ST54

EGFR Gene Mutation Analysis in Non-Small Cell Lung Cancer Using cobas® Assay in FFPE and Plasma Specimen Types

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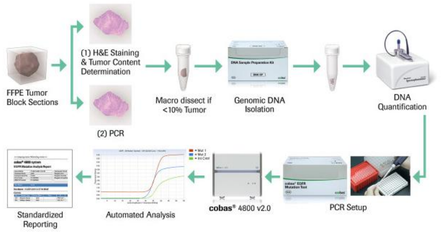
Background

The FDA approved cobas® EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the EGFR gene in DNA derived from formalin-fixed paraffin-embedded (FFPE) or plasma human non-small cell lung cancer (NSCLC) patients. The test is intended to aid in identifying patients with NSCLC whose tumors have defined EGFR mutations and for whom safety and efficacy of a drug have been established. In this study, we have evaluated the clinical and analytical performance features of the assay.

Methods

Genomic DNA was isolated from the tumor specimens using the cobas® DNA Sample Preparation Kit. Mutation detection is achieved through real-time PCR analysis on the cobas® z480 analyzer. DNA from NSCLC specimens were used to evaluate accuracy, repeatability, reproducibility and analytical sensitivity of the assay.



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Results

Of the specimens tested during validation, 20 FFPE specimens with known mutations in EGFR had cobas® results that were 90% concordant and 20 plasma specimens were 100% concordant. The lower concordance in FFPE specimen type was due to borderline positive specimens, specimen degradation and detection sensitivity difference between the two platforms. Repeatability was 90% concordant for FFPE specimens and 100% for plasma specimens; reproducibility was 100% concordant for both FFPE and plasma specimens. The lower concordance for FFPE repeatability was due to borderline positive specimens. This assay can detect 5% of mutant DNA in a background of wild type genomic DNA when the input DNA is 50ng for FFPE specimens and 100 copies for plasma specimens.

The cobas® EGFR Mutation Test v2 has been offered as a clinical test in LabCorp. Of the 389 FFPE specimens tested, 53.47% were negative, 42.67% were positive with 1-3 EGFR mutations. 27.50% specimens had one variant, 11.31% contained exon 19 deletion/T790M variants, 2.83% had T790M/L858R variants, and 1.03% had 3 variants or 2 other variants. Results could not be obtained in 3.85% specimens due to specimen degradation or low DNA yield. Of the 513 plasma specimens tested, 59.84% were negative, 39.96% were positive with 1-3 EGFR mutations. 26.12% specimens had one variant, 7.4% contained exon 19 deletion/T790M variants, 5.46% had T790M/L858R variants, and 0.98% had 3 variants or 2 other variants. Results could not be obtained in 1 (0.19%) specimen. There was no difference in the mutation rate and distribution between males and females. The higher detection sensitivity of the cobas® platform has resulted in the higher mutation detection rate than other platforms.

Plasma Specimen Accuracy Validation Data

Specimen ID	LabCorp cobas results	2nd Laboratory cobas results	Sanger sequencing results
d746	Ex19Del	NA	Ex19Del
T790M	T790M	NA	T790M
S768I	S768I	NA	S768I
G719S	G719X	NA	G719S
L858R	L858R	NA	L858R
V769	Ex20Ins	NA	Ex20Ins
n1	NMD	NA	NMD
n2	NMD	NA	NMD
n3	NMD	NA	NMD
n4	NMD	NA	NMD
n5	NMD	NA	NMD
n6	NMD	NA	NMD
10095	NMD	NMD	NA
19099	L861Q/Ex20Ins	L861Q/Ex20Ins	NA
10096e	NMD	NMD	NA
19100e	L861Q/Ex20Ins	L861Q/Ex20Ins	NA
10197	NMD	NMD	NA
19101	L861Q/Ex20Ins	L861Q/Ex20Ins	NA
19103	L861Q/Ex20Ins	L861Q/Ex20Ins	NA
10130	NMD	NMD	NA

