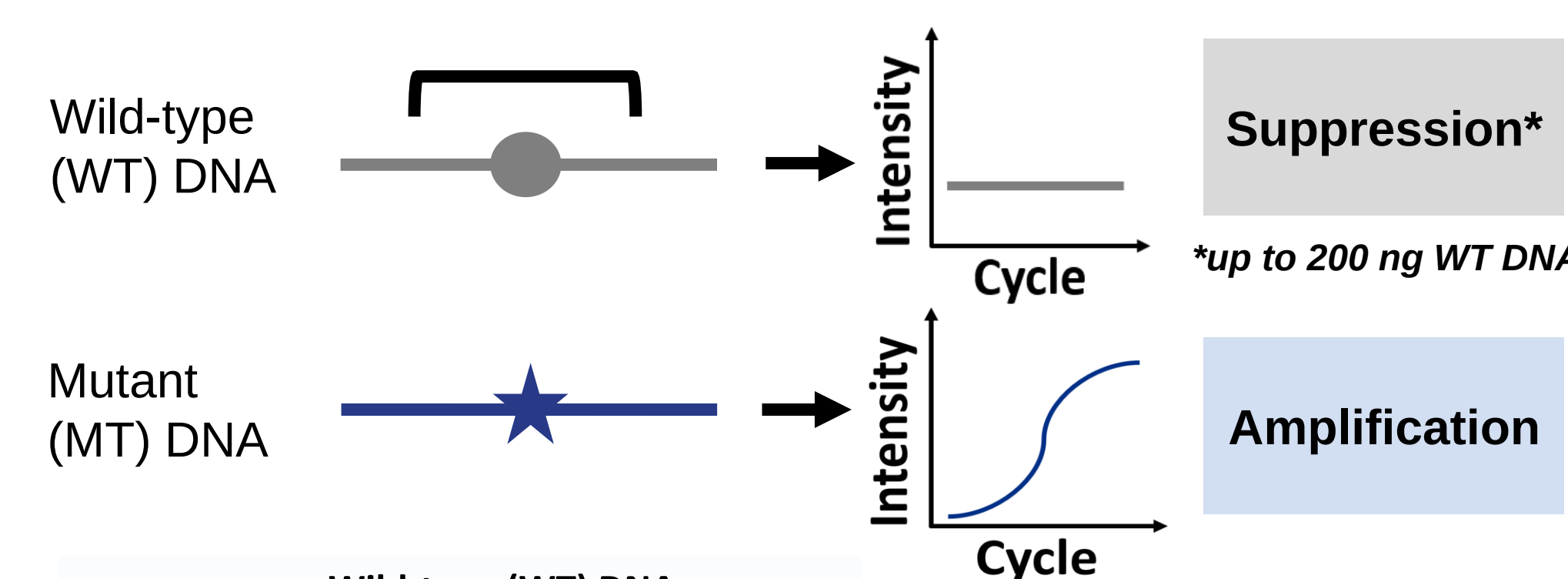
RESULTS

Wild-Type Blocker Suppresses WT Amplification for Direct Ct-Based Interpretation

Without wild-type blocker



With wild-type blocker



Legend:
— Wild-type (WT) DNA
— Mutant (MT) DNA
● Wild-type base
★ Mutant SNP or deletion
— Wild-type blocker oligonucleotide

