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Viga MTB Molecular Diagnostic Kit

Genotyping Real Time-PCR Assay

Molecular detection HPV kit based on Real Time-PCR

For In Vitro Diagnostic Use

By ROJE

Edition, 07/2023

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality, affordable Molecular and diagnostic Kits and reagents comparable to the world leaders to research centers, laboratories, clinics, hospitals, and diagnostic centers all over the world.

Contents

Kit content

Kit Content	25 Preps	100 Preps
Q-ROMAX, 4X	600µl	2400µl
Pro 1 HPV Mix	350µl	1400µl
Pro 2 HPV Mix	350µl	1400µl
Pro 3 HPV Mix	350µl	1400µl
Pro 4 HPV Mix	350µl	1400µl
Positive Control	100µl	600µl
Water (PCR Grade)	150µl	600µl

Storage

ROJETechnologies checked Shipment condition. After arrival, all reagents should be kept in darkness, at -20°C to -25°C temperature. Do not freeze-thaw the Kits frequently. When storage condition is as directed, all reagents are stable until the expiration date, as indicated on the kit box.

The Viga Genotyping HPV Molecular Diagnostic Kit technology is an in vitro nucleic acid TaqMan assay with signal amplification using polymerase chain reaction for the genotyping detection of 14 high and two low-risk types of HPV DNA in cervical or vaginal specimens. The HPV types detected by the test are the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and low-risk types 6, 11. This test is "For Professional Use Only" by trained and validated laboratory personnel. The present kit utilizes three-Channels Multiplex Real-Time PCR and contains internal control that, in addition to improving isolation quality, prevents the false result of PCR inhibition.

Guarantee and Warranty

ROJETechnologies guarantee the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If products do not meet your satisfaction, please contact the technical support team for

reasons other than misuse. If the problem is due to the manufacturing process, the ROJE team will replace the kit for you.

Notice to Purchaser

This product is only for experimental and not for commercial use of any kind. There is no right to resell the kit or any of its components. For information about our licensing or distributors, contact the ROJE business team.

Warning and Precautions

For in vitro diagnostic use

Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Dear users, please follow laboratory safety rules.

Please study the guideline precisely before use.

- Eating, drinking, smoking, chewing gum, applying cosmetics, and taking medicine in laboratories where hazardous materials and human samples are used should be prohibited.
- Consider all samples of patients and positive control potentially infectious.
- The physician's prescription uses the Viga Genotyping HPV Molecular Diagnostic Kit for emergency and in vitro diagnostic use.
- Each step of procedures such as sampling, storage, shipping, and laboratory tests must follow biosafety and molecular laboratory management.
- The clinical laboratories must be equipped with instruments and operators following the rules of the Ministry of Health.
- All kit contents have been developed to test HPV; changing or replacing any kit content will affect its function, in contrast with the product license.
- All pipette tips and microtubes must be sterile and DNase-RNase-free. To avoid contamination, use filter pipette tips, and they must be changed after adding any substance or samples.
- Landfill the waste according to biosafety guidelines. All desks and laboratory instruments must be antisepticised by 70% Ethanol or 10% Sodium Hypochlorite regularly.
- Avoid putting the combination of Pro Mixes in exposure to sunlight.

- Clean and disinfect all spills of specimens using a suitable disinfectant following national and local regulations.
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials following national and local regulations.
- The following hazard and precautionary statements apply to the Viga Genotyping HPV Molecular Diagnostic Kit components.

Quality control

According to clinical and laboratory standards, institute, and WHO, Viga Genotyping HPV Molecular Diagnostic Kit is tested against predetermined experiments on a lot-to-lot basis to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on the web at www.rojetechnoloes.com.

Equipment & Reagents to Be Supplied by User

- DNase-RNase-free microtubes (1.5ml)
- PCR microtube 0.1ml or 0.2ml strip
- Various models of pipette and pipette tip (10µl, 100µl, and 1000µl of filter pipette tips)
- Surface sanitizing solution like RNZO (Cat No: RN983048)
- Disposable Powder- Free gloves and surgical gown
- Three-Channels Multiplex Real-Time PCR Instrument (with green, yellow, and orange channels)
- Vortex
- Cool box

Procedures

Viga Genotyping HPV Molecular Diagnostic Kit testifies a polymerase chain reaction of Real Time-PCR. This kit is designed for genotyping molecular diagnosis of L1, E1, E2, E6, and E7 genes Human papillomavirus. After nucleic acid isolation by using DNall VirAll Kit or other kits that are approved by the Ministry of Health and verified sample combination could be added to the master mix primer/probe mix to perform the reaction. In addition, the Viga Genotyping HPV Molecular Diagnostic Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Yellow of the Real Time-PCR instruments. With the help of its sampling, the quality of sample isolation and the PCR reaction process can be checked and controlled to prevent false-

negative results. The results demonstrated that the LoD of the assay for types 16, 18, 45 is 5 Copies/5µl, for types 6/11, 51, 56/66, 33/52/58, 35, 59, 39 is 50 Copies/5µl and for types 68, 31 is 500 Copies/5µl.

Applications

The Viga Genotyping HPV Molecular Diagnostic Kit technology is an in vitro nucleic acid TaqMan assay with signal amplification using polymerase chain reaction and fluorescent probs (ROX/Texas Red, Yakima Yellow, and FAM) for the genotyping detection of 14 high-risk and two low-risk types of HPV DNA in cervical or vaginal specimens. The HPV types detected by the test are the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and 2 low-risk types 6, 11 in human cervical screening, cytology sample, urine, and paraffin-embedded tissue. This diagnostic test kit utilizes the polymerase chain reaction (PCR) and is configured with three-Channels Multiplex Real-Time PCR instruments.

Features

Table 1: Viga Genotyping HPV Molecular Diagnostic Kit features and specifications

Technology	Real Time-PCR
Type of Analysis	Genotyping
Target Sequence	L1, E1, E2, E6 and E7 genes
Analytical Specificity	high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 low-risk HPV types 6 and 11
Analytical Sensitivity (LOD)	for types 16, 18, 45 is 5 Copies/5µl, for types 6/11, 51, 56/66, 33/52/58, 35, 59, 39 is 50 Copies/5µl .and for types 68, 31 is 500 Copies/5µl
Diagnostic Specificity	100%
Diagnostic Sensitivity	98.52%
Extraction/Inhibition Control	PCR inhibition and DNA extraction efficiency control
Validated Specimen	Cervical screening, Pap smear, Urine and Paraffin-embedded tissue sample
Storage	-20 ± 5°C
Validated Extraction Method	DNall VirAll Kit
Instruments	Rotor-Gene Q, 2plex, Corbett Rotor-Gene 3000&6000, Mic qPCR Cycler, StepOne and StepOne plus Applied Biosystem
Required Detection Channels	Green-Yellow-Orange

Recommended Starting Material

- Before starting any tests, each component must be thawed, vortexed, and centrifuged briefly. Avoid repeated freeze-thaw cycles.
- Always treat samples as infectious and (bio-) hazardous by safe laboratory procedures.
- Sample should be collected from the cervix with the brush or swab, cytology sample, urine, and paraffin-embedded tissue.

Storage and Sample Preparation

Cervical screening, Pap smear, Urine, and Paraffin-embedded tissue sample

The fresh specimen must either be processed immediately as per sample procedure outlined in the section on Sample Processing Protocol or stored frozen at -20°C. Frozen samples must be

brought to room temperature before starting sample processing. Sample Pre-treatment decontaminates the specimen and makes it ready for extraction.

DNA isolation

For nucleic acid, isolation use DNall VirAll Kit or other kits that approve by the Ministry of Health.

Before Start

Take out each component from the kit and place them on the bench top. Allow the reagents to equilibrate to room temperature, then briefly vortex each tube for later use.

Buffer Preparation

Take out each component from the kit and allow the reagents to equilibrate to room temperature. Before use, vortex components briefly. The whole volume of isolated nucleic acid should be 5µl. Follow table 1 to prepare buffers and table 2 for PCR run.

Table 2: preparation of components per single reaction

components	Volume
Q-ROMAX, 4X	6µl
Pro 1 HPV or Pro 2 HPV or Pro 3 HPV or Pro 4 HPV Mix	14µl
Isolated DNA	5µl

Pathogenicity

Human papillomaviruses (HPVs) are small double-stranded DNA viruses infect the cutaneous and mucosal epithelium. Infection by specific HPV types has been linked to the development of cervical carcinoma. HPV infects epithelial cells that undergo terminal differentiation and encode multiple mechanisms to override the normal regulation of differentiation to produce progeny virions, leading to morphological changes resulting in noncancerous tumors in skin so-called "Papilloma".

Human papillomavirus contains 150 strains identified in various types. Almost 75% of papilloma types targets epithelial cells (cutaneous types), and the remaining 25% (around four types) targets mucosal epithelial tissues identified as mucosal or genital types. Cutaneous types mainly

cause noncancerous epithelial warts in different areas of skin, specifically in lips' epithelial cells and mucosal types cause genital warts and cancerous tumors, particularly cervix cancer.

