URODIAG KIT CE-IVD

HANDBOOK

Urine-based test for the surveillance of non-muscle-invasive bladder cancer (NMIBC)

Version V1_2022

CE

IVD

REF UR50N (50 Filters, 60 PCR reactions)
UR50K (50 filters, 50 DNA extraction, 55 bisulfite conversion of DNA, 60 PCR reactions)

Contents

Protocol for urine collection

RECOMMENDATIONS

- **Drink at least 1 liter of water** every day for 3 days before urine collection

- **Collect the first morning urine** (it is recommended not to drink after midnight).

COLLECTION INSTRUCTIONS

Perform on STERILE CONTAINER

- Wash hands thoroughly with soap
- Make a careful local toilet
- Eliminate the 1st urine stream in the toilet
- **Collect 50 to 100 ml of urine** in the sterile container
- Close the bottle by screwing the lid on properly and identify it (name + first name + date and time of collection)

Sample storage time

- Transport the urine container from the patient's home to the competent service (laboratory, hospital), at room temperature and within 2 hours,

- After 2 hours, store the urine sample in the refrigerator (+4°C to +8°C) for up to 72 hours

Protocol for urine samples filtration

Urodiag Urine Filter Kit

50 Filters

Protocol

- Urodiag urine filter: Filters are stored at room temperature (15°C-25°C).
- Expiry time: 10 years from production date.

Equipment and Reagents to Be Supplied by User:

■ Urine sample **(50 ml to 100 ml)**

■ Syringe 50 ml luer-lock (Terumo)

■ Phosphate-buffered saline (PBS 1X), without Mg & Ca (stored at +4°C)

■ Waste bottle containing bleach

1. Remove the plunger from the syringe and connect the **Filter** to the syringe.
2. Introduce **50 ml of urine sample**, then reinsert the piston into the syringe.
3. Apply gentle pressure on the syringe piston to ensure the filtration of the urine sample. The filtrate is collected in a waste container containing bleach.

For urine volumes greater than 50 ml, the operator should disconnect the filter from the syringe after the first 50 ml of urine has been filtered and repeat steps 1 to 3 to filter the remaining volume of the sample.

4. Repeat step 1, introduce 5 ml of PBS 1X, reinsert the piston into the syringe and apply gentle pressure on the syringe piston. The filtrate is collected in the waste bottle containing bleach.
5. Disconnect **the Filter** from the syringe.

The Filter is ready for DNA extraction procedure or can be stored one month at -20°C and shipped on dry ice.

Note: Alternative protocol *using the* ***QIAvac 24 Plus (Qiagen).***
Ensure that the main vacuum valve is closed (protocol in QIAvac 24 Plus Handbook). Switch on the vacuum pump by pressing the power switch. Adjust the needle of the vacuum approximately at ***-300 mbar****. Insert the VacConnector into the luer slot, then the* ***Filter/syringe (without the piston) or Device,*** *on the QIAvac 24 Plus. Introduce the first 50 ml of the urine sample into the Device. Open the main vacuum valve and ensure that the needle is stabilized near -300 mbar. Once all the urine sample has been filtered, wash the filter with 5 ml of PBS 1X and then switch off the vacuum pump. Remove the Device from the vacuum manifold, and discard the VacConnector.*

Protocol for DNA extraction and purification

Protocol

The extraction and purification of DNA are carried out with the QIAamp DNA Mini Kit (QIAGEN). The kit can be stored at room temperature (+15°C to +25°C).

Expiry time: 12 months from delivery date.

Equipment and Reagents to Be Supplied by User:

- QIAamp DNA Mini Kit (Qiagen), Catalog No. 51304 (50 rns) (UR50N) (included with UR50K)
- Absolute ethanol (96-100%) (stored at 4°C)
- Syringe 2.5 ml with needle 21 Gx25 mm (Terumo)
- Micropipets and pipet tips with aerosol barrier
- Microcentrifuge (with rotor for 1.5-2 ml tubes)
- Vortexer
- Water bath or heating block at +56°C
- Phosphate-buffered saline (PBS 1X), without Mg & Ca (stored at +4°C)

Buffer preparation:

Preparation of Wash Buffer- AW1 and **AW2** buffers are supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle. AW1 and AW2 buffers are stable for 1 year when stored closed at room temperature (+15°C to +25°C).

