HB miRDx™ BKV Kit CE IVD

(Revision 4)

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1. Intended use

The HB miRDx™ BKV Kit is an *in vitro* molecular diagnostic test for the quantitation of BK virus-specific miRNA biomarker, bkv-miR-B1-5P, to detect BK virus-specific miRNA in human urine using a reverse transcription quantitative polymerase chain reaction (RT-qPCR).
The HB miRDx™ BKV Kit is intended as an aid in the detection of BK virus infection, together with other clinical and laboratory findings.
Testing with the HB miRDx™ BKV Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures.
The results from HB miRDx™ BKV Kit are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Test results must not be the sole basis for patient management decisions.

2. Background

miRNAs (microRNA) are non-coding RNAs composed of around 22 ribonucleotides that bind to the target site within 3‘-UTR of the target mRNA (messenger RNA), which causes the inhibition of protein synthesis or degradation of mRNA to induce gene silencing. miRNAs control the expression of genes related to cancer, cardiac and neurologic disorders, and insulin secretion in the body, and as they perform an important role as a modulator of the progression of tumors including cancer, they can be used as a biomarker for cancer screening, custom-tailored drug selection, and prediction of prognosis.
The BK virus is a major cause of lethal loss of function in patients with renal transplants, and early diagnosis is crucial. Generally, BK virus nephropathy can be confirmed at around 12 months after renal transplantation, and regular monitoring for the BK virus after transplantation is necessary. The viral infection site is localized in the transplanted kidney and there is a high probability that it is not detected in a biopsy, and serum PCR can test positive without an infection.
This product is a Reverse Transcription Real-time PCR Kit that can detect the miRNA biomarker bkv-miR-B1-5p in the patient’s urine, which is abundantly expressed by the BK virus in infected patients with kidney transplants and thus has a higher diagnostic efficiency, with high sensitivity and specificity.

3. Test Principle

The HB miRDx™ BKV Kit is an *in vitro* molecular diagnostic test for the quantitation of BK virus-specific miRNA biomarker, bkv-miR-B1-5P, to detect BK virus-specific miRNA in human urine using a reverse transcription quantitative polymerase chain reaction (RT-qPCR). BK virus detection using the HB miRDx™ BKV kit is performed based on four steps as follows.

Extraction and Purification of miRNA in human urine
The miRNAs are extracted by using the validated reagents from human urine specimen. Internal Control (IC) could be added to the urine specimen to extract miRNA or mix with the reverse transcription mixture. It is to confirm the presence of a non-specific PCR inhibitor in the sample or to check errors in the miRNA extraction process.

Synthesis of cDNA by Reverse Transcription (RT) of target miRNA
cDNA is synthesized by reverse transcription from the extracted miRNA using a specific primer that directly targets BKV-miR-B1-5p as a miRNA of BKV.

Nucleic Acid Amplification and Target Detection
The specific Extension Sequence primer, universal forward and reverse primers are used to amplify the cDNA from BKV-miR-B1-5p. To detect BKV-miR-B1-5p, dual labeled probe fluorescence FAM is used.

Probe anneals to the location in between forward and reverse primer. Taq polymerase activation leads to hydrolysis of the dual-labeled probe to cleavage quencher from reporter. Quenching refers to any process which decreases the fluorescence intensity of a given substance. Internal control (IC) is used to determine whether the miRNA extraction went without experimental error and non-specific PCR inhibitors are detected.

Note: The assay has been validated on the ABI 7500 FAST and Bio-rad CFX96 touch Real-Time PCR system. Other thermal cyclers require end-user validation.

BKV DNA Quantitation

STD is provided as 10^{10} copies/ul. STD is diluted into 1/10 serial dilution to prepare STD1~5 (10^{9}~10^{5} copies/ul). Create the standard curve with measured STD1~5 CT values. Then use the equation from the IFU to calculate the concentration of the sample.

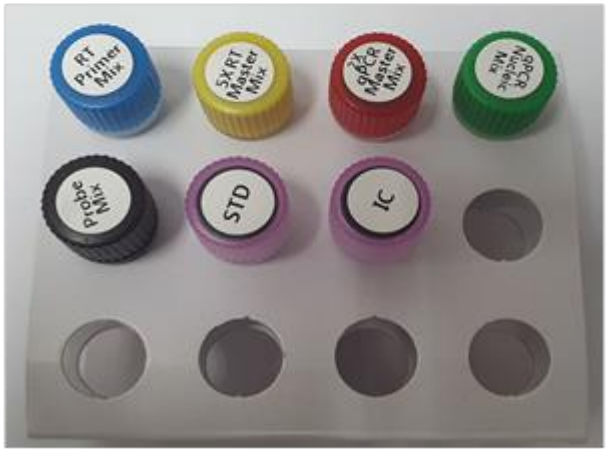
4. Product Description

Product Name	HB miRDx™ BKV Kit	
Trade Name	HB miRDx™ BKV Kit	
Catalogue No	HMIRP-BK101	For 24 rxns
Classification acc. to IVDD	Others	Neither listed in Annex II of IVDD, Nor self-testing
GIVD Code	15.04.40.22	BK virus – NA Reagents
Conformity Assessment Route	Self Declaration according to Annex III of IVDD	
List of applied Harmonised Standard	See Attachment 1. Declaration of Conformity Attachment 2. ER Checklist	