Low-risk HPV

Low-risk HPV strains contain types causing noncancerous and low-risk warts in genital areas both in women and men, but in women appear in internal genitalia, including the vagina and cervix. There are 12 types of papillomaviruses with low cancer risk while can cause warts and morphological changes in the genital area: 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73, and 81. Both 6 and 11 are responsible for 90 %of genital warts.

High-risk HPV

High-risk HPV can cause cancers of the cervix, vagina, and vulva in women, Penis in men, anus, and back of the throat, including the base of the tongue and tonsils (oropharynx), in both men and women. There are 14 types of HPV high-risk virus including: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Around 70% of reported cervix cancers are caused by HPV 16 and 18. In addition, HPV 16 is responsible for 95% of oral, vaginal, and anus cancers. HPV 31 and 45 are considered high-risk cancer strains, contributing to 5%-10% of cervix cancers.

Workstation preparation

All work surfaces, pipettes, and other supplies must be cleaned and sanitized before use. To reduce the risk of nucleic acid contamination, use sanitizers like 70% Ethanol or 10% Sodium Hypochlorite.

Protocols

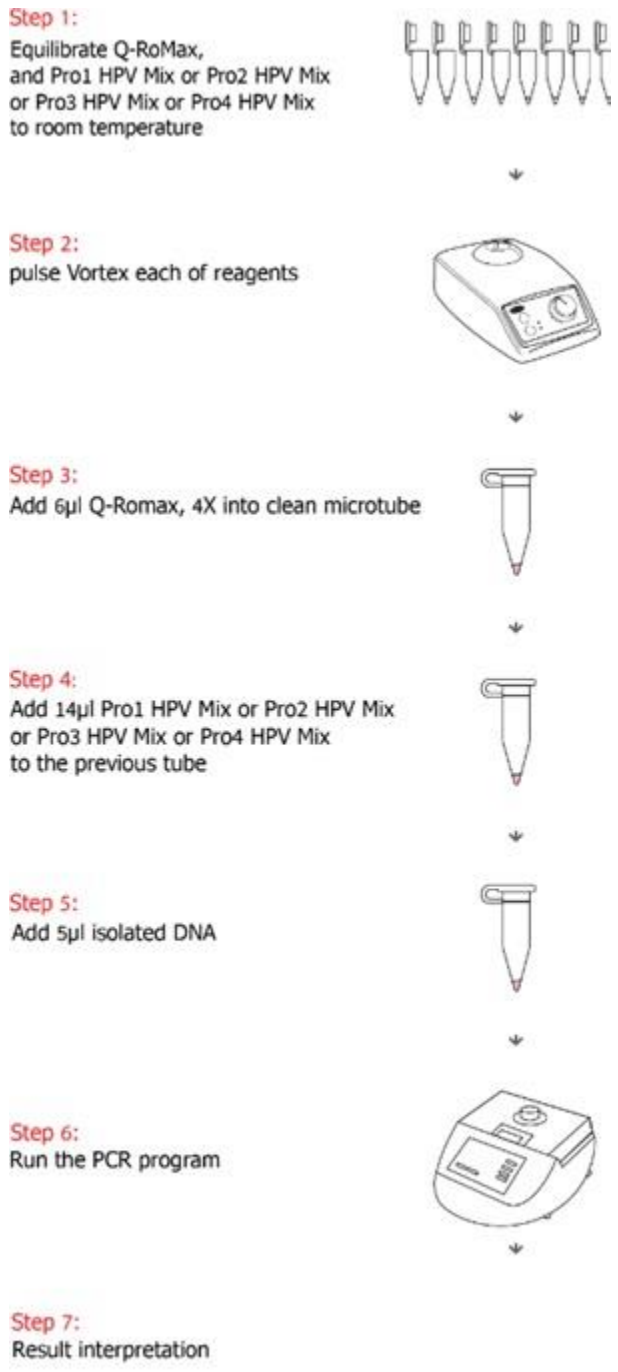

Figure 1: preparation of reagents, PCR run, and interpretation of results.

PCR reaction preparation

Table 3: PCR reaction preparation

Components	Volume
Q-ROMAX, 4X	6µl
Pro1 HPV or Pro2 HPV or Pro3 HPV or Pro4 HPV or Mix	14µl
Isolated DNA	5µl

Thermal Profile

Table 4: Thermal profile for Viga Genotyping HPV Molecular Diagnostic Kit

	Temperature	Incubation Time	Cycle Numbers
Pre-Denaturation	95 °C	1 min	1
Denaturation	95 °C	10 sec	45
Annealing and acquisition on channel Green, Yellow and Orange	57°C	30 sec	

Results Interpretation

Data analysis for each type should be performed separately using a manual threshold. Use the following table for results interpretation, showing that Pro1 HPV to Pro4 HPV mixes are detectable in identified channels.

For detection of L1, E1, E2, E6 ₉ E7 genes fluorophore FAM (green), Yakima Yellow (Yellow), and ROX/Texas Red (orange) are identified for Pro1 HPV Mix to Pro4 HPV Mix and Yakima Yellow (Yellow) for IC gene in Pro2 HPV Mix.

Table 5: Specific fluorescent channels identified for HPV types.

ProMIX	Green	Yellow	Orange
Pro1 HPV	45	16	18
Pro2 HPV	51	Internal Control	56/66
Pro3 HPV	35/39	33/52/58	6/11
Pro4 HPV	31	68	59

A negative control is used as contamination control. The magnitude increase of the Fluorescence curve in the negative control does not cross the threshold. If Ct is less than 35 (Ct<35), it is considered as possible contamination. Strong signals above 35 in the NTC can be PCR artifacts, which in these cases, the shape of the curve can be considered (the S-shaped curve is typical for a positive result).

Internal control should be positive for all clinical specimens at Ct 35 or less than 35, indicating sufficient nucleic acid from the human gene and the sample has acceptable quality.

Internal control curve with Ct>37 or without Ct indicates low sample concentration or inhibitors in the reaction (the isolated sample is recommended to dilute at least ½). If the test result is not acceptable again during the retest, another new sample should be taken from the patient, and the test must be repeated.

A positive clinical specimen should have Ct≤37 for gene.

If the expected positive reaction is not achieved (typical S-shaped curve), the performed test is not acceptable. The test must be repeated based on kit instructions accessible in the kit catalog.

Determine the reason for the failure of positive control, take corrective action, and document correctional action results.

For more information about positive and negative specimens, refer to table 3.

Table 6: Control conditions for a valid PCR Run

ProMIX	ROX/Texas Red	Yakima Yellow	FAM	results
Pro 1 HPV	+	+	+	Positive:45 Positive: 16 Positive:18
Pro 2 HPV	+	It is not considered	+	Positive:51 Positive:56/65
Pro 3 HPV	+	+	+	Positive:35/39 Positive:33/52/58 Positive:6/11
Pro 4 HPV	+	+	+	Positive:31 Positive:68 Positive:59
Pro 2 HPV	-	+	-	Negative Result
Pro 1 HPV or Pro 2 HPV or Pro 3 HPV or Pro 4 HPV	-	-	-	Invalid results

Test Limitations

- Low virus titers in patients' specimens, improper transportation, and low-quality DNA isolation can cause false-negative results.
- All related controls should be checked before result interpretation. Otherwise, results are comparable.
- The limit of detection of the present kit demonstrated Ct≤37, and the typical S shape curve must appear for all positive specimens.
- Improper storage can lead to false-negative results.
- The product is to be used by personnel specially instructed and trained in the in-vitro diagnostics only as individual errors can compromise the results.
- The patient is diagnosed as infected with Human papillomavirus in cases with test positive results accompanied with clinical symptoms, so the appropriate treatment is

conducted based on diagnostic kits result, medical condition backgrounds, and response to remedy.

- Limit of detection (LoD) determined for types 16, 18, 45 is 5 Copies/5µl, for types 6/11, 51, 56/66, 33/52/58, 35, 59, 39 is 50 Copies/5µl and for types 68, 31 is 500 Copies/5µl.

Evaluation and Qualification Analysis

Limit of Detection (LoD)-Analytical sensitivity

LoD studies were used to determine the lowest detectable concentration of HPV DNA, at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples. The analytical sensitivity in consideration of the purification (DNall VirAll Kit) of the Viga Genotyping HPV Molecular Diagnostic Kit was determined using a dilution series of the standards from 5 to nominal 50 and 500 HPV genome equivalents (GE)/5µl spiked in clinical cervical specimens.

The LoD of each test was then confirmed by testing 20 replicates with a dilution series (500, 50, 5 genome equivalents (GE)/5µl) at the tentative limit of detection. The final LoD of each test was determined to be the lowest dilution series resulting in positive detection of 19 out of 20 replicates.