Preparation of Cell Lysis Buffer

1. Introduce 220 µl **Buffer PBS 1X** and 22 µl QIAGEN **Proteinase K** in 1.5 ml microcentrifuge tube.
2. Mix by pulse-vortexing for 1 second and briefly centrifuge the tube for 2 seconds.
3. Add 220 µl **Buffer AL**. Mix by pulse-vortexing for 2-5 seconds and briefly centrifuge the tube for 2 seconds.

   Note: *Mix Buffer AL thoroughly by shaking before use. Do not add Proteinase K directly to Buffer AL.*

4. Disconnect the needle and the piston from the syringe.
5. Connect the **Filter** to the syringe, then to the needle, and placed the system in 2 ml microcentrifuge tube.

   Note: *If the Filter has been stored at -20°C, leave it on the bench 5 min at room temperature before the lysis step*

6. Introduce 462 µl of **Cell Lysis Buffer** into the syringe, then reinsert the piston.
7. Pass the lysate 3 times through the filter by aspiration/discharge operation and pushing/pulling the piston slowly.

   Note: *If foaming occurs, we recommend to briefly centrifuge of the tube for 2 seconds.*

8. Incubate the sample at 56°C for 15 minutes (water bath or heating block).

9. Briefly centrifuge the 2 ml microcentrifuge for 2 seconds to remove drops from the lid or sides.

10. Add 220 µl of **Ethanol** to the sample and mix by pulse-vortexing for 2 seconds. After mixing, briefly centrifuge the 2 ml microcentrifuge for 2 seconds to remove drops from the lid or sides.

11. Introduce the mixture (682 µl) onto the **QIAamp Mini spin column** (in a 2 ml collection tube). Close the cap, and centrifuge at full speed (14,000 rpm, 16,800 rcf) for 1 min. Discard the flow-through and collection tube.

12. Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500 µl **Buffer AW1.** Close the cap, and centrifuge at full speed (14,000 rpm, 16,800 rcf) for 1 min. Discard the flow-through and collection tube.

13. Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500 µl **Buffer AW2.** Close the cap, and centrifuge at full speed (14,000 rpm, 16,800 rcf) for 3 min. Discard the flow-through and collection tube.

14. Place the spin column into a 1.5 ml microcentrifuge tube and centrifuge at full speed for 1 min to eliminate possible traces of Buffer AW2, which will be removed by pipetting.

15. Add 50 µl of **AE Elution Buffer** directly to the column matrix, and incubate at room temperature for 5 minutes. Centrifuge at full speed for 1 min to elute the DNA.

16. Determine the concentration of DNA by fluorometry (i.e. Qubit® fluorometer)

17. Dilute the DNA solution to **1.25 ng/µl in Buffer AE**.

Note: *If the DNA solution is less than 1.25 ng/µl, it should be concentrated by heating to +50°C using a heating block or a DNA concentrator (speed vacuum). The volume of DNA to be evaporated is calculated to obtain the concentration of 1.25 ng/µl.*

18. The DNA solution is ready for immediately analysis or can be stored at -20°C for up to 1month.

The Mutation assay *requires a quantity of* ***10 ng of DNA (1.25 ng/µl)***

The Methylation assay *requires a quantity of* ***15 ng to 30 ng of DNA (1.25 ng/µl);*** *It is recommended to perform the Methylation test from 30 ng of DNA.*