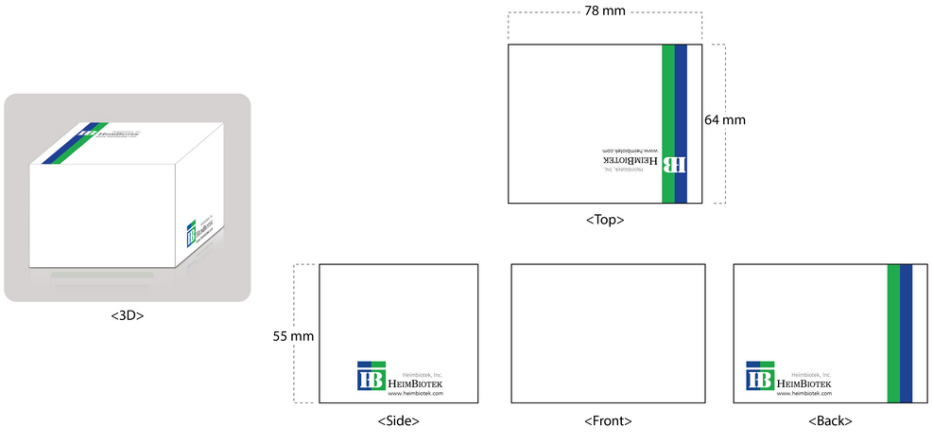
4.1 Kit Components

No	Components	Volume	Q'ty	Appearance features
1	RT Primer Mix	24 ul	1	Colorless transparent solution in a colorless plastic tube with a blue lid
2	5X RT Master Mix	96 ul	1	Colorless transparent solution in a colorless plastic tube with a yellow lid
3	2X qPCR Master Mix	240 ul	1	Colorless transparent solution in a colorless plastic tube with a red lid
4	qPCR Nucleic Mix	72 ul	1	Colorless transparent solution in a colorless plastic tube with a green lid
5	Dual-labeled Probe Mix	24 ul	1	A light red solution in a dark brown plastic tube with a dark brown lid.
6	Standard (STD)	5 ul	1	Colorless transparent solution in a colorless plastic tube with a purple lid
7	Internal Control (IC)	30 ul	1	Colorless transparent solution in a colorless plastic tube with a purple lid

Component	RT Primer Mix	5X RT Master Mix	2X qPCR Master Mix	qPCR Nucleic Mix	Dual-labeled Probe Mix	Standard (STD)	Internal Control (IC)
Imager							
Size	Dimension X High ≒ 1.0 X 5.0 ㎝						
Product Name	HB miRDx™ BKV Kit						
Image	Package Box Image						



Drawing

	
Size	W x L x H ≒ 7.8 X 6.4 X 5.5 ㎝

4.2 Materials required but not provided

4.2.1. RNA Extraction Kits / Equipment

For the HB miRDx™ BKV Kit, it is recommended to use the miRNeasy Serum/Plasma Kit [217184] (QIAGEN, Germen) for miRNA extraction from the sample.

4.2.2. Real-time PCR equipment

HB miRDx™ BKV Kit was developed for use with the following devices.
– Bio-Rad CFX96 Touch™ Real-Time PCR Detection System
– Applied Biosystem® 7500 Fast Real-Time PCR System

4.2.3. Other equipment and consumables

– PCR hood
– Laboratory freezers (-10 °C to -30 °C, ≤ -70 °C)
– Vortex mixer
– Centrifuge with a rotor for microplates
– Microcentrifuge
– Micropipettes (2.5 µl, 10 µl, 200 µl, 1000 µl)
– Racks for 1.5 mL microcentrifuge tubes or PCR tubes
– Microplates or 8-strip PCR tube
– 2 x 96-well -20 °C cold blocks
– 0 °C Cold block or ice
– Molecular grade nuclease-free water

4.3. Sample Preparation and Test Method

4.3.1. Sample preparation

① All clinical samples should be treated as infectious substances. Urine is the recommended sample for the HB miRDx™ BKV Kit

② When collecting a urine sample, pass the first urine and use a standard container provided by the hospital to collect the midstream urine, which is less likely to be contaminated by microorganisms, etc.

③ Centrifuge the urine samples 4°C for 5 min at 1,500~ 2,000 rpm.

④ Collect the supernatant from the tube by pipetting.

⑤ Samples should be extracted immediately or, if this is not possible, kept refrigerated (no more than a week) or frozen (for a month).

⑥ Samples should be transported in unbreakable containers to prevent contamination by cracks in the sample container.

4.3.2. Test preparation

① miRNA extraction of urine samples is recommended using the miRNeasy Serum/Plasma Kit [217184] (QIAGEN, Germany). For miRNA extraction, add the Internal Control (IC) 1 µl/sample provided in the HB miRDx™ BKV Kit to the sample after the Lysis stage before proceeding to the next stage.