FFPE Specimen Accuracy Validation Data

Sample ID	cobas results	SNaPshot results
224-225-20020	Ex20Ins	Ex20Ins
233-225-26080	G719X/S768I	G719C/S768I
349-844-62880	Ex19Del	Ex19Del
031-225-40030	Ex19Del/Ex20Ins	E19Del
067-225-23520	G719X	G719A
068-402-78350	NMD	NMD
069-225-40190	Ex20Ins	NMD
118-430-85030	NMD	NMD
137-225-40070	NMD	L858R
138-844-64160	S768I/G719X	S768I/G719A
148-143-80561	NMD	NMD
149-143-85960	NMD	NMD
192-614-00010	Ex19Del	Ex19Del
163-225-40080	NMD	NMD
164-225-04530	S768I/G719X	S768I/G719C
174-225-40100	Ex19Del/T790M	Ex19Del/T790M
180-256-90120	NMD	NMD
191-071-01660	NMD	NMD
Horizon	L861Q	L861Q
Horizon	L858R	L858R

03122540030, cobas assay detected E19del and E20ins, SNaPshot assay detected E19del; 06922540190, cobas assay detected E20ins, SNaPshot assay did not detect this variant, the discrepancy could due to higher detection sensitivity of cobas assay or tumor heterogeneity. 13722540070, cobas assay had no mutation detected, SNaPshot assay detected L858R mutation at low level. The discrepancy could due to tumor heterogeneity.

FFPE Specimen Detection Sensitivity

Sample ID	% mutation	replicate 1	replicate 2	replicate 3
Horizon Control (T790M)	20	T790M	T790M	T790M
	10	T790M	T790M	T790M
	5	T790M	T790M	T790M
	1	no mutation detected	no mutation detected	no mutation detected
Horizon Control (L861Q)	20	L861Q	L861Q	L861Q
	10	L861Q	L861Q	L861Q
	5	L861Q	L861Q	L861Q
	1	no mutation detected	no mutation detected	no mutation detected
Horizon Control (L858R)*	20	L858R	L858R	L858R
	10	L858R	L858R	L858R
	5	L858R	L858R	L858R
	1	L858R	L858R	L858R
Horizon Control (E746: Ex19del)	20	Ex19Del	Ex19Del	Ex19Del
	10	Ex19Del	Ex19Del	Ex19Del
	5	Ex19Del	Ex19Del	Ex19Del
	1	no mutation detected	no mutation detected	no mutation detected

Percentage of mutation detected was determined by diluting 4 Horizon FFPE samples with known WT samples to achieve 20, 10, 5 and 1% mutations; all dilutions were run in triplicate. The 5% mutation sensitivity claim by the manufacturer was met with the L861Q, Ex19del L858R and T790M mutations.

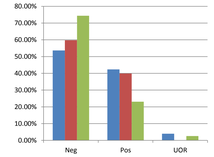
Plasma Specimen Detection Sensitivity

Horizon ID	Replicate	Result
HD258	1	T790M
	2	T790M
	3	T790M
HD254	1	L858R
	2	L858R
	3	L858R
HD251	1	Ex19Del
	2	Ex19Del
	3	Ex19Del

Percentage of mutation detected was determined by diluting 4 Horizon FFPE samples with known WT samples to achieve 20, 10, 5 and 1% mutations; all dilutions were run in triplicate. This assay could detect down to 100 copies of mutant in the background of wild type. The manufacturer's sensitivity levels were met.

Clinical Testing Specimen Mutation Distribution

Specimen Type	Neg	Pos	UOR
FFPE	204	161	15
	53.68%	42.37%	3.95%
Plasma	307	205	1
	59.73%	39.88%	0.19%



Specimen Type	E19del	L858R	Other 1 Mutation	E19del/T790M	L858R/T790M	Other 2 mutations	3 mutations
FFPE	57	36	10	42	10	3	3
	35.40%	22.36%	6.21%	26.09%	6.21%	1.86%	1.86%
Plasma	75	47	12	40	27	2	2
	36.59%	22.93%	5.85%	19.51%	13.17%	0.98%	0.98%

Conclusions

The cobas® EGFR Mutation Test v2 is a robust, reproducible, sensitive, and fast assay for molecular diagnostic utilization in NSCLC using FFPE or plasma specimen types.

References

1. cobas® EGFR Mutation Test v2 Package Insert. Rev. 3.0 2016.