A NEW IDH1/2 PCR ASSAY FOR ONE-STEP DETECTION OF 12 IDH1 AND IDH2 MUTATIONS IN GLIOMA

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BACKGROUND

• Isocitrate dehydrogenase (IDH) mutational status is a strong diagnostic and prognostic marker in glioma which will probably be introduced in the next WHO classification system.

• Current IDH mutations screening is performed with an IHC assay specific for IDH1 R132H, the most common mutation. Sequencing is recommended as a second-step test for IHC-negative or –equivocal cases. However, sequencing is not readily accessible in all centers, and its use generally leads to additional delay in providing a comprehensive IDH1/2 mutational status assessment. Moreover, both IHC and Sanger sequencing lack of standardization can lead to inter-laboratory variability.

• Beyond IDH1 R132H, 11 other IDH mutations, 6 IDH1 and 5 IDH2, have been reported so far in literature, in large cohorts of gliomas.

• Recent data indicate that mutant specific IDH1-inhibitors impair growth of mutant-IDH1 gliomas in mice.

• A real-time PCR assay was designed to detect the 12 IDH1/2 mutations in one single step in FFPE samples, and identify the most frequent ones

OBJECTIVES

• Establish the analytical performance of the new IDH1/2 PCR assay

• Validate the IDH1/2 PCR assay performance on FFPE glioma clinical samples by comparing PCR IDH mutational status to IHC and Sanger sequencing.

MATERIAL & METHODS

IDH1/2 one-step qPCR assay:

• PCR Clamping was used for the qualitative detection of IDH1 R132H and 11 additional IDH1/2 mutations. ARMS PCR technology was combined to selectively identify the most frequent IDH1 (R132H / R132C) and IDH2 (R172K) mutations (**Table 1**).

Evaluation of analytical sensitivity:

• Limit of Detection (LOD) (min. % mutant DNA detected in a WT background) was determined following CLSI/NCCLS EP17-A guidelines.
• 5 low positive samples (2-5-10-15 and 20%) obtained by mixing IDH mutant plasmid DNA with glioma IDH1/2 WT DNA were tested per mutation (n= 30 to 110 measurements per mutation and mutation percentage)

Validation of the IDH1/2 PCR assay:

• 171 FFPE glioma samples series: 121 samples retrospectively collected in a reverse chronological order from 2 academic centers (n1=102; n2=19) and 50 additional commercial samples. No specific selection criteria beyond tumor characteristics assessed by local pathologists.
• Samples selection: < 10 yrs; ≥ 50mm² tissue area with ≥ 40% tumoral cells.
• DNA extracted from 10 µm FFPE sections using the QIAamp DNA FFPE Tissue Kit (Qiagen)
• qPCRs performed on 25 ng DNA acc. to *therascreen* IDH1/2 RGQ PCR Kit protocol (Qiagen) and run on the Rotor-Gene Q 5plex HRM instrument (Qiagen)
• IHC was performed locally using the IDH1 R132H monoclonal antibody Clone H09 (Dianova)
• Bidirectional Sequencing (central) using recommended primers for IDH1 codon 132 and IDH2 codon 172 [3] and newly designed primers for IDH1 codon 100.

• 22 Synthetic samples (30% and 40% Mutant DNA in WT DNA) were additionally tested for the 11 rare mutations. Synthetic samples were processed similarly to clinical samples

RESULTS

IDH1/2 ASSAY CONTENT

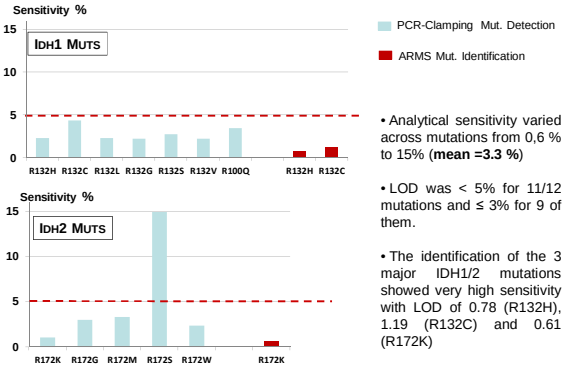
Gene / Codon	Mutation	Base change
IDH1 / R132	R132H*	395 G>A
	R132C*	394 C>T
	R132S	394 C>A
	R132G	394 C>G
	R132L	394 G>T
	R132V	394_395 CG>GT
IDH1 / R100	R100Q	299 G>A
IDH2 / R172	R172K*	515 G>A
	R172M	515 G>T
	R172W	514 A>T
	R172S	516 G>T
	R172G	514 A>G

Table 1 - IDH1/2 mutations detected and identified * with the IDH1/2 PCR Assay

➢ Detection of 12 mutations (6 within IDH1 codon R132, 5 within the homologous codon 172 of IDH2, and one within IDH1 codon 100)