Note: Alternative DNA purification protocol using QIAvac 24 Plus (Qiagen).
Steps 11, 12 and 13 can be performed using the QIAvac 24 Plus. Turn on the vacuum pump by pressing the power switch. Adjust the vacuum needle to ***approximately -600 mbar****.*
Connect the VacConnector device / QIAamp Mini spin column of the QIAvac 24 Plus. Add the sample from step 10 ***(682 µl)*** *to the QIAamp Mini spin column and leave the column cover open.*
Open the main vacuum valve and make sure the needle is stabilized near -600 mbar. Once all of the lysate has been aspirated through the spin column, turn off the vacuum pump.
Add 750 µl of Buffer AW1 *washing solution to the column and turn on the vacuum pump. Once all of the Buffer AW1 solution has been aspirated through the column, turn off the vacuum pump.*
Add 750 µl of Buffer AW2 *washing solution to the column and turn on the vacuum pump. Once all of the AW2 solution has been drawn through the column, turn off the vacuum pump. Close the column, remove it from the QIAvac suction system and discard the VacConnector.*

Protocol for DNA conversion with sodium bisulfite

Protocol

The bisulfite conversion of DNA is carried out with the EZ DNA Modification kit (Zymo Research). The kit can be stored at room temperature (+15°C to +25°C).

Expiry date: 12 months from delivery date.

Equipment and Reagents to Be Supplied by User:

■ EZ DNA Methylation Kit (Zymo Research), Catalog No. D5001 (55 reactions) (UR50N) (included with ref. UR50K)

■ Absolule ethanol (96-100%)

■1.5 ml microcentrifuge tubes and MicroAmp 8-Tube strip, 0.2 ml, catalog No. N8010580 (ThermoFisher Scientific) and 8-Cap Strip, Catalog No. N8010535 (ThermoFisher Scientific)

■ Micropipets and Pipet tips with aerosol barrier

■ Microcentrifuge (with rotor for 1.5-2 ml tubes)

■ Vortex

■ Heating block (+37°C)

■ Incubator at +50°C in thermocycler (recommended) or in heating block

■ Water PCR grade (DNase, RNase free)

Buffer preparation:

1. **Preparation of the conversion solution (CT-Conversion tube) for 11 reactions –**The **CT Conversion** tube contains a solid mixture. The conversion solution must be prepared as follows:

- Add 750 µl sterile water and 210 µl of **M-Dilution Buffer** to a tube of **CT Conversion** tube
- Mix at room temperature (15°C-25°C) with frequent vortexing for 5-10 minutes.

Note: *It is normal to see traces of undissolved reagent in the CT-Conversion tube. The CT-Conversion solution can be used immediately or stored for one week at +4°C or one month at -20°C.*

2. **Preparation of M-Wash Buffer–** Add 24 ml of ethanol to the 6 ml **M-Wash Buffer** concentrate

3. **Preparation of DNA-** Introduce in a sterile microtube (1.5 ml) the different volumes indicated in the table below:

At each experience (run)	C+ meth (purple cap)*	Patient
DNA sample (1.25 ng/µl)	24 µl (30 ng)	X µl (15 to 30 ng**)
Water PCR grade	21 µl	X µl (adjust the volume to 50 µl)
M-Dilution Buffer	5 µl	5 µl
Mix the sample by pipetting up and down and centrifuge the tube 2 seconds		

***Tube C+ meth of Urodiag® Multiplex PCR Kit (purple cap**) = Methylated human control DNA, Catalog No. ZD5011 (Zymo Research)

**** The quantity of **30 ng of DNA** is recommended

4. Incubate the sample in heating block for 15 minutes at +37°C.

5. Centrifuge briefly the tube for 2 seconds to remove drops from the lid or sides.

6. Add 100 µl of the conversion solution to DNA sample (50 µl) and mix by pipetting up and down 5 times.

7. Transfer the DNA sample (150 µl) contained in the 1.5 ml microtube to a 0.2 ml PCR tube (e.g., strip of 8 MicroAmp tubes and strip of 8 caps, applied biosystems).

8. Incubate the DNA sample in a thermocycler for 15 hours and 30 minutes at +50°C and then hold at +4°C.

9. The tube can be stored at +4°C for 10 minutes to 20 hours.

Note: Alternative incubation condition*. Step 6: The DNA sample (150 µl) contained in the 1.5 ml microtube is incubated in a heating block at 50°C for 15 hours and 30 minutes, then stored at +4°C for 10 minutes to 20 hours.* Centrifuge the tube for 2 seconds to remove drops from the lid or sides.