② Thaw frozen reagents by allowing to stand at 4℃ or on ice.

4.3.3. Test method

① Reverse Transcription

a. To ensure accurate results, briefly vortex and spin down the reagents and Positive Control, except for the dual-labeled probe. Tap the dual-labeled probe.

b. Prepare the required amount of the RT Master Mixture by mixing 4 µl of the 5X RT Master Mix and 1 µl of the RT Primer Mix. (See Table 1. RT Reaction Mixture)

[Table 1. RT Reaction Mixture]

No. of / Sample Reagent	1 test
5X RT Master Mix	4 µl
RT Primer Mix	1 µl
DEPC-DW	14 µl
Total Volume	19 µl

c. Dilute the STD material provided in the HB miRDx™ BKV Kit to prepare STDs 1–5, as shown in Table 2, to be used as templates. (Refer to Table 2. Standard (STD) serial dilution)

[Table 2. Standard (STD) serial dilution]

Input	STD n (µl)	DEPC (µl)	Output
STD	2	18	STD 1
STD 1	2	18	STD 2
STD 2	2	18	STD 3
STD 3	2	18	STD 4
STD 4	2	18	STD 5

* The STD material is for dilution, and should not be used before dilution

* Sufficient tapping and spin-down is needed for accurate dilution

d. Transfer 19µl of the prepared mixture to each PCR tube.
e. Add 1µl of the sample or diluted STD 1–5 or IC and close the lids.
f. Transfer to the real-time PCR machine and allow to react. (See Table 3. BKV RT Condition)

[Table 3. BKV RT Condition]

Temperature	Times
37℃	60 min
95℃	5 min

② Real-time PCR

a. To ensure accurate results, briefly vortex and spin down all reagents before use and the cDNA after RT.

b. Prepare the required amount of the Real-time PCR Master Mixture by mixing 10 µl of 2X qPCR Master Mix, 3 µl of qPCR Nucleic Mix, and 1 µl of the dual-labeled probe Mix. (See Table 4. qPCR Reaction Mixture)

[Table 4. qPCR Reaction Mixture]

No. of Sample Reagent	1 test
2X qPCR Master Mix	10 µl
qPCR Nucleic Mix	3 µl
Dual-labeled Probe Mix	1 µl
DEPC DW	4 µl
Total Volume	19 µl

[Table 5. qPCR Plate Layout Example]

	1	2	3	4	5	6	7	8	9	10	11	12
A	#STD 1	#1	#9	#17								
B	#STD 2	#2	#10	#18								
C	#STD 3	#3	#11	#19								
D	#STD 4	#4	#12	#20								
E	#STD 5	#5	#13	#21								
F	#IC	#6	#14	#22								
G	-	#7	#15	#23								
H	#NC	#8	#16	#24								

*Example of qPCR plate layout for 24 urinary RNA samples.
*NC : Negative Control or None-Template Control
*NC is recommend separate from STD and IC to avoid cross-contamination

c. Transfer 19µl of the prepared mixture to each qPCR strip tube.
d. Add 1µl of cDNA and close the lids.
e. Transfer to the real-time PCR machine and allow to react. (See Table 6. BKV qPCR Condition)

*Plate scan of instruments must be includes two fluorescence (FAM to BKV, Cy5 to IC).

[Table 6. BKV qPCR Condition]

Temperature / Times	Cycles
50℃ / 3 min	1 cycle
95℃ / 10 min	1 cycle
95℃ / 15 sec 60℃ / 60 sec	40 cycles

4.3.4. qPCR results check

① After real-time PCR is finished, adjust the FAM and Cy5 threshold.

② Analyze the data obtained from the results using the software of each real-time PCR system.

③ For detailed instructions on how to use the system software, see the system user manual.

④ Set the Copy number of the STD in the software. (Refer to Table 7. Define and set up standards)

[Table 7. Define and set up standards]

Instruments / STD n	BioRad CFX96™	ABI 7500/Fast
STD 1	9.66E+09	# of Points: 5 # of Replicates: 1 Starting quantity: 9.66+E09 Serial Factor: 1:10
STD 2	9.66E+08	
STD 3	9.66E+07	
STD 4	9.66E+06	
STD 5	9.66E+05	

4.4. Interpretation

4.4.1. Test validation criteria

① Threshold line Setting

Instrument	Threshold		Baseline	
	B1-5p (FAM)	IC (Cy5)	Begin	End
Bio-Rad CFX96	100	100	3	15
ABI 7500 Fast	5000	5000	3	15

② Standard Curve

- If the slope and coefficient of determination do not conform to the criteria below

Items	Standard value
Slope	-3.3 ± 0.5
Coefficient of determination (R^2)	0.98 or greater

③ IC & NC

Instrument	Standard Value (Ct Value)	
	IC	NC
Bio-Rad CFX96	Ct < 40	Ct (B1-5p) > 37
ABI 7500 Fast	Ct < 40	Ct (B1-5p) > 35

④ Validation of target sample

- If the sample conforms to the standard values of the standard curve and IC and NC standards, and is BKV miR Positive in the table below, calculate the copy number of the BKV-miR-B1-5p using the Ct value of the sample from the standard curve.
- If it is BKV miR Positive, it is considered negative and calculation of the copy number using the standard curve is unnecessary.