Most Mutation Targets Demonstrated LoD₉₅ ≤1% MAF

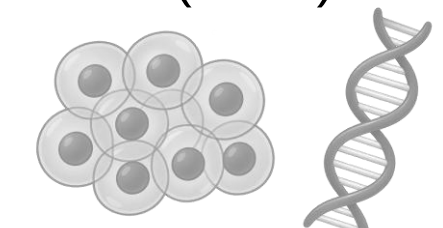
No	Exon	Mutation	Base Change	LoD ₉₅ (%MAF)
1	21	L858R	2573T>G	0.5%
2		E746_A750del (1)	2235_2249del15	0.25%
3		E746_A750del (2)	2236_2250del15	1%
4		L747_P753>S	2240_2257del18	2%
5		E746_T751>I	2235_2252>AAT(complex)	1.75%
6		E746_T751del	2236_2253del18	0.5%
7		E746_T751>A	2237_2251del15	1%
8		E746_S752>A	2237_2254del18	1%
9		E746_S752>V	2237_2255>T(complex)	0.5%
10		E746_S752>D	2238_2255del18	0.75%
11	19	L747_A750>P	2238_2252>GCA(complex)	0.5%
12		L747_T751>Q	2238_2252>GCA(complex)	1%
13		L747_E749del	2239_2247del9	15%
14		L747_T751del	2239_2253del15	0.5%
15		L747_S752del	2239_2256del18	0.75%
16		L747_A750>P	2239_2248TTAAGAGAAG>C(complex)	1%
17		L747_P753>Q	2239_2258>CA(complex)	0.75%
18		L747_T751>S	2240_2251del12	0.5%
19		L747_T751del	2240_2254del15	0.75%
20		L747_T751>P	2239_2251>C(complex)	0.5%
21		G719C	2155G>T	4.75%
22	18	G719A	2156G>C	7.5%
23		G719S	2155G>A	4.5%
24	20	S768I	2303G>T	1%
25	21	L861Q	2582T>A	0.85%

METHODS

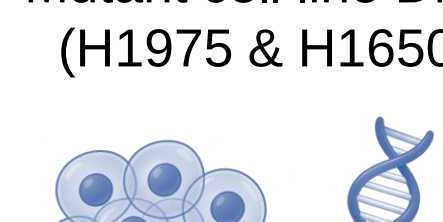
Analytical Sensitivity: Limit of Detection Evaluation

REFERENCE MATERIALS

Wild-type cell line DNA (A549)



Mutant cell line DNA (H1975 & H1650)

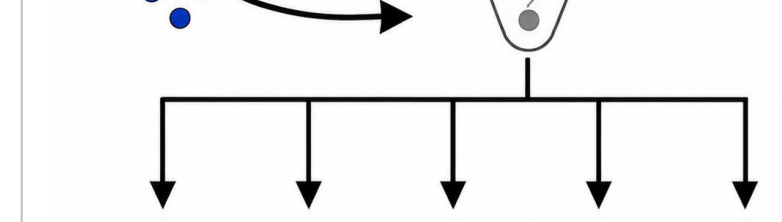


Synthetic DNA representing each mutation.

SERIAL DILUTION IN WT BACKGROUND

Wild-Type (WT) DNA (A549)

Mutant DNA (from cell lines or synthetic DNA)



Mutant Allele Frequency (MAF)

10% 5% 1% 0.5% 0.1%

REAL-TIME PCR TEST (ASSAY EVALUATION)



20 replicates per tested concentration

High MAF (e.g., 10%)

Low MAF (e.g., 1%)

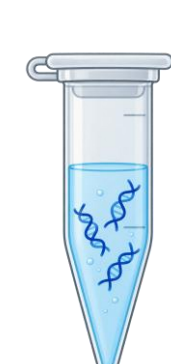
Very low MAF (e.g., 0.1%)

Fluorescence

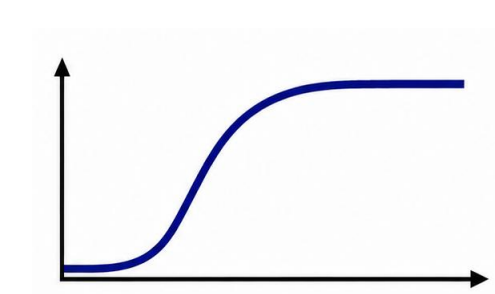
Cycle

LoD₉₅: the lowest %MAF consistently detected in ≥95% of replicates

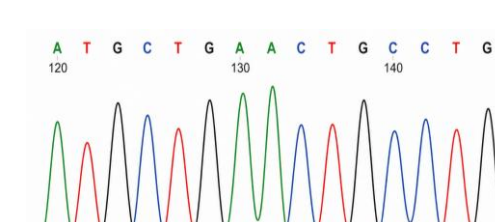
Clinical Performance Evaluation (N = 98)



Leftover NSCLC DNA Isolates
Cytology: 56 | FFPE: 42



Real-Time PCR Assay Evaluation (DIAGO)



Sanger Sequencing Exon 18, 19, 20, 21 (Reference Method)

REFERENCES

References

- Heriyanto, D. S., Trisnawati, I.,... & Chuang, H. C. 2025. Spectrum of rare EGFR mutations in Indonesian lung adenocarcinoma: Findings from an 8-year analysis of 4,778 cases highlighting the need for advanced targeted therapies. *Narra J*, 5(2), e1721.
- Syahrudin, E., Wulandari, L.,... & Utomo, A. R. H. 2018. Uncommon EGFR mutations in cytological specimens of 1,874 newly diagnosed Indonesian lung cancer patients. *Lung Cancer: Targets and Therapy*, 25-34.