10. Add 400 µl of **M-Binding Buffer** to a **Zymo-Spin IC Column** and place the column into a provided **Collection tube**.

11. Load the sample (~150 µl) into the Zymo-Spin IC Column containing the M-Binding Buffer. Close the column and mix by inverting the column at least 5 times.

12. Centrifuge the column at full speed (14,000 rpm, ≥10,000 x g) for 30 seconds. Discard the flow-through.

13. Add 100 µl of **M-Wash Buffer** to the column. Close the column and centrifuge at full speed (14,000 rpm, ≥10,000 x g) for 30 seconds.

14. Add 200 µl of **M-Desulphonation Buffer** to the column and let stand at room temperature for 20 minutes. After the incubation, centrifuge the column at full speed (14,000 rpm, ≥10,000 x g) for 30 seconds.

15. Add 200 µl of **M-Wash Buffer** to the column. Close the column and centrifuge at full speed (14,000 rpm, ≥10,000 x g) for 30 seconds. Add another 200 µl of **M-Wash Buffer** to the column and centrifuge at full speed (14,000 rpm, ≥10,000 x g) for an additional 30 seconds.

16. Place the column into a 1.5 ml microcentrifuge tube and centrifuge at full speed (14,000 rpm, ≥10,000 x g) for 30 seconds to eliminate possible traces of M-Wash Buffer, which will be removed by pipetting.

17. Add 10 µl of **M-Elution Buffer** directly to the column matrix, and let stand at room temperature for 5 minutes. Centrifuge the microcentrifuge tube at full speed (14,000 rpm, ≥10,000 x g) for 30 seconds to elute DNA.

The bisulfite-converted DNA solution is ready for immediate analysis or can be stored at -20°C for one week.

Multipex PCR protocol

Urodiag® Multiplex PCR Kit

The Urodiag® Multiplex PCR Kit is an in vitro diagnostic test for the surveillance of patients with non-muscle-invasive bladder cancer (NMIBC).

The procedure for multiplex PCR reactions is carried out with the StepOnePlus Real-Time system (applied biosystems, ThermoFisher Scientific). The kit can be stored in the freezer (-15°C to -30°C) for 12 months. It is not recommended to freeze and thaw the kit more than 5 times.

Expiry time: 12 months from production date.

Equipment and Reagents to Be Supplied by User:

■ StepOnePlus Real-Time system

■ MicroAmp Fast Optical 96-Well Reaction Plate, 0.1 ml, Catalog No. 4346907 (ThermoFisher Scientific) and Optical Adhesive Covers, Catalog No. 4360954 (ThermoFisher Scientific) or MicroAmp Fast 8-Tube Strip, 0.1 ml, Catalog No. 4358293 (ThermoFisher Scientific) and MicroAmp Optical 8-Cap Strip, Catalog No. 4323032 (ThermoFisher Scientific)

■ Micropipets and pipet tips with aerosol barrier

■ Microcentrifuge for PCR plates

■ Vortexer

■ Cooling block

■ Water, PCR grade (DNase, RNase free)

Kit contents

The kit is designed for 50 PCR tests corresponding to 120 PCR reactions for 5 runs (maximum 10 patients/run). Each test PCR or run consists of 24 PCR reactions including 4 for Quality Control and 20 PCR reactions for 10 patients.

It is designed for the detection of 4 mutations of the FGFR3 gene (G372C, R248C, S249C, Y375C) and the quantification of 3 DNA methylation markers (HS3ST2, SEPTIN9, SLIT2) by multiplex PCR from patients' urine DNA.

The kit is composed of 8 tubes: 5 tubes for the Mutation assay (blue, green and white cap), 3 tubes for the Methylation assay (red, orange and purple cap). Each tube contains all the components (PCR master mix, primers and probes) necessary to carry out the Mutation and Methylation assays.