BKV-miR-B1-5p (FAM)	IC (Cy5)	Interpretation
+	+	BKV miR Positive
+	-	BKV miR Positive *
-	+	BKV miR Negative
-	-	Experimental Fail **

* If the target miRNA is excessively detected, the PCR reaction of the IC is inhibited and may not be detected. In this case, it is not considered an Experimental Fail and copy number calculation proceeds because it is BKV miR Positive.

* In the case of an Experimental Fail, the test is performed again since it is due to an error in the extraction process or inclusion of large amounts of PCR Inhibitor in the extract.

4.4.2. Quantification

① Standard curve

- The standard curve is expressed in the following form.

$$Y = aX + b \quad ---------- eq.\ (a)$$

Y: Ct value, X: $\log_{10}(Copy\ Number)$

a: Slope, b: Y-intercept

② Using the standard curve, calculate X from the Ct value of the sample.

$$X = \frac{Y - b}{a} \quad ----------eq.(b)$$

As $X\ is\ \log_{10}(Copy\ Number)$,

$$\therefore Copy\ Number = 10^X \quad ---------- eq.\ (c)$$

③ Calculation of concentration from the original sample

- The copy number obtained from the standard curve is calculated based on the amount put in the RT-qPCR reaction after extraction, so it should be expressed as the concentration of the original sample before extraction.

$$Result\ (copy/ml) = \frac{Copy\ Number(copy/\mu l)\ \times Elution\ Volume\ (\mu l)}{Sample\ Volume\ (ml)} \quad ---eq.(d)$$

4.4.3. Determination by cut-off

Determine according to the $log_{10}(Result)$ value as shown below.

If $log_{10}(Result) \geq 6.70$, BKVN positive
If $log_{10}(Result) < 6.70$, BKVN negative

4.4.4. Determination example

① Calculation of Copy Number by the Standard Curve

- The Y-intercept and slope obtained from the standard curve were 50.4 and -3.5, respectively, and if the Ct value of the test sample is 22, the copy number is calculated as shown below.

$$X = \frac{22 - (50.4)}{-3.5} = 8.1$$

$$\therefore Copy\ Number = 10^{X} = 10^{8.1}$$

② Calculation of concentration of the original sample

- Since the amount of original sample is 0.2ml and the amount of sample eluate after extraction is 14μl,

$$Result\ (copy/ml) = \frac{10^{8.1} \times 14}{0.2} = 70 \text{ X } 10^{8.1} = 10^{9.95}$$

③ BKNV determination by cut-off

- Therefore, $log_{10}10^{9.95} = 9.95$, it is over the BKVN cut-off value 6.70 and is considered as BKVN positive

4.5. Quality Control

Local regulations typically specify that the laboratory is responsible for control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, approved test system.

4.6. Precautions for Use

4.6.1. Safety information

① This product is an in vitro diagnostic medical device.

② This product should only be used by professionals.

③ When handling specimens, personal protective equipment such as gloves, eye protection, and lab coat should be worn, and then RNA should be extracted from the specimen and tested using the HB miRDx™ BKV Kit.

④ All specimens are handled under the assumption that they are infectious, and laboratory safety rules are followed.

⑤ Unused reagents and human specimens should be autoclaved and disposed of in accordance with biosafety guidelines.

⑥ HB miRDx™ BKV Kit testing is performed in a laboratory with an appropriate environment by experienced experimenters who have been trained in the relevant technical and safety procedures.

4.6.2 Handling and procedural requirements

① Please read the instruction manual carefully before inspection.

② Perform viral RNA extraction and purification in a place separate from the laboratory to prevent contamination of reagents.

③ In order to prevent cross-contamination, STDs should be processed in a location separate from the sample (RNA) and kit components during testing.

④ Open the tube cap carefully to avoid contamination.

⑤ No reagent dilution to reduce cost.

⑥ Follow the PCR conditions in the manual for PCR conditions and temperature settings. If adjusted incorrectly, results are not guaranteed.

⑦ Do not leave the kit components and PCR mixture at room temperature for a long time. Results are not guaranteed in this case.

⑧ If the amount of RNA in the sample is not sufficient, the PCR results may differ from the actual conditions.

⑨ Since it is a test that includes viral RNA extraction and PCR amplification, be careful not to contaminate the kit's amplification reaction mixture. Regularly check for laboratory contamination.

⑩ Be careful not to change the sample during RNA extraction.

⑪ If reagents from different lots are used, the test results may be wrong.

⑫ If you make a mistake, restart the test.

⑬ Inappropriate specimen collection, transportation, storage, and processing may lead to erroneous results.

⑭ Incorrect test results may occur due to inadequate sample (RNA) dilution.