The LoD of the Viga Genotyping HPV Molecular Diagnostic Kit was established using DNall VirAll Kit. The results demonstrated that the LoD of an assay for types 16, 18, 45 is 5 Copies/5µl, for types 6/11, 51, 56/66, 33/52/58, 35, 59, 39 is 50 Copies/5µl and for types 68, 31 is 500 Copies/5µl.

Table 7: Detection Results of Viga Genotyping HPV Molecular Diagnostic Kit Using DNall VirAll Kit.

Test No	**(Copies/5µl)**		
	HPV type 16		
	500	**50**	**5**
1	32.14	34.04	36.04
2	32.51	35.14	36.14
3	32.57	35.37	36.47
4	32.01	34.57	35.41

5	32.11	34.91	34.95
6	31.75	34.87	35.21
7	32.67	34.18	36.74
8	32.84	35.77	37.54
9	31.47	35.24	36.25
10	32.55	34.44	37.64
11	31.94	34.15	Undetermined
12	31.27	34.27	35.73
13	31.53	35.04	36.28
14	31.84	35.21	37.42
15	31.95	35.11	36.08
16	32.01	35.29	36.47
17	32.43	34.77	37.51
18	31.47	34.69	37.69
19	32.63	34.94	36.73
20	32.77	34.23	36.47
Positive percentage in each concentration	**100%**	**100%**	**95%**

Test No	**(Copies/5µl)**		
	HPV type 18		
	500	**50**	**5**
1	33.19	35.18	37.10
2	34.33	35.46	36.87
3	33.61	36.35	36.49
4	33.12	36.49	Undetermined

5	34.40	35.13	36.77
6	33.07	35.02	37.14
7	33.41	35.49	37.04
8	34.34	36.20	37.24
9	33.53	35.82	38.08
10	33.89	35.61	37.53
11	34.06	36.63	36.94
12	33.38	36.36	38.91
13	33.29	35.98	36.28
14	34.72	36.62	37.10
15	33.76	35.15	37.27
16	34.79	35.11	37.24
17	33.78	36.46	37.91
18	33.22	35.53	37.21
19	34.44	36.06	37.66
20	33.69	36.15	37.14
Positive percentage in each concentration	**100%**	**100%**	**95%**

Test No	**(Copies/5µl)**		
	HPV type 45		
	500	**50**	**5**
1	33.19	35.18	37.10
2	34.33	35.46	36.87
3	33.61	36.35	36.49
4	33.12	36.49	Undetermined

5	34.40	35.13	36.77
6	33.07	35.02	37.14
7	33.41	35.49	37.04
8	34.34	36.20	37.24
9	33.53	35.82	38.08
10	33.89	35.61	37.53
11	34.06	36.63	36.94
12	33.38	36.36	38.91
13	33.29	35.98	36.28
14	34.72	36.62	37.10
15	33.76	35.15	37.27
16	34.79	35.11	37.24
17	33.78	36.46	37.91
18	33.22	35.53	37.21
19	34.44	36.06	37.66
20	33.69	36.15	37.14
Positive percentage in each concentration	**100%**	**100%**	**95%**

Test No	**(Copies/5µl)**		
	HPV type 33/52/58		
	500	**50**	**5**
1	36.17	37.16	Undetermined
2	35.42	38.06	39.38
3	35.61	37.61	39.49
4	35.52	37.82	39.52

5	35.49	38.13	41.01
6	36.07	38.41	41.48
7	35.41	37.49	41.59
8	35.37	37.84	Undetermined
9	35.53	37.71	Undetermined
10	35.89	37.61	Undetermined
11	36.06	37.88	Undetermined
12	35.36	37.46	40.91
13	35.52	38.02	41.28
14	35.92	37.49	41.60
15	35.76	37.92	Undetermined
16	35.62	37.91	Undetermined
17	36.71	37.77	Undetermined
18	36.47	37.15	Undetermined
19	36.51	38.08	Undetermined
20	36.01	38.25	Undetermined
Positive percentage in each concentration	**100%**	**100%**	**45%**

Test No	**(Copies/5µl)**		
	HPV type 39		
	500	**50**	**5**
1	36.61	38.11	Undetermined
2	37.37	Undetermined	Undetermined
3	37.88	38.15	Undetermined
4	37.02	38.19	Undetermined

5	37.59	38.72	Undetermined
6	37.07	38.02	Undetermined
7	37.41	37.82	41.04
8	37.92	37.60	Undetermined
9	36.53	37.42	41.58
10	36.89	37.72	40.83
11	37.06	37.53	Undetermined
12	37.41	37.73	40.91
13	37.28	37.92	41.48
14	37.01	38.16	Undetermined
15	36.03	38.35	Undetermined
16	37.38	37.91	Undetermined
17	36.79	38.12	Undetermined
18	37.12	38.02	41.31
19	36.66	38.27	Undetermined
20	37.50	37.85	Undetermined
Positive percentage in each concentration	**100%**	**95%**	**30%**

Test No	**(Copies/5µl)**		
	HPV type 59		
	500	**50**	**5**
1	Undetermined	37.78	40.10
2	35.44	38.06	40.17
3	35.83	37.66	41.59
4	36.02	37.92	40.58

5	35.89	Undetermined	40.44
6	36.07	37.02	Undetermined
7	35.48	37.19	41.04
8	35.55	38.60	Undetermined
9	35.53	38.12	40.58
10	35.89	37.51	41.53
11	36.06	38.12	Undetermined
12	36.16	38.66	41.91
13	36.28	37.98	42.28
14	35.92	37.66	40.60
15	35.16	38.15	Undetermined
16	35.76	39.94	Undetermined
17	35.66	38.46	Undetermined
18	36.22	39.09	Undetermined
19	36.04	37.88	40.15
20	36.44	37.52	Undetermined
Positive percentage in each concentration	**95%**	**95%**	**60%**

Test No	**(Copies/5µl)**		
	HPV type 35		
	500	**50**	**5**
1	36.27	38.28	41.01
2	37.20	39.06	41.52
3	37.61	38.15	41.49
4	36.02	39.79	37.04

5	Undetermined	39.13	39.22
6	37.07	40.02	Undetermined
7	37.40	40.49	39.04
8	37.37	40.60	Undetermined
9	36.53	39.42	40.58
10	37.89	Undetermined	Undetermined
11	38.06	40.63	Undetermined
12	37.36	39.86	41.21
13	38.18	39.98	Undetermined
14	36.92	39.56	40.10
15	37.76	40.15	Undetermined
16	36.66	41.01	Undetermined
17	37.72	40.46	Undetermined
18	36.21	39.33	Undetermined
19	37.44	39.06	Undetermined
20	37.31	39.09	Undetermined
Positive percentage in each concentration	**95%**	**95%**	**45%**

Test No	**(Copies/5µl)**		
	HPV type 68		
	500	**50**	**5**
1	37.27	Undetermined	Undetermined
2	37.12	Undetermined	Undetermined
3	37.71	40.13	Undetermined
4	38.92	40.49	Undetermined

5	Undetermined	39.81	Undetermined
6	39.07	40.21	Undetermined
7	37.41	Undetermined	Undetermined
8	38.37	39.21	Undetermined
9	37.13	40.12	Undetermined
10	38.89	Undetermined	Undetermined
11	38.12	Undetermined	Undetermined
12	37.31	39.56	40.91
13	38.28	40.18	40.32
14	38.92	Undetermined	41.04
15	38.16	Undetermined	Undetermined
16	37.23	Undetermined	Undetermined
17	37.72	Undetermined	Undetermined
18	38.12	Undetermined	39.78
19	38.54	Undetermined	39.17
20	38.29	Undetermined	Undetermined
Positive percentage in each concentration	**95%**	**40%**	**2%5**

Test No	**(Copies/5µl)**		
	HPV type 6/11		
	500	**50**	**5**
1	35.27	37.28	Undetermined
2	35.87	38.06	41.57
3	35.61	37.84	41.49
4	36.02	37.14	Undetermined

5	35.49	Undetermined	Undetermined
6	37.07	37.02	Undetermined
7	35.41	37.49	40.04
8	35.37	38.60	40.24
9	36.53	37.42	40.58
10	35.89	38.47	41.47
11	37.06	39.62	Undetermined
12	35.16	36.46	40.41
13	35.28	38.48	41.28
14	35.92	38.66	40.60
15	36.76	38.17	40.17
16	36.12	37.91	Undetermined
17	36.04	38.74	41.28
18	37.01	37.19	41.41
19	36.57	37.11	40.65
20	36.77	37.25	Undetermined
Positive percentage in each concentration	**100%**	**95%**	**65**

Test No	**(Copies/5µl)**		
	HPV type 66/56		
	500	**50**	**5**
1	39.71	Undetermined	Undetermined
2	39.30	39.06	Undetermined
3	39.41	39.35	Undetermined
4	37.02	40.49	Undetermined