	Cap	Label	Description
Mutation assay		**MM mut 1** Lot 0000X Exp. Store at: -15 to -30°C Light sensitive Vol. 1000 µL	**Master Mix mutation1:** The tube contains all components (PCR master mix, primers and probes) necessary to detect S249C, Y375C mutations and the GLOBIN gene
		**C+ mut 1** Lot 0000X Exp. Store at: -15 to -30°C Vol. 22 µL	**Positive control mutation 1:** The tube contains a mixture of two DNA solutions: (i) synthetic FGFR3 sequences comprising the mutations S249C and Y375C inserted in a plasmid vector, (ii) human control DNA to detect the GLOBIN gene (internal control)
		**MM mut 2** Lot 0000X Exp. Store at: -15 to -30°C Light sensitive Vol. 1000 µL	**Master Mix mutation 2:** The tube contains all components (PCR master mix, primers and probes) necessary to detect G372C, R248C mutations and the GLOBIN gene
		**C+ mut 2** Lot 0000X Exp Store at: -15 to -30°C Vol. 22 µL	**Positive control mutation 2:** The tube contains a mixture of two DNA solutions: (i) synthetic FGFR3 sequences comprising the mutations S249C and Y375C inserted in a plasmid vector, (ii) human control DNA to detect the GLOBIN gene (internal control)
		C- mut Lot 0000X Exp. Store at: -15 to -30°C Vol. 44 µL	**Negative Control mutation:** human DNA to detect the GLOBIN gene (internal control)
Methylation assay		**MM meth A** Lot 0000X Exp. Store at: -15 to -30°C Light sensitive Vol. 1000 µL	**Master Mix methylation A:** The tube contains all components (PCR master mix, primers and probes) necessary to detect and quantify, ALBUMIN (unmethylated allele) and SEPTIN9 (methylated allele)
		**MM meth B** Lot 0000X Exp. Store at: -15 to -30°C Light sensitive Vol. 1000 µL	**Master Mix methylation B:** The tube contains all components (PCR master mix, primers and probes) necessary to detect and quantify, HS3ST2 and SLIT2 (methylated alleles)
		**C+ meth** Lot 0000X Exp. Store at: -15 to -30°C Vol. 126 µL	**Positive control methylation:** 100% methylated control DNA (human). The DNA solution is converted with sodium bisulfite before use.

Protocol

Step 1: Sample preparation

1. Thaw tubes in a cooling block (recommended).
2. Homogenize the master mix (MM mut 1 and MM mut 2) tubes by inverting 5 and the DNA control tubes (C+ and C-) by tapping.
3. Briefly centrifuge (2 seconds) all tubes to collect solutions at the bottom of the tubes.

- **Mutation assay**

It is recommended to carry out the Mutation Assay from **10 ng of DNA** with 5 ng of DNA for the detection of S249C and Y375C mutations and 5 ng of DNA for the detection of G372C and R248C mutations.

Prepare samples according to recommendations in Table 1 below.

Tube	Control				Patient	
	Control 1		Contrôle 2		Test 1	Test 2
	Positive	Négative	Positive	Négative		
MM mut 1	16 µl	16 µl	/	/	16 µl	/
C+ mut 1	4 µl	/	/	/	/	/
MM mut 2	/	/	16 µl	16 µl	/	16 µl
C+ mut 2	/	/	4 µl	/	/	/
C- mut	/	4 µl	/	4 µl	/	/
DNA patient	/	/	/	/	4 µl	4 µl
Total volume	20 µl					

- **Methylation assay**

It is recommended to carry out the methylation assay with **30 ng of bisulfite-converted DNA**, (15 ng of bisulfite-converted DNA for the quantification of ALBUMIN et SEPTIN9 and 15 ng of bisulfite-converted DNA for the quantification of HS3ST2 and SLIT2).