⑮ After each experiment, to minimize the risk of nucleic acid contamination, wash the workbench, pipette, and centrifuge with a cleaning agent (e.g DNA/RNA Remover, Ethanol, 10% bleach) to prevent contamination.

4.6.3 Limitations

① Please read the instruction manual carefully before inspection.

② The HB miRDx™ BKV Kit is designed to use the Bio-Rad CFX96™ Dx System and Applied Biosystem® 7500 FAST Dx Real-Time PCR System® equipment and miRNAs extracted from urine samples.

③ For inspection, the procedure in the user manual should be followed. Any changes in the procedure could result in inspection failures or incorrect results.

④ It is recommended that experienced experimenters use the kit to ensure the performance of the kit.

⑤ During use and storage of kit components, frequent monitoring should be performed to prevent contamination.

⑥ Interpretation of results should take into account the possibility of false-negative and false-positive results.

- Causes of false negatives
  a. Improper collection, handling, or storage of specimens
  b. Use of unvalidated miRNA extraction kits or PCR platforms
  c. Presence of RT-PCR inhibitors
  d. In case the IFU (Instruction for use) is not followed
- Causes of false positives
  a. Cross-contamination during sample handling or preparation
  b. Cross-contamination between patient samples
  c. Improper handling of amplified products (caps opened after PCR, etc.)

⑦ All results should be discussed with a healthcare professional in relation to the patient's medical history and clinical symptoms.

⑧ Analysis results should be used to confirm the expression of BKV virus, and other infection information should not be provided.

⑨ A negative result of this test does not absolutely rule out the possibility of being positive.

4.7. Package

- 24 rxns/Kit.

4.8. Storage

- HB miRDx™ BKV Kit storage at below -17℃.

4.9. Expiry Date

- From the date of the manufacturing, Twelve (12) months.

5. Performance characteristics

5.1. Analytical Performance Test

5.1.1. Analytic sensitivity

① Limit of detection

To confirm the LOD (Limit of detection) of the HB miRDx™ BKV Kit, prepare a mimic miRNA with the same sequence as the BKV miRNA. The prepared mimic miRNA was diluted and the experiment was performed with the copy number shown in the following table. A test was repeated for 20 times using the CFX96 Touch Real-Time PCR Detection System and ABI7500 FAST. As a result of Probit Analysis, the detection limit was 4.27 X 10^3 copies (95% CI: 3.63 X 10^3~4.48 X 10^3) in Bio-Rad CFX96 equipment and 1.93 X 10^3 copies (95% CI: 1.64 X 10^3~ 2.02 X 10^3) in ABI 7500 equipment.

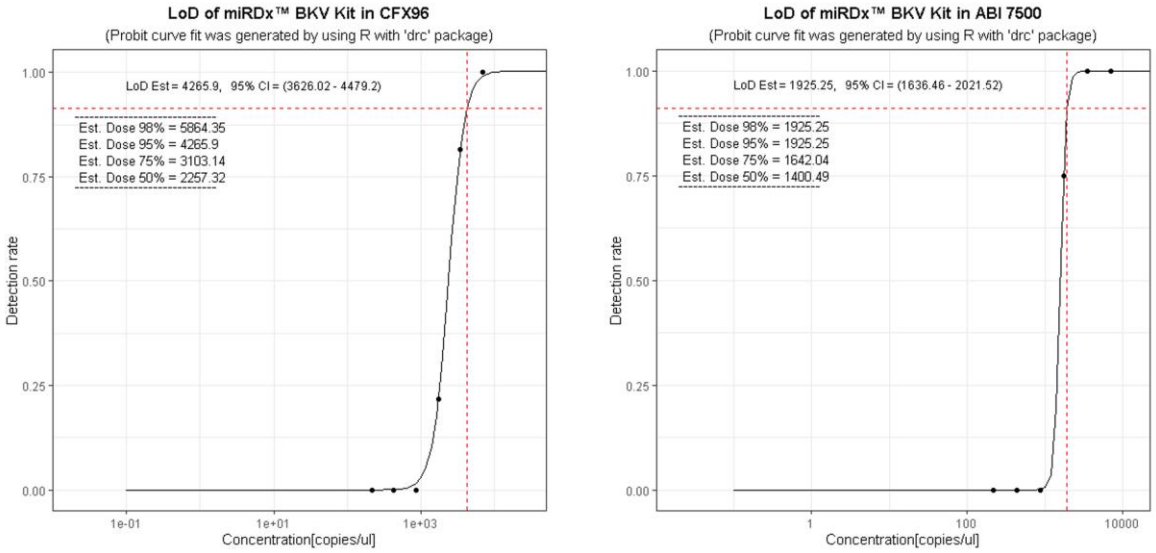
miRNA	Overall Mean Concentration (Copies/㎕)	Bio-Rad CFX96 Touch		ABI 7500 FAST	
		Detection Rate	Ct mean	Detection Rate	Ct mean
B1-5p	6.94E+05	(60/60) 100%	27.39	(60/60) 100%	25.93
	6.94E+04	(60/60) 100%	31.52	(60/60) 100%	29.11
	6.94E+03	(60/60) 100%	35.45	(60/60) 100%	32.53