5	38.49	41.13	41.34
6	38.64	41.02	Undetermined
7	37.14	40.49	42.05
8	37.37	39.60	Undetermined
9	38.53	39.42	40.28
10	37.89	40.61	Undetermined
11	38.06	39.48	Undetermined
12	37.36	41.46	40.91
13	37.78	40.48	40.28
14	33.92	40.66	40.60
15	34.76	39.15	Undetermined
16	34.76	39.97	40.38
17	35.72	39.47	Undetermined
18	38.71	39.41	40.25
19	37.97	38.67	41.25
20	37.57	39.24	Undetermined
Positive percentage in each concentration	**100%**	**95%**	**45%**

Test No	**(Copies/5µl)**		
	HPV type 51		
	500	**50**	**5**
1	34.73	39.28	Undetermined
2	34.57	39.06	Undetermined
3	35.21	40.35	Undetermined
4	35.47	39.27	Undetermined

5	34.89	39.13	Undetermined
6	36.07	39.27	Undetermined
7	35.41	39.57	41.37
8	35.37	39.97	40.97
9	34.98	Undetermined	40.58
10	35.74	39.61	40.53
11	35.57	39.62	Undetermined
12	36.36	39.67	40.91
13	35.28	39.17	Undetermined
14	34.92	39.69	39.60
15	34.73	39.18	Undetermined
16	34.47	39.91	Undetermined
17	35.01	40.46	39.27
18	37.27	40.19	40.41
19	36.12	39.57	40.65
20	35.74	39.95	Undetermined
Positive percentage in each concentration	**100%**	**95%**	**45%**

Inclusivity (analytical sensitivity)

The inclusivity of the primer/probe set used in the Viga Genotyping HPV Molecular Diagnostic Kit was analyzed in silico based on HPV sequences from the NCBI database accessed on September 26, 2021. The primer/probe sets for L1, E1, E2, E6, and E7 genes sequence alignment analysis demonstrate 100% inclusivity for HPV sequences identified from patient samples. The table shows the representative alignment results for L1, E1, E2, E6, and E

Table 8: Alignment test result for different HPV types gene

Strain- 18	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Human papillomavirus isolate HPV18	E1	KY501976.1	100	100	100
Human papillomavirus isolate HPV18	E1	KY501973.1	100	100	100
Human papillomavirus isolate HPV18 P1-20	E1	KY501967.1	100	100	100
Human papillomavirus isolate HPV18 P1	E1	KY501965.1	100	100	100
Human papillomavirus isolate HPV18 P2	E1	KY501971.1	100	100	100
Human papillomavirus isolate HPV18 P1-50	E1	KY501970.1	100	100	100
Human papillomavirus isolate HPV18 P2-30	E1	KY501974.1	100	100	100
Human papillomavirus isolate HPV18 P1-40	E1	KY501969.1	100	100	100

Strain- 45	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Human papillomavirus type 45 isolate LNS8825361_HPV45	E1	LR862061.1.1	100	100	100
Human papillomavirus type 45 isolate Qv34163	E1	KC470260.1	100	100	100
Human papillomavirus type 45 isolate Qv30712	E1	>KC470259.1.1	100	100	100
Human papillomavirus type 45 isolate Qv20214	E1	EF202156.1	100	100	100
Human papillomavirus type 45 isolate LNS8825361_HPV45	E1	LR862061.1.1	100	100	100
Human papillomavirus type 45 isolate Qv27565	E1	EF202157.1	100	100	100
Human papillomavirus type 45 isolate Qv33330	E1	EF202158.1	100	100	100
Human papillomavirus type 45 isolate Qv34178	E1	EF202159.1	100	100	100

Strain- 31	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Human papillomavirus 31 isolate VBD13/14	E2	MW814876.1	100	100	100
Human papillomavirus type 31 isolate LNS8465006_HPV31	E2	LR862053.1	100	100	100
Human papillomavirus type 31 isolate LNS8357921_HPV31	E2	LR862049.1	100	100	100
Human papillomavirus type 31 isolate LNS7548544_HPV31	E2	LR862026.1	100	100	100
Human papillomavirus type 31 isolate LNS7384732	E2	LR862018.1	100	100	100
Human papillomavirus type 31 isolate LNS7358029	E2	LR862015.1	100	100	100
Human papillomavirus type	E2	LR861970.1	100	100	100

31 isolate LNS6001593					
Human papillomavirus type 31 isolate LNS0937074	E2	LR861951.1	100	100	100
Human papillomavirus type 31 isolate IARC366181RW	E2	MT752571.1	100	100	100
Human papillomavirus type 31 isolate IARC366076RW	E2	MT752570.1	100	100	100
Human papillomavirus type 31 isolate PAP293744	E2	MT752505.1	100	100	100
Human papillomavirus type 31 isolate PAP287325	E2	MT752449.1	100	100	100

Strain- 35	**Target**	**Accession**	**% Homology Test Forward primer%**	**% Homology Test Reverse primer%**	**% Homology Test Probe%**
Human papillomavirus type 35 isolate LNS0838993	E6/E7	LR861946.1	100	100	100
Human papillomavirus type 35 isolate PAP294578	E6/E7	MT217995.1	100	100	100
Human papillomavirus type 35 isolate PAP282649	E6/E7	MT217962.1	100	100	100
Human papillomavirus type 35 isolate PAP163773	E6/E7	MT217679.1	100	100	100
Human papillomavirus type 35 isolate PAP162942	E6/E7	MT217678.1	100	100	100
Human papillomavirus type 35 isolate PAP145045	E6/E7	MT217620.1	100	100	100
Human papillomavirus type 35 isolate IARC1220350CH	E6/E7	MT217487.1	100	100	100
Human papillomavirus type 35 isolate IARC1101761NI	E6/E7	MT217470.1	100	100	100
Human papillomavirus type 35 isolate IARC1251067MO	E6/E7	MT217441.1	100	100	100
Human papillomavirus type 35 isolate IARC166070014RW	E6/E7	MT217402.1	100	100	100
Human papillomavirus type 35 isolate IARC1110712GU	E6/E7	MT217344.1	100	100	100
Human papillomavirus type 35 isolate IARC311027GU	E6/E7	MT217305.1	100	100	100

Strain- 59	**Target**	**Accession**	**%**	**%**	**%**

			Homology Test Forward primer%	Homology Test Reverse primer%	Homology Test Probe%
Human papillomavirus 59 strain kyd-s0359	E6	MT783417.1	100	100	100
Human papillomavirus type 59 isolate LNS9786324	E6	LR862080.1	100	100	100
Human papillomavirus type 59 isolate LNS3279856	E6	LR861868.1	100	100	100
Human papillomavirus type 59 isolate Qv23880	E6	KC470263.1	100	100	100
Human papillomavirus type 59 isolate LZod68-59	E6	EU918767.1	100	100	100
Human papillomavirus type 59 isolate LNS5859951	E6	LR861933.1	100	100	100
Human papilloma virus type 59	E6	X77858.1	100	100	100
Human papillomavirus type 59 isolate Qv25652	E6	KC470261.1	100	100	100
Human papillomavirus type 59 isolate Qv25808	E6	KC470264.1	100	100	100
Human papillomavirus type 59 isolate Qv33993	E6	KC470265.1	100	100	100
Human papillomavirus type 59 isolate 32A.59	E6	KU298921.1	100	100	100
Human papillomavirus type 59 isolate LNS2917513_HPV59	E6	LR861860.1	100	100	100

Strain- 39	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Human papillomavirus type 39 isolate LNS9068892	L1	LR862071.1	100	100	100
Human papillomavirus type 39 isolate LNS8643648	L1	LR862054.1	100	100	100
Human papillomavirus type 39 isolate LNS7329845	L1	LR862014.1	100	100	100
Human papillomavirus type 39 isolate 39PL03 L1	L1	MK344660.1	100	100	100
Human papillomavirus type 39 isolate 39PL02 L1	L1	MK344659.1	100	100	100
Human papillomavirus type 39 isolate 16B	L1	KX514417.1	100	100	100
Human papillomavirus type	L1	KC470243.1	100	100	100

39 isolate Tw562					
Human papillomavirus type 39 isolate Qv25609	L1	KC470242.1	100	100	100
Human papillomavirus type 39 isolate Qv25959	L1	KC470240.1	100	100	100
Human papillomavirus type 39 isolate Qv27715	L1	KC470237.1	100	100	100
Human papillomavirus type 39 isolate LNS4492831	L1	LR861904.1	100	100	100
Human papillomavirus type 39 clone 39L1.A major capsid protein	L1	JN104068.1	100	100	100