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Q&A



Scan Me!

Results

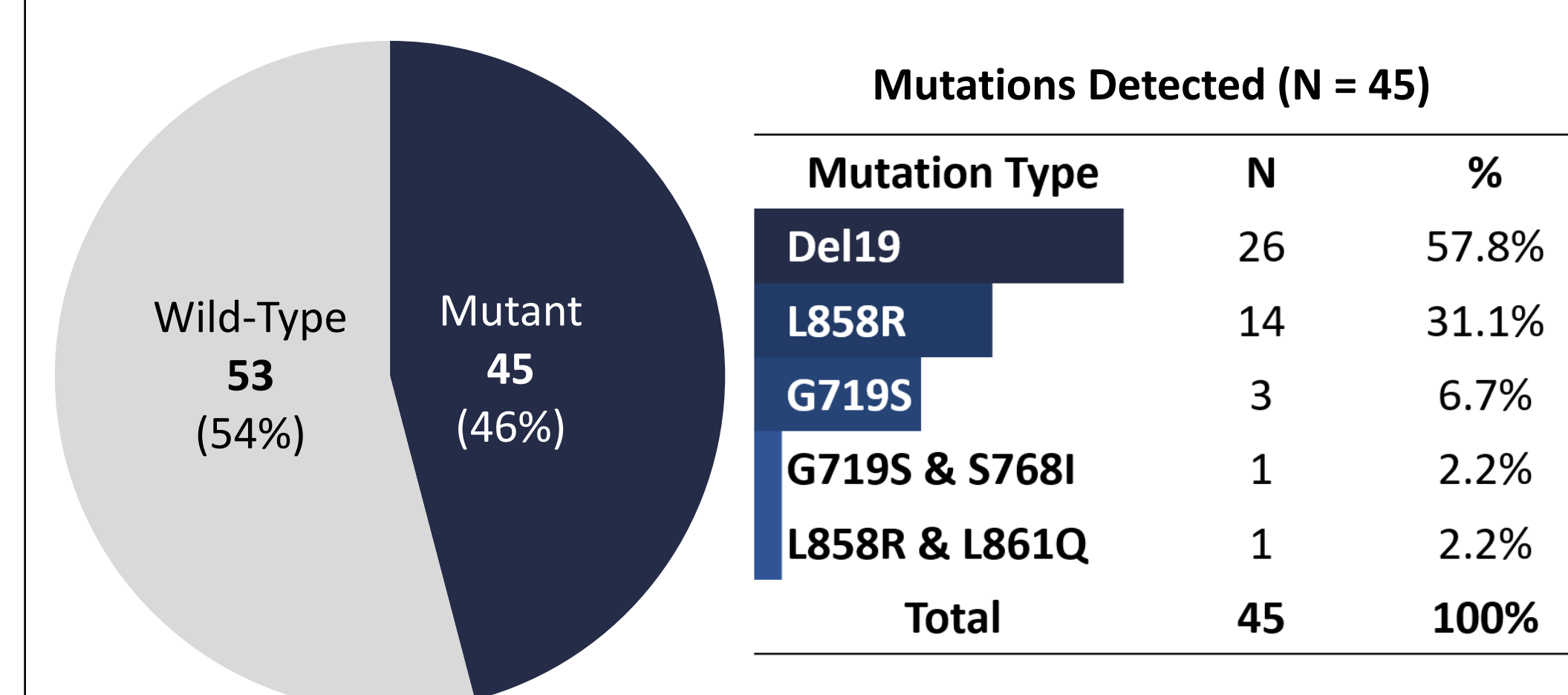


Contact Information

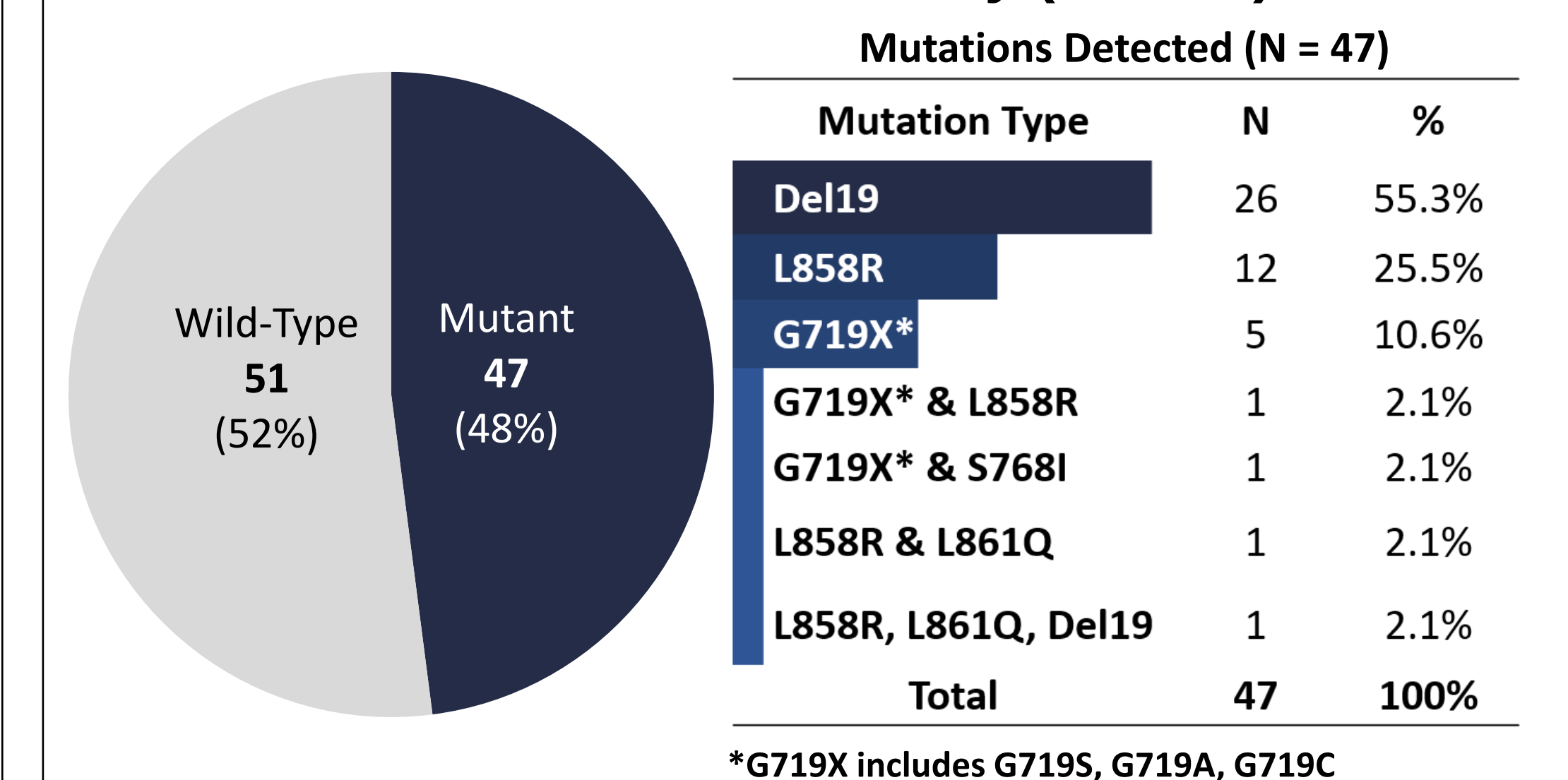
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The Real-Time PCR Assay Demonstrated 100% Sensitivity and 96.23% Specificity

Sanger Sequencing (Reference)



Real-Time PCR Assay (DIAGO)



Comparison Matrix

		Sanger Sequencing		Total
		Mutant	Wild-Type	
Real-Time PCR Assay (DIAGO)	Mutant	45	2*	47
	Wild-Type	0	51	51
Total		45	53	98

*Discordant cases: 2 (Sanger: wild-type | DIAGO: G719X)

Clinical Performance

Sensitivity
100%
(45/45)

95% CI = 92.13% to 100%

Specificity
96.23%
(51/53)

95% CI = 87.02% to 99.54%