	3.47E+03	(49/60) 81.67%	36.55	(60/60) 100%	33.49
	1.74E+03	(13/60) 21.67 %	37.59	(45/60) 75%	34.62
	8.68E+02	(0/60) 0%	38.64	(0/60) 0%	36.48
	4.34E+02	(0/60) 0%	39.10	(0/60) 0%	38.49
	2.17E+02	(0/60) 0%	-	(0/60) 0%	-

* - : Not-detected

② Measurement range

To confirm the measurement range and linearity, the mimic miRNA was serially diluted 10-fold from 6.941 X 10^5 copy number to 6.941 X 10^3 copy number and was serially diluted from 6.941 X 10^3 copy number to 2 times. Using the HB miRDx™ BKV Kit, 12 or 13 repeated measurements were performed for each instrument and concentration. It was measured by diluting to a concentration at which detection is zero.



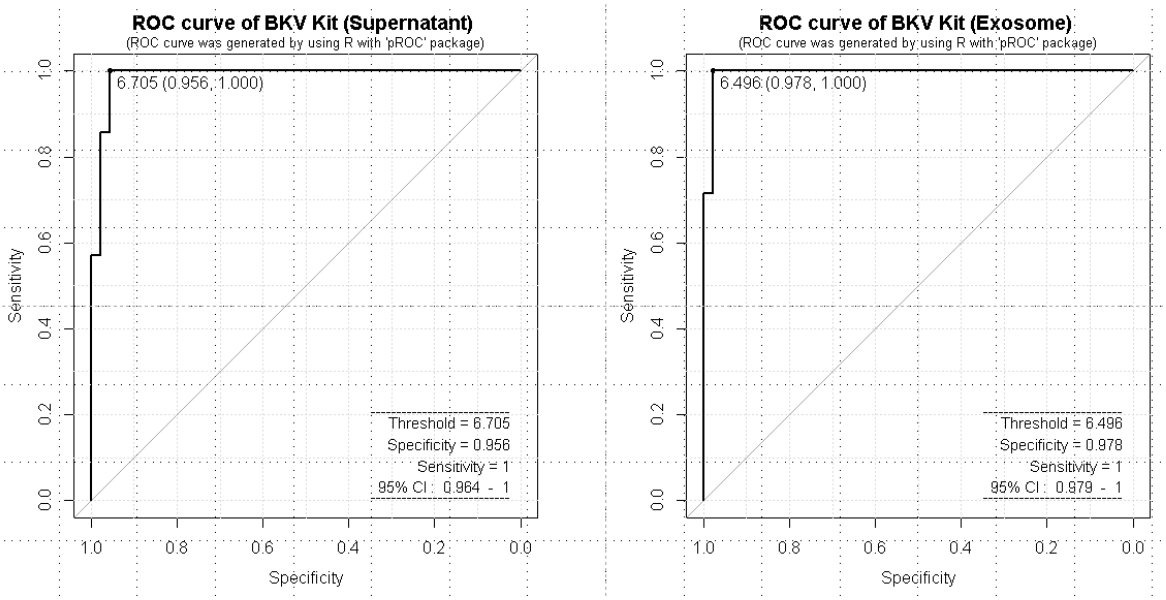
< Probit Analysis according to miR B1-5p concentration in the sample>

③ Cut-off value

In the clinical trial, miRNAs were extracted from exosomes and supernatant from 1 ml of Urine from 52 patients (7 BKVN confirmed patients and 45 non-BKVN patients including the following diseases*) and tested using the HB miRDx™ BKV Kit.

* NP (Normal Pathology), TCMR (T-Cell Mediated Rejection), ABMR (Antibody-Mediated Rejection), LGS (Long-term Graft Survival), CAD (Chronic Allograft Dysfunction), BKVN (BK Virus Nephropathy) and OGIs (Other Graft)

As a result of ROC analysis, the cut-off was set at 6.50 $\log_{10}$ copies/mL (sensitivity 100%, specificity 98%) for exosomes, and 6.70 $\log_{10}$ copies/mL (sensitivity 100%, specificity 96%) for the supernatant.



<ROC analysis according to miR B1-5p detection concentration in urine supernatant (left) and exosome (right)>

5.1.2. Analytic specificity

① Cross-Reactivity

In order to confirm the cross-reactivity of the HB miRDx™ BKV Kit, 7 viral mimic miRNAs and 13 human miRNAs that can come from urine and plasma were diluted to 9.661 X 10^9 copy number and tested. As a result of each repeated test 3 times, no cross-reactivity was observed.

