A novel ultra-sensitive urine based FGFR3 mutation assay for diagnosis and recurrence detection of non-muscle-invasive bladder cancer (NMIBC) – Design of the Urodiag® Kit

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Abstract

Background

We have recently developed a highly accurate urine-based test, named Urodiag[®], associating FGFR3 mutation and DNA methylation assays for recurrence surveillance in patients with low-, intermediate-, and high-risk NMIBC. Previously, the detection of four FGFR3 mutations (G372C, R248C, S249C and Y375C) required the amplification steps and the PCR products were analyzed by capillary electrophoresis (Allele Specific-PCR, AS-PCR), which was expensive and time-consuming. Here, we present the development a novel ultra-sensitive multiplex PCR assay as called “Mutated Allele Specific Oligonucleotide-PCR (MASO-PCR)”, generating cost-effective, simple, fast and clinically applicable assay for the detection of FGFR3 mutations in voided urine.

Methods

Comparative clinical performances of MASO-PCR and AS-PCR technologies were performed from 263 urine DNA samples (87 FGFR3 mutated and 176 FGFR3 wild type). In the development of Urodiag[®] Kit, we studied the stability and reproducibility of each all-in-one PCR master mix (single reaction mixture including all the necessary PCR components) for MASO-PCR and QM-MSPCR (Quantitative Multiplex Methylation-Specific PCR to co-amplify SEPTIN9, HS3ST2 and SLIT2 methylated genes) assays.

Results

Complete concordance (100%) was observed between the MASO-PCR and AS-PCR results. Each PCR master mix displayed excellent reproducibility and stability after 12 months of storage at -20°C, with intra-assay standard deviations lower than 0.3 Ct and coefficient of variations (CV) lower than 1%. The limit of detection (LoD) of MASO-PCR was 5% mutant detection in a 95% of wild-type background. The limit of quantification (LoQ) of QM-MSPCR was 10 pg of bisulfite-converted DNA.

Conclusions

We developed and clinically validated the MASO-PCR assay, generating cost-effective, simple, fast and clinically applicable assay for the detection of FGFR3 mutations in urine. We also designed the Urodiag[®] Kit, which includes the MASO-PCR and QM-MSPCR assays. Adapted to routine clinical

laboratory (simplicity, accuracy), the kit will be a great help to urologists for recurrence surveillance in patients at low-, intermediate- and high-risk NMIBC. Reducing the number of unnecessary cystoscopies, it will have extremely beneficial effects for patients (painless) and for the healthcare systems (low cost).

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Tables

Due to technical limitations, Tables 1-3 are provided in the Supplementary Files section.

Additional Files

Additional file 1: Figure S1. Construction of mutated FGFR3 plasmids

Additional file 2: Figure S2. LoD for the Mutation assay

Additional file 3: Figure S3. LoQ for the Methylation assay

Figures

Figure 1

DNA integrity assessed by PCR amplification of Globin gene DNA concentrations were determined by fluorometry. The Globin gene was amplified with an amount of urine DNA comprised between 10 and 18 ng (4 μl of DNA sample) from each filter (F). Amplification curves are shown from Pool 1 to Pool 4, respectively.

a

b

Figure 2

2a-2b Design of Mutation and Methylation PCR assays

Supplementary Files

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[Additional file 2-Figure S2.pdf](#)

[Additional file 3-Figure S3.pdf](#)

[Table 3.pdf](#)

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