Strain- 6/11	**Target**	**Accession**	**% Homology Test Forward primer%**	**% Homology Test Reverse primer%**	**% Homology Test Probe%**
Human papillomavirus type 11 isolate HPV11-gw-1108	L1	MK463919.1	100	100	100
Human papillomavirus type 11 isolate HPV11-gw-1111	L1	MK463922.1	100	100	100
Human papillomavirus type 11 isolate HPV11-gw-1110	L1	MK463921.1	100	100	100
Human papillomavirus type 11 isolate HPV11-gw-1109	L1	MK463920.1	100	100	100
Human papillomavirus type 6 isolate HPV6-gw-0611	L1	MK463909.1	100	100	100
Human papillomavirus type 6 isolate HPV6-gw-0602	L1	MK463905.1	100	100	100
Human papillomavirus type 6 isolate ASCUS1/HPV6	L1	MK313781.1	100	100	100
Human papillomavirus type 6 isolate CAC1/HPV6	L1	MK313778.1	100	100	100
Human papillomavirus type 6 isolate JO-RRP2/HPV6	L1	MK313777.1	100	100	100
Human papillomavirus type 6 isolate AO-RRP7/HPV6	L1	MK313775.1	100	100	100
Human papillomavirus type 6 isolate AO-RRP5/HPV6	L1	MK313773.1	100	100	100
Human papillomavirus type 6 isolate AO-RRP3/HPV6	L1	MK313771.1	100	100	100
Human papillomavirus type 6 isolate AO-RRP1/HPV6	L1	MK313769.1	100	100	100
Human papillomavirus type	L1	KX514423.1	100	100	100

6 isolate 111B					
Human papillomavirus type 6 isolate J-50	L1	KU721785.1	100	100	100

Strain- 51	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Human papillomavirus type 51 isolate LNS9024319	L1	LR862069.1	100	100	100
Human papillomavirus type 51 isolate LNS8077695	L1	LR862046.1	100	100	100
Human papillomavirus type 51 isolate LNS0570411_HPV51	L1	LR861939.1	100	100	100
Human papillomavirus type 51 isolate LNS4020261	L1	LR861893.1	100	100	100
Human papillomavirus type 51 isolate LNS3425544	L1	LR861869.1	100	100	100
Human papillomavirus type 51 isolate LNS9888540_HPV51	L1	LR861810.1	100	100	100
Human papillomavirus type 51 isolate CL17 L1	L1	MH577961.1	100	100	100
Human papillomavirus type 51 isolate CL16	L1	MH577960.1	100	100	100
Human papillomavirus type 51 isolate CL10	L1	MH577954.1	100	100	100
Human papillomavirus type 51 isolate 118A.51	L1	KU298905.1	100	100	100

Strain- 66	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Human papillomavirus type 66 isolate NGSk294-66	E6	LR861964.1	100	100	100
Human papillomavirus type 66 isolate LNS4474748_HPV66	E6	LR861902.1	100	100	100
Human papillomavirus type 66 isolate LNS2720744	E6	LR861851.1	100	100	100

Human papillomavirus type 66 isolate 110	E6	JN661435.1	100	100	100
Human papillomavirus type 66 isolate 100	E6	JN661426.1	100	100	100
Human papillomavirus type 66 isolate 95	E6	JN661421.1	100	100	100
Human papillomavirus type 66 isolate 92	E6	JN661418.1	100	100	100
Human papillomavirus type 66 isolate 90	E6	JN661416.1	100	100	100
Human papillomavirus type 66 isolate Bsb-107	E6	HM585479.1	100	100	100
Human papillomavirus type 66 clone Qv11088	E6	EF177190.1	100	100	100

Strain- 68	**Target**	**Accession**	**% Homology Test Forward primer%**	**% Homology Test Reverse primer%**	**% Homology Test Probe%**
Human papillomavirus type 68 isolate 55-86-21	E6/E7	MK874671.1	100	100	100
Human papillomavirus type 68 isolate 21-54-34	E6/E7	MK874669.1	100	100	100
Human papillomavirus type 68 isolate 21-54-24	E6/E7	MK874668.1	100	100	100
Human papillomavirus type 68 isolate 21-54-18	E6/E7	MK874666.1	100	100	100
Human papillomavirus type 68 isolate 1-20-9	E6/E7	MK874662.1	100	100	100
Human papillomavirus type 68 isolate M180	E6/E7	MN047810.1	100	100	100
Human papillomavirus type 68 isolate 268	E6/E7	KX514428.1	100	100	100
Human papillomavirus type 68 isolate P141607	E6/E7	KU195243.1	100	100	100
Human papillomavirus type 68 isolate Rw826	E6/E7	KC470281.1	100	100	100
Human papillomavirus type 68 isolate Qv25395	E6/E7	KC470279.1	100	100	100

Strain- 56/66	**Target**	**Accession**	**% Homology Test**	**% Homology Test**	**% Homology Test**

			Forward primer%	Reverse primer%	Probe%
Human papillomavirus type 56 isolate 56SE-23	E6/E7	KX645764.1	100	100	100
Human papillomavirus type 56 isolate 56SE-21	E6/E7	KX645762.1	100	100	100
Human papillomavirus type 56 isolate 56SE-19	E6/E7	KX645760.1	100	100	100
Human papillomavirus type 56 isolate 56SE-09	E6/E7	KX645750.1	100	100	100
Human papillomavirus type 56 isolate 60B	E6/E7	KX514418.1	100	100	100
Human papillomavirus type 56 isolate 87A.56	E6/E7	KU298916.1	100	100	100
Human papillomavirus type 56 clone Qv22608	E6/E7	EF177179.1	100	100	100
Human papillomavirus type 66 isolate LNS7384732	E6/E7	LR862020.1	100	100	100
Human papillomavirus type 66 isolate LNS1839688	E6/E7	LR861964.1	100	100	100
Human papillomavirus type 66 isolate LNS5491243	E6/E7	LR861922.1	100	100	100
Human papillomavirus type 66 isolate LNS3785675	E6/E7	LR861879.1	100	100	100
Human papillomavirus type 66 isolate 106	E6/E7	JN661479.1	100	100	100
Human papillomavirus type 66 isolate 95	E6/E7	JN661470.1	100	100	100
Human papillomavirus type 66 isolate 85	E6/E7	JN661460.1	100	100	100
Human papillomavirus type 66 isolate Qv32783	E6/E7	EF546480.1	100	100	100
Human papillomavirus type 66 clone Qv11088	E6/E7	EF177190.1	100	100	100

Strain- 33/52/ 58	Target	Accession	% Homology	% Homology	%
			Test Forward primer%	Test Reverse primer%	Homology Test Probe%
Human papillomavirus type 33 isolate LNS9453833	E1/E2	LR862077.1	100	100	100

Human papillomavirus type 33 isolate LNS3510974	E1/E2	LR861872.1	100	100	100
Human papillomavirus type 33 isolate 67B.33	E1/E2	KU298894.1	100	100	100
Human papillomavirus type 33 isolate 65C.33	E1/E2	KU298893.1	100	100	100
Human papillomavirus type 33 isolate BF375	E1/E2	KF436865.1	100	100	100
Human papillomavirus type 33	E1/E2	M12732.1	100	100	100
Human papillomavirus type 33 isolate LZcc12-33	E1/E2	EU918766.1	100	100	100
Human papillomavirus type 33 isolate Qv32494	E1/E2	HQ537688.1	100	100	100
Human papillomavirus 52 isolate PAP3497	E1/E2	MZ374448.1	100	100	100
Human papillomavirus 52 isolate PAP1639	E1/E2	MZ374436.1	100	100	100
Human papillomavirus 52 isolate PAP1189	E1/E2	MZ374435.1	100	100	100
Human papillomavirus 52 isolate PAP2912	E1/E2	MZ374431.1	100	100	100
Human papillomavirus 52 isolate PAP2357	E1/E2	MZ374425.1	100	100	100
Human papillomavirus 52 isolate PAP2484	E1/E2	MZ374419.1	100	100	100
Human papillomavirus 52 isolate PAP1917	E1/E2	MZ374415.1	100	100	100
Human papillomavirus 52 isolate PAP2698	E1/E2	MZ374413.1	100	100	100
Human papillomavirus type 52 isolate LNS9453833	E1/E2	LR862077.1	100	100	100
Human papillomavirus type 52 isolate LNS6759684	E1/E2	LR861998.1	100	100	100
Human papillomavirus type 58 isolate LNS6048641	E1/E2	LR861973.1	100	100	100
Human papillomavirus type 58 isolate LNS0838993	E1/E2	LR861950.1	100	100	100
Human papillomavirus type 58 isolate LNS5390598	E1/E2	LR861919.1	100	100	100
Human papillomavirus type 58 isolate LNS4582218	E1/E2	LR861906.1	100	100	100

Clinical Sensitivity

The wet testing of inclusivity using the DNall VirAll Kit was evaluated as supplemental data by testing three HPV positive specimens from WHO HPV Lab Net. These specimens were confirmed positive by Viga Genotyping HPV Molecular Diagnostic Kit. Each specimen was diluted to (≤3log10 LOD, ≤2log10 LOD, ≤1log10 in negative specimen matrix (Cervical swab) and tested in the tenth replicate. (Below table).