Note: *For patient, the minimum amount of DNA is 15 ng, i.e. 7.5 ng of bisulfite-converted DNA for the quantification of ALBUMIN and SEPTIN9 and 7.5 ng of bisulfite-converted DNA for the quantification of HS3ST2 and SLIT2.*

Prepare samples according to recommendations in Table 2 below.

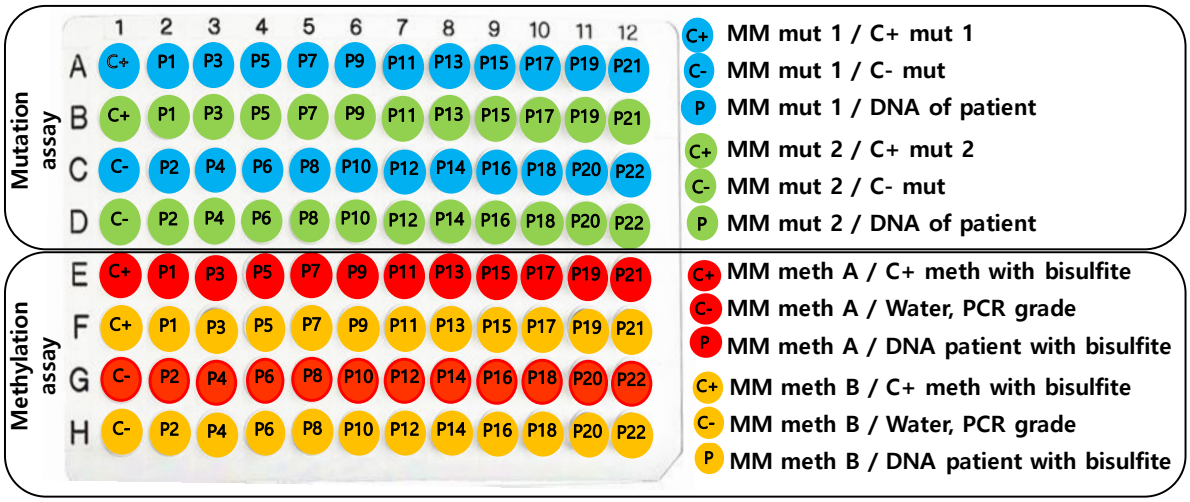
Tube	Control				Patient	
	Control 1		Control 2		Test 1	Test 2
	Positive	Negative	Positive	Negative		
MM meth A	16 µl	16 µl	/	/	16 µl	/
MM meth B	/	/	16 µl	16 µl	/	16 µl
C+ meth*	4 µl	/	4 µl	/	/	/
Patient DNA*	/	/	/	/	4 µl	4 µl
Water, PCR grade	/	4 µl	/	4 µl	/	/
Total volume	20 µl					

* Bisulfite-converted DNA

Step 2: Loading of samples in MicroAmp Fast Optical 96-well plate or MicroAmp Fast Reaction (8-tubes/Strip)

As below, the position of the DNA samples on a 96-well PCR plate or PCR tubes (8-reaction tubes/strip, 0.1 ml) to carry out the URODIAG test of 22 patients.

1- Dispense appropriate volumes (reagents/samples) into corresponding wells or tubes, according Tables 1 & 2.



2- After covering the 96-well plate with the optical film or closing the PCR tubes with the caps, homogenize the reaction mixture by vortexing for 2 seconds.

3- Centrifuge the 96-well PCR plate or the PCR tubes using a microplate rotor for 10-15 seconds at approximately 1000 x g (3000 rpm).

Step 3: Programming the StepOnePlus Real-Time system

The PCR reaction configuration is stored in the file URODIAG_TEMPLATE_PCR.edt.

The operator should import the URODIAG_TEMPLATE_PCR.edt file into the StepOnePlus.

Place the 96-well PCR plate or PCR tubes in the PCR machine and start the PCR program by selecting RUN.