No	Target	Results
1	bkv-miR-B1-5p	Ct Value: 13±1
2	cmv-US22a-5p	None
3	cmv-US25-2-5p	None
4	cmv-US33-5p	None
5	cmv-US112-3p	None
6	cmv-US25-1-5p	None
7	ebv-BART-2-5p	None
8	ebv-BART-7-3p	None
9	ebv-BART-9-3p	None
10	hsa-let-7b-5p	None
11	hsa-let-7d-5p	None
12	hsa-let-7e-5p	None
13	hsa-miR-10a-5p	None
14	hsa-miR-21-5p	None
15	Has-miR-23a-5p	None
16	hsa-miR-23a-3p	None
17	hsa-miR-23c-3p	None
18	hsa-miR-31-5p	None
19	hsa-miR-133a-3p	None
20	hsa-miR-200b-3p	None
21	hsa-miR-302a-3p	None
22	hsa-miR-302c-3p	None
23	hsa-miR-302d-3p	None

② Interference reactions

In order to confirm the effects of substances included in the sample that may affect the PCR reaction, the immunosuppressants 10 ng/ml Tacrolimus, 200 ng/ml Cyclosporine, 10 ng/ml Sirolimus, and 10 ng/ml Mycophenolate were tested. In order to confirm the effect of the interfering substance, three copy numbers (6.941 X 10^9, 6.941 X 10^6, 6.941 X 10^3) of BKV mimic miRNA were used and repeated three times with and without the interference substance. As a result of testing using the HB miRDx™ BKV Kit, no interference reaction was observed.

Materials	Conc.	Category	Copy number		
			6.941 X 10^9	6.941 X 10^6	6.941 X 10^3
Without interference (D.W.)	N/A	Aver.	13.00	23.43	34.46
		SD	0.08	0.18	0.16
		CV (%)	0.63	0.75	0.46
Tacrolimus	10 ng/㎖	Aver.	12.95	23.56	34.36
		SD	0.15	0.08	0.19
		CV (%)	1.13	0.35	0.54
Cyclosporine	200 ng/㎖	Aver.	13.06	23.57	34.46
		SD	0.10	0.17	0.16
		CV (%)	0.80	0.73	0.45
Sirolimus	10 ng/㎖	Aver.	13.09	23.53	34.48
		SD	0.06	0.19	0.20
		CV (%)	0.48	0.80	0.59
Mycophenolate	10 ng/㎖	Aver.	13.08	23.51	34.50
		SD	0.05	0.16	0.15
		CV (%)	0.41	0.68	0.42

5.1.3. Precision

① Repeatability and reproducibility

Three copy numbers (6.941 X 10^9, 6.941 X 10^6, 6.941 X 10^3) were tested for 3 lots, 3 inspectors, 2 sites, for 20 days, twice daily, 3 times for each test. The statistically analyzed test results are shown in the table below.

Assay	Category		Lot 1			Lot 2			Lot 3		
			Conc. (copies/ul)			Conc. (copies/ul)			Conc. (copies/ul)		
			6.941X 10^9	6.941X 10^6	6.941X 10^3	6.941X 10^9	6.941X 10^6	6.941X 10^3	6.941X 10^9	6.941X 10^6	6.941X 10^3
Between site (QC Room)	1st	Aver.	13.42	23.45	35.40	13.47	24.01	35.34	13.48	24.07	35.44
		SD	0.13	2.42	0.14	0.14	0.24	0.16	0.15	0.28	0.16
		CV (%)	0.98	10.30	0.38	1.06	0.98	0.46	1.09	1.18	0.45
	2nd	Aver.	13.48	24.07	35.44	13.42	24.05	35.36	13.45	24.14	35.40
		SD	0.16	0.31	0.21	0.13	0.31	0.20	0.13	0.33	0.12
		CV (%)	1.20	1.28	0.60	0.97	1.28	0.57	0.99	1.38	0.33
Between site (R&D Room)	1st	Aver.	13.41	23.53	35.41	13.41	23.55	35.37	13.47	24.07	35.37
		SD	0.12	2.42	0.21	0.13	2.41	0.15	0.14	0.28	0.15
		CV (%)	0.90	10.29	0.58	0.94	10.23	0.42	1.06	1.15	0.42
	2nd	Aver.	13.44	24.10	35.39	13.46	24.02	35.35	13.43	24.04	35.28
		SD	0.10	0.35	0.16	0.12	0.27	0.16	0.15	0.26	0.13
		CV (%)	0.78	1.46	0.44	0.90	1.11	0.45	1.08	1.10	0.36

Assay	Category		Lot 1			Lot 2			Lot 3		
			Conc. (copies/ul)			Conc. (copies/ul)			Conc. (copies/ul)		
			6.941X 10^9	6.941X 10^6	6.941X 10^3	6.941X 10^9	6.941X 10^6	6.941X 10^3	6.941X 10^9	6.941X 10^6	6.941X 10^3
Between inspectors	1st	Aver.	13.40	23.71	35.38	13.45	23.76	35.31	13.47	23.77	35.37
		SD	0.14	0.38	0.14	0.14	0.32	0.20	0.16	0.38	0.17
		CV (%)	1.04	1.58	0.40	1.07	1.34	0.55	1.18	1.62	0.48
	2nd	Aver.	13.41	23.82	35.35	13.41	23.77	35.33	13.40	23.84	35.33
		SD	0.17	0.36	0.24	0.15	0.38	0.18	0.16	0.42	0.15
		CV (%)	1.30	1.51	0.67	1.14	1.60	0.52	1.23	1.77	0.41