Table 9: the results of diagnostic sensitivity of Viga Quantitative HPV Molecular Diagnostic Kit ROJETechnologies

Dilution series	Copies/5 µl	Ct
3log10> LOD	50.000	24.55
		24.25
		25.05
		25.13
		24.71
		24.87
		24.18
		24.46
		25.14
		25.23
2log10 > LOD	5.000	28.44
		28.48
		29.31
		28.64
		28.78
		28.73
		29.08
		29.17
		29.08
		28.87
1log10 > LOD	500	34.84
		33.82
		34.52
		33.88
		34.17
		35.27
		35.37
		35.76
		35.78
		35.37

Cross-reactivity (analytical specificity)

Cross-reactivity of the Viga Genotyping HPV Molecular Diagnostic Kit was evaluated in silico analysis and by wet testing potentially cross-reactive whole pathogens or purified nucleic acid from clinical specimens. No cross-reactivity was detected. The in-silico mapping analysis of each primer/probe against several pathogens is based on the NCBI nr/nt database accessed January 9, 2022, using the online BLASTN 2.10.0+, and the representative results are shown below the table. No cross-reactivity was observed for other listed pathogens in silico and wet-testing.

Table 10: The In-Silico Specificity Analysis of Primer and Probe Set for Other pathogens.

Strain- 18 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	HIV-1 isolate 002211_C07	MT033226.1	59%	72%	62%
Hepatitis C virus subtype 1a	10jsszGP017	JQ303503.1	63%	55%	62%
HSV-1	s17pp22a	MN159382.1	70%	50%	66%
Hepatitis B virus	isolate 2793	KF169309.1	50%	66%	59%
HSV-2	strain 2009-24855_S55_L001	MH790632.1	45%	61%	70%
Mycoplasma genitalium	M2321 MgPar 9	CP003773.1	45%	55%	51%
Chlamydia trachomatis	TC0350	CP042803.1	66%	61%	77%
Streptococcus agalactiae	Sag27	CP031556.1	81%	77%	48%
Human T-cell leukemia virus type I	057-OL	MN453165.1	50%	88%	68%
Human gamma herpesvirus	HC-0036	LR813015.1	63%	63%	62%
Human alpha herpesvirus	s17pp22a	MN159382.1	95%	55%	66%
Human T-lymphotropic virus 2	HTLV-2/Japan/NIID/001/2020	K01670.1	50%	44%	51%
Human parvovirus B19	911NewF	MN088124.1	33%	55%	40%
JC polyomavirus	UJC005	MW587996.1	72%	50%	88%
Neisseria	TUM16691	AP023075.1	54%	55%	44%

gonorrhoeae					
Trichomonas vaginalis	TVAG_290210	XM_001312003.1	59%	72%	62%

Strain- 16 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	YBF319	KT198590.1	52%	63%	65%
Hepatitis C virus subtype 1a	HCV-1b/US/BID-V377/2006	EU256065.1	48%	40%	55%
HSV-1	2007-16123	MG999881.1	36%	33%	50%
Hepatitis B virus	B127W	MH051987.1	48%	40%	50%
HSV-2	2006-15095	MH899846.1	55%	40%	72%
Mycoplasma genitalium	1206KLM26 MgpC adhesin	MT439445.1	44%	53%	50%
Chlamydia trachomatis	TC0411:TncL2/tetR-104	CP042798.1	75%	86%	75%
Streptococcus agalactiae	2013-1366	CP051844.1	70%	84%	44%
Human T-cell leukemia virus type I	Aus-CS	KF242506.1	60%	45%	30%
Human gammaherpesvirus	21_AJOUA	MT648662.1	55%	48%	43%
Human alphaherpesvirus	HSV1-ORIGINAL-H1	MH160367.1	36%	33%	50%
Human T-lymphotropic virus 2	(HTLV-II AA	X76030.1	40%	72%	43%
Human parvovirus B19	HZ13 NS1	KT389459.1	43%	73%	75%
JC polyomavirus	IRN-26H	MN163027.1	70%	36%	66%
Neisseria gonorrhoeae	NG196	CP043872.1	40%	40%	44%
Trichomonas vaginalis	TVAG_231320	XM_001313720.1	52%	46%	60%

Strain- 31 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	A09.Q2.20M9D1	MH264318.1	63%	63%	68%
Hepatitis C virus subtype 1a	AV67	EU484132.1	58%	75%	52%
HSV-1	HSV-H1412	MH999851.1	56%	87%	45%
Hepatitis B virus	551.31	KR013975.1	70%	58%	52%
HSV-2	HSV2_19	MH697440.1	64%	87%	52%
Mycoplasma genitalium	1493B26 MgpB	MT439517.1	52%	56%	42%
Chlamydia trachomatis	TC0657:TnxL2/tetR-403	CP042757.1	87%	42%	54%
Streptococcus agalactiae	CM45/1	CP088933.1	55%	87%	62%
Human T-cell leukemia virus type I	IR (94) bZIP	MN453136.1	57%	47%	52%
Human gammaherpesvirus	20_IHOM	MT648661.1	78%	48%	58%
Human alphaherpesvirus	CM1v8	KX791821.1	56%	87%	56%
Human T-lymphotropic virus 2	N1232	KY493238.1	50%	75%	42%
Human parvovirus B19	NA121	MG765334.1	50%	56%	78%
JC polyomavirus	SA38527_04 VP1	EU835186.1	42%	42%	87%
Neisseria gonorrhoeae	SRRSH205	CP048907.1	78%	68%	62%
Trichomonas vaginalis	TVAG_449190	XM_001308315.1	62%	63%	68%

Strain- 35 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	OU072	OK180789.1	48%	44%	56%
Hepatitis C virus	EA03T0	AM271169.1	47%	39%	43%

subtype 1a					
HSV-1	2011-12741	MG999893.1	43%	74%	47%
Hepatitis B virus	CHB-47-PS	KY428722.1	40%	37%	43%
HSV-2	2012-5447	MH790637.1	56%	43%	52%
Mycoplasma genitalium	1491KLM26	MT439589.1	33%	52%	44%
Chlamydia trachomatis	TC0411: TnxD/tetR-103	CP042802.1	54%	81%	48%
Streptococcus agalactiae	SA627	CP019837.1	43%	52%	33%
Human T-cell leukemia virus type I	BHP00140	MG388047.1	52%	43%	44%
Human gammaherpesvirus	19_ELTW	MT648660.1	40%	48%	47%
Human alphaherpesvirus	2011-17239	MG999896.1	43%	74%	47%
Human T-lymphotropic virus 2	BBD_7003	FJ911659.1	51%	29%	59%
Human parvovirus B19	D1599 NS1	DQ234775.2	40%	55%	59%
JC polyomavirus	J007 VP1	MK477564.1	37%	88%	33%
Neisseria gonorrhoeae	TUM19853	AP023067.1	47%	77%	52%
Trichomonas vaginalis	TVAG_196200	XM_001312988.1	51%	66%	52%

Strain-33/52/58 Pathogen (Taxonomy ID)	**Strain**	**GenBank Acc#**	**% Homology Test FP**	**% Homology Test RP**	**% Homology Test Probe**
Human immunodeficiency virus	AA075a_WG7	JX447542.1	63%	66%	**59%**
Hepatitis C virus subtype 1a	PATNO72	AB285080.1	45%	62%	**41%**
HSV-1	2004-63623	MG999877.1	62%	59%	**72%**
Hepatitis B virus	OHBV-HIV137	MF618348.1	45%	45%	**50%**

HSV-2	2006-18003CAM	MH790593.1	41%	59%	**62%**
Mycoplasma genitalium	10467 MgpB	KP318824.1	50%	63%	**40%**
Chlamydia trachomatis	TC0189:TncL2/tetR-102	CP042734.1	87%	59%	**63%**
Streptococcus agalactiae	NEM316	AL766847.1	45%	87%	**50%**
Human T-cell leukemia virus type I	BHP00005	MG388044.1	75%	59%	**54%**
Human gammaherpesvirus	sLCL-T3.27	LC573551.1	59%	50%	**45%**
Human alphaherpesvirus	2003-15756	MG999872.1	75%	68%	**62%**
Human T-lymphotropic virus 2	NIID18001	LC440555.1	36%	41%	**31%**
Human parvovirus B19	BN58.3	DQ408302.1	54%	77%	**66%**
JC polyomavirus	IRN-72H	MN163031.1	45%	37%	**63%**
Neisseria gonorrhoeae	SS3160	AP019853.2	41%	58%	**45%**
Trichomonas vaginalis	TVAG_308110	XM_001320393.1	40%	58%	**72%**

Strain- 59 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	505_1982a	MG197155.1	60%	52%	68%
Hepatitis C virus subtype 1a	GZ52540	KC844051.1	35%	42%	46%
HSV-1	11_DOCK8	MN401208.1	64%	40%	42%
Hepatitis B virus	NOA_142	MG098582.1	48%	40%	44%
HSV-2	HJ12	MN187895.1	40%	36%	52%
Mycoplasma genitalium	M2288	CP003773.1	44%	56%	40%
Chlamydia trachomatis	TC0350:TncL2/tetR-936	CP042803.1	44%	40%	64%
Streptococcus agalactiae	NEM316	AL766845.1	56%	40%	36%
Human T-cell leukemia virus type I	PH1511	MN781156.1	40%	56%	40%
Human gammaherpesvirus	HKHD138	MH590507.1	64%	44%	76%
Human alphaherpesvirus	2007-16123	MG999881.1	64%	40%	36%
Human T-lymphotropic virus 2	BBD_3079	FJ911654.1	36%	40%	32%
Human parvovirus B19	HZ13 NS1	KT389459.1	40%	36%	76%
JC polyomavirus	Tn-19 VP1	JQ433658.1	36%	32%	48%
Neisseria gonorrhoeae	TUM16691	AP023075.1	44%	76%	52%
Trichomonas vaginalis	TVAG_149710	XM_001308182.1	60%	52%	56%