- **Parameters**

Mutation assay		
TaqMan reagents		
Mode Quantitation –Comparative Ct		
Reaction volume per well)		**20 µl**
Ramp speed		**Fast**
Ramp rate		**100%**
Threshold (ΔRn)	R248C G372C S249C Y375C *GLOBIN*	**0.24** **0.15** **0.15** **0.15** **0.15**
Baseline		**Auto**
Passive reference		**ROX**

Methylation assay		
TaqMan reagents		
Mode Quantitation –Comparative Ct		
Reaction volume per well)		**20 µl**
Ramp speed		**Fast**
Ramp rate		**100%**
Threshold (ΔRn)	*ALBUMIN* *HS3ST2* *SEPTIN9* *SLIT2*	**0.10** **0.10** **0.10** **0.10**
Baseline		**Auto**
Passive reference		**ROX**

- **Dye (Reporter)**

Mutation assay		
Detection	**Reporter**	**Quencher**
G372C	VIC	(none)
R248C	FAM	(none)
S249C	FAM	(none)
Y375C	VIC	(none)
GLOBIN	NED	(none)

Methylation assay		
Detection	**Reporter**	**Quencher**
ALBUMIN	VIC	(none)
HS3ST2	FAM	(none)
SEPTIN9	FAM	(none)
SLIT2	VIC	(none)

- **Multiplex PCR cycling conditions**

Steps	**Number of cycles**	**Time**	**Temperature**	**Fluorescence data collection**
Initial PCR activation step (hot start)	1	5 min	95°C	-
Denaturation	40	45 s	95°C	-
Annealing/ extension		45 s	60°C	✓

Step 4: Rendering of results

Confidence intervals (min and max Ct values) as well as threslods (ΔRn) were set for a highest accuracy of the Urodiag test in terms of sensitivity and specificity, respectively.

- **Quality Control (QC)**

Mutation assay			**Ct values**		**Rendering**
			Min	**Max**	
Control	**Positive**	GLOBIN	28	32	PASSED
		FGFR3 mutations: G372C, R248C, S249C and Y375C	28	33	PASSED
	Negative	GLOBIN	28	32	PASSED
		FGFR3 mutations: G372C, R248C, S249C and Y375C	No amplification		PASSED
Patient	**Positive**	GLOBIN	28	32	PASSED

Methylation assay			Ct values		Rendering
			Min	Max	
Control	**Positive**	All targets: ALBUMIN, HS3ST2, SEPTIN9 and SLIT2	26	30	PASSED
	Negative	All targets: ALBUMIN, HS3ST2, SEPTIN9 and SLIT2	No amplification		PASSED
Patient	**Positive**	ALBUMIN	26	30	PASSED