Assay	Category		Lot 1			Lot 2			Lot 3		
			Conc. (copies/ul)			Conc. (copies/ul)			Conc. (copies/ul)		
			6.941X 10^9	6.941X 10^6	6.941X 10^3	6.941X 10^9	6.941X 10^6	6.941X 10^3	6.941X 10^9	6.941X 10^6	6.941X 10^3
Between instruments (Bio-rad)	1st	Aver.	13.39	23.47	35.28	13.45	23.55	35.33	13.39	23.52	35.21
		SD	0.17	0.17	0.17	0.19	0.24	0.16	0.14	0.18	0.17
		CV (%)	1.25	0.73	0.49	1.43	1.02	0.46	1.07	0.78	0.48
	2nd	Aver.	13.42	23.55	35.25	13.37	23.55	35.28	13.36	23.53	35.34
		SD	0.16	0.24	0.17	0.14	0.16	0.16	0.14	0.18	0.16
		CV	1.22	1.01	0.49	1.05	0.70	0.46	1.05	0.77	0.44

		(%)									
Between instruments (ABI-7500 FAST)	1st	Aver.	12.10	22.47	32.40	12.14	22.50	32.46	12.11	22.49	32.45
		SD	0.08	0.15	0.25	0.07	0.17	0.20	0.66	0.20	0.22
		CV (%)	0.62	0.67	0.79	0.55	0.74	0.61	0.52	0.90	0.69
	2nd	Aver.	12.10	22.45	32.44	12.08	22.52	32.49	12.11	22.53	32.48
		SD	0.06	0.14	0.23	0.06	0.17	0.23	0.07	0.16	0.28
		CV (%)	0.50	0.62	0.72	0.53	0.74	0.72	0.57	0.71	0.85

5.2. Clinical performance test

Urine samples from a total of 24 patients performed under transplant kidney biopsy were tested.As a result of testing with miRNA extracted from exosomes from the same urine and miRNA extracted from the supernatant of urine, the clinical performance was as follows.

- Test results using BK virus exosomal miRNA extracted from urine exosome fraction

Results		BKVAN confirmed by biopsy		Total
		POS	NEG	
HB miRDx™ BKV Kit	POS	5	0	5
	NEG	0	19	19
Total		5	19	24

Cut-off: 6.5
Sensitivity: 100.0%
Specificity: 100.0%
kappa: 1.0

- Test results using BK virus miRNA extracted from urine supernatant

Results		BKVAN confirmed by biopsy		Total
		POS	NEG	
HB miRDx™ BKV Kit	POS	5	3	8
	NEG	0	16	16
Total		5	19	24

Cut-off: 6.7
Sensitivity: 100.0%
Specificity: 84.2%
kappa: 0.690

- Test results using plasma BK virus DNA in the licensed product from other company

Results		BKVAN confirmed by biopsy		Total
		POS	NEG	
BKV DNA PCR	POS	4	2	6
	NEG	1	17	18
Total		5	19	24

Cut-off: 4.0
Sensitivity: 80.0%
Specificity: 89.5%
kappa: 0.647

Please refer to Attachment 8. Performance Evaluation Summary.

6. Manufacturer and Factory Information

6.1. Manufacturer and Factory Name

6.1.1. Manufacturer name: Heimbiotek, Inc.

6.1.2. Factory name: Same as manufacturer.

6.2. Manufacturer and Factory Address

6.2.1. Manufacturer address: Pangyo Silicon Park A-201,35, Pangyo-ro 255beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do, 13486 Republic of Korea.

6.2.2. Factory name: Same as manufacturer.

6.3. Manufacturer and Factory Telephone Number

6.3.1. Telephone number: +82 (31) 548-2130.

6.3.2. Fax number: +82 (31) 548-2135.

6.4. Manufacturer and Factory Internet Home Page (URL) and E-mail Address

6.4.1. Internet home Page (URL): www.heimbiotek.com

6.4.2. E-mail address: info@heimbiotek.com

7. Authorized Representative Information

7.1. Authorized Representative Name

7.1.1. Authorized representative name: Qarad EC-REP BY

7.2. Authorized Representative Address

7.2.1. Address: Pas 257 2440 Geel, Belgium

8. symbols Information

8.1. Indication of Symbols

Symbols	Information
	Catalogue number
	Lot (Batch) number
	*In vitro* diagnostic medical device
	Use by Date
	Storage temperature limitation
	Indicates the need for the user to consult the instructions for use
	Do not reuse
	Indicates the need for the user to consult the instructions for use for important cautionary information
	Indicates the total number of IVD tests that can be performed with the IVD
	Manufacturer
	Authorised Representative in the European Community
	Components List

RT PRIMER	RT Primer Mix Components
5x RT MASTER	5x RT Master Mix Components
2x qPCR MASTER	2X qPCR Master Mix Components
qPCR NUCLEIC	qPCR Nucleic Mix Components
PROBE MIX	Dual-labeled Probe Mix Components
STD	Standard (STD) Components
INTERNAL CONTROL	Internal Control (IC) Components

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