Strain- 39 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	505_1982a	MG197155.1	60%	52%	68%
Hepatitis C virus	GZ52540	KC844051.1	35%	42%	46%

subtype 1a					
HSV-1	11_DOCK8	MN401208.1	64%	40%	42%
Hepatitis B virus	NOA_142	MG098582.1	48%	40%	44%
HSV-2	HJ12	MN187895.1	40%	36%	52%
Mycoplasma genitalium	M2288	CP003773.1	44%	56%	40%
Chlamydia trachomatis	TC0350:TncL2/tetR-936	CP042803.1	44%	40%	64%
Streptococcus agalactiae	NEM316	AL766845.1	56%	40%	36%
Human T-cell leukemia virus type I	PH1511	MN781156.1	40%	56%	40%
Human gammaherpesvirus	HKHD138	MH590507.1	64%	44%	76%
Human alphaherpesvirus	2007-16123	MG999881.1	64%	40%	36%
Human T-lymphotropic virus 2	BBD_3079	FJ911654.1	36%	40%	32%
Human parvovirus B19	HZ13 NS1	KT389459.1	40%	36%	76%
JC polyomavirus	Tn-19 VP1	JQ433658.1	36%	32%	48%
Neisseria gonorrhoeae	TUM16691	AP023075.1	44%	76%	52%
Trichomonas vaginalis	TVAG_149710	XM_001308182.1	60%	52%	56%

Strain- 66 Pathogen (Taxonomy ID)	**Strain**	**GenBank Acc#**	**% Homology Test FP**	**% Homology Test RP**	**% Homology Test Probe**
Human immunodeficiency virus	2303-08PT46	MH832382.1	50%	35%	46%
Hepatitis C virus subtype 1a	EG06T1ak	AM708703.1	35%	42%	39%
HSV-1	H166	KM222726.1	35%	39%	53%
Hepatitis B virus	124_CA_Kal	MH580631.1	39%	35%	42%
HSV-2	HJ12	MN187895.1	35%	50%	57%
Mycoplasma genitalium	199 MgPar 7	EF117299.2	57%	42%	39%
Chlamydia trachomatis	KU2043c768-137	CP042789.1	32%	39%	35%
Streptococcus agalactiae	NY84115	JF270524.1	35%	39%	42%
Human T-cell leukemia virus type I	Gab1014FC	EU444097.1	80%	50%	38%
Human gammaherpesviru	DOCK8	MN401207.1	35%	42%	53%

s					
Human alphaherpesvirus	E25	HM585506.2	50%	57%	42%
Human T-lymphotropic virus 2	HTLV-2/Japan/NIID/001/2020	LC534557.1	57%	50%	73%
Human parvovirus B19	BX2	MH201456.1	34%	30%	65%
JC polyomavirus	VP1	EF369493.1	61%	69%	42%
Neisseria gonorrhoeae	SW0236	CP061485.1	61%	53%	30%
Trichomonas vaginalis	TVAG_402520	XM_001580933.1	42%	69%	50%

Strain- 68 Pathogen (Taxonomy ID)	**Strain**	**GenBank Acc#**	**% Homology Test FP**	**% Homology Test RP**	**% Homology Test Probe**
Human immunodeficiency virus	BA855	MK732623.1	57%	76%	50%
Hepatitis C virus subtype 1a	9419KBS/12	MH627077.1	50%	53%	50%
HSV-1	DOCK8	MN401208.1	46%	61%	73%
Hepatitis B virus	BB1618958	MH247244.1	69%	42%	57%
HSV-2	HJ12	MN187895.1	61%		
Mycoplasma genitalium	TC0350:TncL2/tetR-936	CP042803.1	35%	39%	57%
Chlamydia trachomatis	01173	CP053027.1	42%	69%	42%
Streptococcus agalactiae	IL1657	KF202322.1	61%	36%	34%
Human T-cell leukemia virus type I	IT1036	KF202320.1	34%	32%	38%
Human gammaherpesvirus	HKHD44	MH590413.1	42%	38%	69%
Human alphaherpesvirus	BB1618958_p	MH247244.1	30%	61%	50%
Human T-lymphotropic virus 2	HTLV-2/Japan/NIID/001/2020	LC534557.1	69%	34%	30%
Human parvovirus B19	R0416	DQ234769.2	38%	50%	42%
JC polyomavirus	JCV144CSFRRC	JF425684.1	65%	46%	57%
Neisseria gonorrhoeae	TUM16691	AP023075.1	42%	65%	46%
Trichomonas vaginalis	TVAG_454270	XM_001329699.1	46%	50%	42%

Strain- 6/11 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency virus	7864_6	MW189577.1	54%	41%	50%
Hepatitis C virus subtype 1a	1b.NR.022	KM580649.1	66%	50%	54%
HSV-1	Sc16	MN159383.1	54%	45%	50%
Hepatitis B virus	cxx16	MZ244221.1	50%	41%	
HSV-2	HJ12	MN187895.1	45%	45%	54%
Mycoplasma genitalium	M2288	CP003773.1	62%	54%	41%
Chlamydia trachomatis	TC0693:TncL2/tetR-3509	CP042693.1	50%		41%
Streptococcus agalactiae	QMA0271	CP029632.1	50%	54%	66%
Human T-cell leukemia virus type I	mel5	L02534.1	54%	41%	45%
Human gammaherpesvirus	RK_LCL_L3	MG298914.1	66%	45%	54%
Human alphaherpesvirus	McKrae	MN136524.1	37%	50%	70%
Human T-lymphotropic virus 2	HTLV-2/Japan/NIID/001/2020	LC534557.1	70%	37%	66%
Human parvovirus B19	057214	JN211130.1	29%	62%	45%
JC polyomavirus	NIID11-53	LC164352.1	70%	33%	37%
Neisseria gonorrhoeae	TUM16691	AP023075.1	66%	37%	45%
Trichomonas vaginalis	TVAG_439770	XM_001306803.1	62%	29%	37%

Strain- 56/66 Pathogen (Taxonomy ID)	Strain	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human immunodeficiency	RF (HAT-3)	M17451.1	83%	50%	58%

virus					
Hepatitis C virus subtype 1a	Pt9.Seg8-Con	KX084702.1	50%	75%	34%
HSV-1	2339/2009	JQ352258.1	45%	70%	66%
Hepatitis B virus	HBV_NWFD_AHB111	MZ090870.1	75%	54%	58%
HSV-2	M2288	CP003773.1	58%	50%	
Mycoplasma genitalium	TC0411: TnxD/tetR-103	CP042802.1	75%	62%	58%
Chlamydia trachomatis	TC0411: TnxD/tetR-103	CP042802.1	50%	75%	45%
Streptococcus agalactiae	SG-M25	CP021867.1	58%	54%	41%
Human T-cell leukemia virus type I	AC038	LC192501.1	41%	54%	58%
Human gammaherpesvirus	eBL-Tumor-0014	LR813032.1	30%	41%	
Human alphaherpesvirus	2339/2009	JQ352258.1	62%	56%	50%
Human T-lymphotropic virus 2	HTLV-2/Japan/NIID/001/2020	LC534557.1	41%	50%	45%
Human parvovirus B19	HZ92	KT389469.1	34%	30%	58%
JC polyomavirus	RKAB15 LTAg	LC650363.1	35%	42%	61%
Neisseria gonorrhoeae	FA6140	CP012027.1	34%	62%	58%
Trichomonas vaginalis	TVAG_480160	XM_001307664.1	50%	30%	62%

Pathogen / 51 (Taxonomy ID)	**Strain**	**GenBank Acc#**	**% Homology Test FP**	**% Homology Test RP**	**% Homology Test Probe**
Human immunodeficiency virus	20263v08	MN791407.1	54%	65%	50%
Hepatitis C virus subtype 1a	HCV-1b/US/BID-V152/2003	EU155224.2	45%	50%	50%
Hepatitis B virus	94T.13.	KR812069.1	54%	50%	54%
HSV-1	2018-5971	MH813987.1	87%	45%	59%
Human papillomavirus	NCI_230248	MG849960.1	40%	45%	45%
HSV-2	1996-45091	MH790670.1	66%	83%	45%

Mycoplasma genitalium	M6285 MgPa	GU226202.1	59%	45%	41%
Chlamydia trachomatis	TC0411: TnxD/tetR-296	CP042800.1	50%	63%	81%
Streptococcus agalactiae	SG-M25	CP021867.1	68%	65%	83%
Human T-cell leukemia virus type I	012BR_HAM107	KF797884.1	41%	50%	40%
Human gammaherpesvirus	HC-0020	LR813006.1	54%	85%	58%
Human alphaherpesvirus	Y033 UL40	AY240581.1	45%	87%	50%
Human T-lymphotropic virus 2	NIID18002	LC440556.1	40%	45%	45%
Human parvovirus B19	CAEN.FRA 21.09/1	FN669504.1	37%	40%	45%
JC polyomavirus	PE-21	AB081027.1	33%	45%	40%
Neisseria gonorrhoeae	AUSMDU00010541	CP045832.1	63%	55%	45%
Trichomonas vaginalis	TVAG_308030	XM_001579636.1	59%	55%	50%

Cross-Reactivity (clinical specificity) of Viga Genotyping HPV Molecular Diagnostic Kit

To check the clinical specificity of the nucleic acid of other pathogens in a matrix of a negative sample (Cervical negative swab) diluted with a specific concentration. Then the samples were extracted, and using Viga Genotyping HPV Molecular Diagnostic Kit was tested. No cross-reactivity was observed for other listed pathogens in the following table.