➢ Identification of 3 major IDH1/2 mutations (IDH1 R132H / R132C and IDH2 R172K)

ANALYTICAL SENSITIVITY

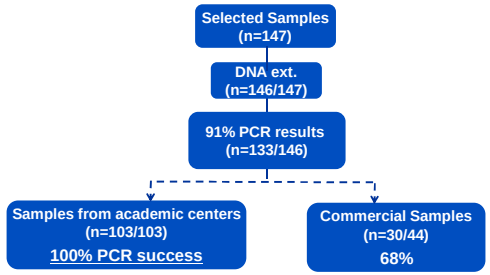


• Analytical sensitivity varied across mutations from 0,6 % to 15% (**mean =3.3 %**)

• LOD was < 5% for 11/12 mutations and ≤ 3% for 9 of them.

• The identification of the 3 major IDH1/2 mutations showed very high sensitivity with LOD of 0.78 (R132H), 1.19 (R132C) and 0.61 (R172K)

CLINICAL VALIDATION COHORT



• 147 samples met the selection criteria - The histological subtype distribution reflected typical clinical ditribution with > 40% GBMs.

• IDH1/2 PCR success was 100% on samples collected in academic centers

CLINICAL PERFORMANCE

Table 2 - Agreement between IHC and IDH1/2 PCR for detection of IDH1 R132H mutation.

		IHC R132H POS	IHC R132H NEG	TOTAL
IDH1/2 PCR	R132H	60	0	60
	NON-R132H	1	72	73
	TOTAL	61	72	133

Table 3 - Agreement between Sequencing and IDH1/2 PCR for IDH1/2 mutation status assessment.

		SANGER SEQUENCING IDH1/2 MUT +	SANGER SEQUENCING IDH1/2 MUT -	TOTAL
IDH1/2 PCR	IDH1/2 MUT +	67	5	72
	IDH1/2 MUT -	0	61	61
	TOTAL	67	66	133

Table 4 - Number and types of IDH mutations detected by IHC, Sequencing or IDH1/2 PCR (n=133)

	IDH1 R132H	IDH1 R132C	IDH1 R132 OTHER	IDH1 R100	IDH2 R172K	IDH2 R172 OTHER	WT	% Mutated Cases
IHC	61	0	0	0	0	0	72	46 %
SEQUENCING	58	2	6*	0	1	0	66	50 %
IDH1/2 PCR	60	3	7	0	1	1	61	54 %

* 3 R132S; 2 R132G; 1 R132L

➢ Overall concordance between IHC and PCR for IDH1 R132H detection was 99% (**Tab 2**) - The only PCR/IHC discordant case was a sample of the commercial series.

➢ Comparison to the reference Sanger Sequencing with a typical LOD of 15-20 % showed a high overall concordance of 96% - PCR detected 5 additional mutated cases (2 IDH1 R132H, 1 IDH1 R132C, 1 IDH1 R132, 1 IDH2 172) not detected by Sequencing (**Tab 3**)

➢ Positive agreement between PCR and IHC was 98.4 % [91.3;99.7] and 100% between PCR and Sequencing [94.6;100] thus exceeding the target (PPA ≥95% with lower confidence limit ≥90%)

➢ All but one IHC-positive cases (n=61) were concordantly identified as IDH1 R132H by PCR whereas 3 (incl. the PCR-neg case) were not detected by Sequencing (**Tab 2-4**)

➢ Out of the IHC-negative cases (n=72), 9 rare mutations were additionally detected by Sequencing (12 %) and 12 (10 IDH1, 2 IDH2) by PCR (17 %) (**Tab 4**)

SYNTHETIC SAMPLES

• The IDH1/2 assay correctly detected the 11 IDH1/2 rare mutations at the two tested mutation-rates (30% and 40%).

SUMMARY AND CONCLUSION

• The newly developed **IDH1/2 PCR assay** showed a **high technical success.**

• The PCR assay **analytical sensitivity** (different for each mutation) was **high** - **LOD** was **< 5%** for all but one (rare IDH2) mutations, making this assay more sensitive than published references for sequencing techniques.

• Positive concordance with IHC (R132H) and Sequencing was high (98% & 100% resp.)

• The PCR assay detected 12 rare IDH1/2 mutations (9%)

• Additional work is ongoing to confirm the rare mutations identified with the kit with more sensitive sequencing techniques

• The **IDH1/2 PCR assay** can reliably be performed on **FFPE** glioma samples of less than 10 yrs of age - Analysis of a small series of samples > 10 yrs (n=17) also indicated a high technical success rate of IDH1/2 PCR (94%) and a high concordance between PCR with IHC (15/16) and Sequencing (14/16).

• This new highly standardized IDH1/2 assay is able to detect the major IDH1R132H mutation and 11 rare IDH1/2 mutations in **one step** and should facilitate the implementation of IDH testing in pathological laboratories to optimize diagnosis of glioma in routine clinical setting

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