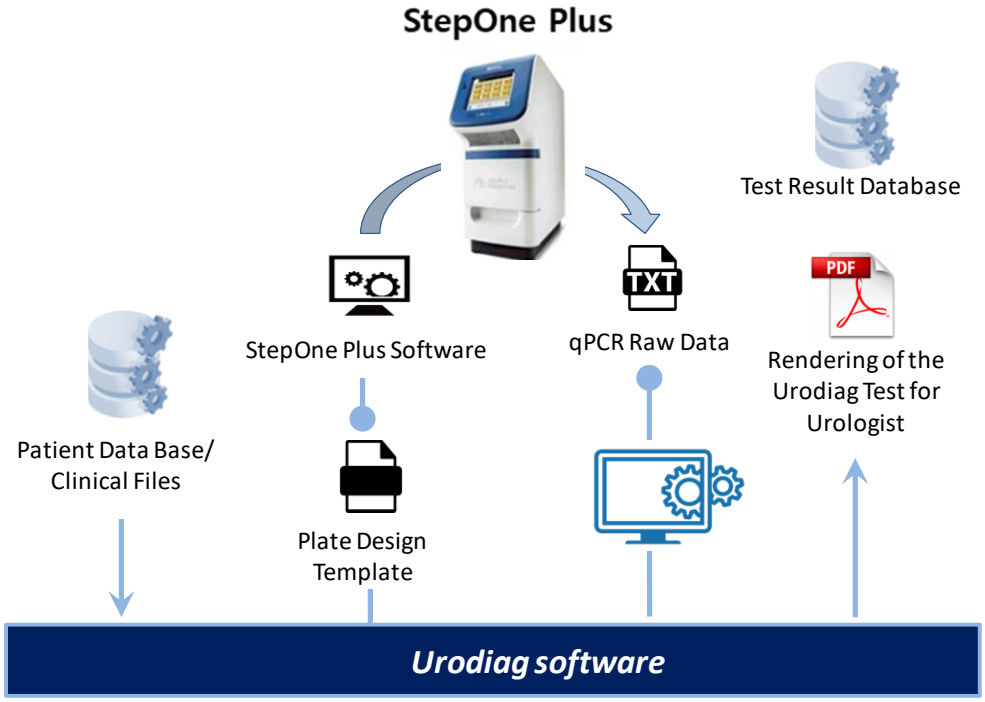
- **Patient**

Mutation assay		Ct values		Interpretation	Result
		Min	Max		
Patient	S249C mutation	25	40	DNA mutated for S249C	POSITIVE
	Y375C mutation			DNA mutated for Y375C	POSITIVE
	G372C mutation			DNA mutated for G372C	POSITIVE
	R248C mutation			DNA mutated for R248C	POSITIVE

Methylation assay		Ct values		Interpretation	
		Min	Max		
Patient	HS3ST2	25	40	Presence of methylated alleles of the *HS3ST2* gene	Determination of the methylation degree of the 3 target genes
	SEPTIN9			Presence of methylated alleles of the *SEPTIN9* gene	
	SLIT2			Presence of methylated alleles of the *SLIT2* gene	

Urodiag test result

The Urodiag software was co-developed by Oncodiag and Biomaneo (https://biomaneo.fr). The software allows the analysis of PCR data, interpretation, result management and rendering of Urodiag test to urologists. Streamlined representation of the workflow is shown below:



It will be provided to customer:

- Urodiag software
- User's manual
- Installation and training
- Maintenance

- **Urodiag test result**

MUTATION ASSAY	METHYLATION ASSAY	URODIAG TEST RESULT
POSITIVE	POSITIVE	POSITIVE
POSITIVE	NEGATIVE	
NEGATIVE	POSITIVE	
NEGATIVE	NEGATIVE	NEGATIVE

- **Rendering of the Urodiag test for Urologist**

Address

Urologist
Street
City

Date :

Subject: Rendering of the test result for patient 01

The result of the Urodiag test on July 01, 2019 is as follows:

NEGATIVE

For information, below is the table giving the Urodiag results during the patient monitoring:

Date	Mutation	Methylation	Urodiag result
01/01/2019	POSITIVE	1st analysis serving as reference	
01/04/2019	NEGATIVE	NEGATIVE	NEGATIVE
01/07/2019	NEGATIVE	NEGATIVE	NEGATIVE
01/10/2019	NEGATIVE	NEGATIVE	NEGATIVE
01/01/2020	NEGATIVE	NEGATIVE	NEGATIVE
01/04/2020	POSITIVE	NEGATIVE	POSITIVE
01/07/2020	NEGATIVE	NEGATIVE	NEGATIVE

Lab manager

Performance characteristics

Limit of detection (LOD)

- Mutation assay = 5% (mutated allele/unmutated allele ratio) corresponds to the limit of detection of FGFR3 mutations.
- Methylation assay = 10 pg is the smallest quantity of control DNA (fully methylated DNA converted by sodium bisulfite solution) necessary for the detection of methylated SEPTIN9, HS3ST2 and SLIT2 genes.

Analytical specificity

The oligonucleotide sequences of each target were defined using NCBI database (National Center for Biotechnology Information).

Stability of components of the Urodiag Kit

- Urodiag Urine Filter Kit: 10 years from production date
- Urodiag Multiplex PCR Kit: 12 months from production date
- Extraction and purification DNA by QIAamp DNA Mini Kit (QIAGEN): 12 months from delivery date
- DNA conversion by EZ DNA Modification kit (Zymo Research): 12 months from delivery date