Table 12: Investigation of the cross-reactivity of the HPV using Viga Genotyping HPV Molecular Diagnostic Kit

Virus/Bacteria/Parasite	Source/ Sample type	Ct Value
Human immunodeficiency virus-1	Clinical sample	-/-
Hepatitis C virus	Clinical sample	-/-
Cytomegalovirus	Clinical sample	-/-
Herpes simplex virus type 1	Clinical sample	-/-

Herpes simplex virus type 2	Clinical sample	-/-
Human papillomavirus	Clinical sample	-/-
Epstein-Barr virus	Clinical sample	-/-
Adenovirus	Clinical sample	-/-
Influenza A	Clinical sample	-/-
Influenza B	Clinical sample	-/-
Legionella pneumophila	Clinical sample	-/-
Cryptococcus neoformans	Clinical sample	-/-
Chlamydia pneumonia	Clinical sample	-/-
Streptococcus pneumonia	Clinical sample	-/-
Respiratory Syncytial Virus	Clinical sample	-/-
Mycoplasma pneumonia	Clinical sample	-/-
Streptococcus pyogenes	Clinical sample	-/-
Mycobacterium tuberculosis	Clinical sample	-/-
10 Pooled human genomes	Clinical sample	-/-

Clinical Evaluation

The clinical performance of the Viga Genotyping HPV Molecular Diagnostic Kit was established using 206 cervical specimens, Liquid-based cytology specimen, Urine, Paraffin-embedded tissue collected from patients suspected of HPV. The comparator method was the HPV 3.5 LCD-Array Kits (Chipron GmbH) and Cobas® HPV test (Roche), which received CE-IVD. The extraction method was the DNall VirAll Kit. The results are summarized in the analysis and demonstrated a PPA of 96.47% and NPA of 100%, compared to the HPV 3.5 LCD-Array Kits (Chipron GmbH) kit and PPA of 94.4% and NPA of 100%, compared to the Cobas® HPV test (Roche) kit.

Table 13: Results of the Viga Genotyping HPV Molecular Diagnostic Kit (ROJETechnologies) and the HPV 3.5 LCD-Array Kits (Chipron GmbH) and Cobas® HPV test (Roche).

	HPV 3.5 LCD-Array Kits (Chipron GmbH)	**Viga Genotyping HPV Molecular Diagnostic Kit (ROJETechnologies)**
Number	**Result**	**Result**
1	11	6/11
2	6/16	6/11, 16
3	51	-
4	16	16
5	51	51
6	6	6/11 33/52/58
7	6/59	6/11
8	16/18	16/18
9	16	16
10	45	45
11	31	31
12	45	45
13	45	45
14	45	45
15	18	18
16	18	18
17	18	18
18	45	45
19	45	45
20	16	16
21	16	16
22	16	16
23	16	16
24	16	16
25	16	16
26	16	16
27	51	51
28	51	51
29	51	51
30	51	51
31	51	51
32	56	56/66
33	56	56/66
34	56	56/66
35	56	56/66

36	56	56/66
37	56	56/66
38	56	56/66
39	66	56/66
40	66	56/66
41	66	56/66
42	66	56/66
43	66	56/66
44	66	56/66
45	66	56/66
46	35	35/39
47	35	35/39
48	35	35/39
49	35	35/39
50	35	35/39
51	35	35/39
52	35	35/39
53	35	-
54	59	59
55	59	59
56	59	59
57	59	59
58	59	59
59	59	59
60	59	-
61	6	6/11
62	6	6/11
63	11	6/11
64	11	6/11
65	11	6/11
66	52	33/52/58
67	52	33/52/58
68	52	33/52/58
69	52	33/52/58
70	52	33/52/58
71	52	33/52/58
72	58	33/52/58
73	58	33/52/58
74	58	33/52/58
75	58	33/52/58
76	58	33/52/58

77	58/33	33/52/58
78	58/33	33/52/58
79	33	33/52/58
80	33	33/52/58
81	33	33/52/58
82	33	33/52/58
	Cobas® HPV test (Roche)	**Viga Genotyping HPV Molecular Diagnostic Kit (ROJETechnologies)**
1	16	16
2	16	16
3	16	16
4	16	16
5	16	16
6	16	16
7	16	16
8	16	16
9	16	16
10	16	16
11	16	16
12	16	-
13	18	18
14	18	18
15	18	18
16	18	18
17	18	18
18	18	18
19	18	18
20	18	18
21	18	18
22	18	-
23	High Risk	33/52/58, 56/66
24	High Risk	6/11, 33/52/58, 51
25	High Risk	6/11, 16, 51
26	High Risk	6/11, 51
27	High Risk	6/11, 33/52/58, 68, 51
28	High Risk	33/52/58, 51
29	High Risk	59
30	High Risk	33/52/58, 51
31	High Risk	33/52/58, 68, 51
32	High Risk	39, 33/52/58, 35, 68

33	High Risk	68, 51, 56/66
34	High Risk	51, 56/66

The results of Clinical Evaluation

Table 14: Clinical Evaluation of Viga Genotyping HPV Molecular Diagnostic Kit compared to the HPV 3.5 LCD-Array Kits (Chipron GmbH)

Test		**HPV 3.5 LCD-Array Kits (Chipron GmbH)**		**Total**
		Positive	**Negative**	
Viga Genotyping HPV Molecular Diagnostic Kit (ROJETechnologies)	**Positive**	82	0	82
	Negative	3	100	103
Total		85	100	185

- Positive Agreement Rate: 82/85 ×100%=96.47 %
- Negative Agreement Rate: 100/100×100%= 100%
- Overall rates of agreement: (100+82) / (82+0+100+3) ×%100=98.37%

Table 15: Clinical Evaluation of Viga Genotyping HPV Molecular Diagnostic Kit compared to the Cobas® HPV test (Roche)

Test		**Cobas® HPV test (Roche)**		**Total**
		Positive	**Negative**	
Viga Genotyping HPV Molecular Diagnostic Kit (Roje Technology)	**Positive**	34	0	34
	Negative	2	100	102
Total		36	100	136

- Positive Agreement Rate: 34/36 ×100%=94.44 %
- Negative Agreement Rate: 100/100×100%= 100%

- Overall rates of agreement: (100+34) / (34+0+100+2) ×%100=98.52%

symbols

Tables 16: symbols on the Kit Label

symbols	meaning	symbols	meaning
	Date of manufacture		manufacturer
	Expiration Date		Temperature limitation
IVD	In Vitro Diagnostics	LOT	Lot number
		REF	Reference number

Troubleshooting

Here we try to cover as many problems as you may see in using this product; however, scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control.	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during the preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the Real-time PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats and replace test tubes and tips in use.
	PCR tube not properly sealed	Ensure plates are sealed correctly
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of DNA samples carrying interferences	Repeat the test with the neat extracted DNA and 1:2 dilution of the extracted DNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

Ordering Information

category	Product name	Preps no.	Cat No.
Molecular diagnostic Kit	Viga Genotyping HPV Molecular Diagnostic Kit	25 Preps	MD003060
	The Viga Genotyping HPV Molecular Diagnostic Kit	100 Preps	MD003061
Related product	DNall VirAll Kit	100 Preps	DN983053

Technical assistance

ROJETechnologies guarantees your complete satisfaction. ROJE technical support team composed of highly trained experienced scientists, who are able to troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select suitable product.

Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE technical support team from our website (www.rojetechnologies.com).
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Factory address

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130- phone: 02191070705

References

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3- Lei et al. (2020) HPV Vaccination and the Risk of Invasive Cervical Cancer. N Engl J Med 2020; 383:1340-8. DOI: 10.1056/NEJMoa1917338

4- World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem https://www.who.int/publications/i/item/9789240014107

ROJETECHNOLOGIES has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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