LabTurbo

LabTurbo AIO Human papillomavirus (HPV) PCR Detection Reagents User Manual

Catalog number: AHPV1209611

Real-time PCR detection reagents
For research use only (RUO)

Version 1.0

Intended use:

The LabTurbo AIO Human papillomavirus (HPV) PCR Detection Reagents are intended for the presumptive qualitative detection of nucleic acid from the HPV-16 and HPV-18 in human vaginal/genital/oral swab specimens.

Results are for the identification of the DNA of the aforementioned pathogens, which is generally detectable in urine during the acute phase of infection. Positive results are indicative of the presence of nucleic acids of the aforementioned bacteria. Positive results do not rule out the presence of other bacteria or pathogens.

Negative results do not preclude HPV-16 or HPV-18 infection and should not be used as the sole basis for presence of the aforementioned pathogens.

The LabTurbo AIO Human papillomavirus (HPV) PCR Detection Reagents are intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Summary and pathogen explanation:

Introduction:

Testing for HPV-16 and HPV-18 is a pivotal component of cervical cancer prevention. These high-risk human papillomavirus (HPV) strains are strongly associated with the development of cervical and anogenital cancers. Detecting the presence of HPV-16 and HPV-18 through specialized tests, such as HPV DNA testing, plays a vital role in early intervention, risk assessment, and monitoring. This handbook provides essential information on the significance of HPV-16 and HPV-18 testing for healthcare professionals and individuals seeking to understand the importance of early detection in preventing cervical cancer.

Prevalence of HPV-16 and HPV-18:

In the United States, HPV-16 and HPV-18 are among the most prevalent high-risk HPV types, with HPV-16 being the most commonly associated type with cervical cancer. The Centers for Disease Control and Prevention (CDC) recognizes the critical role of HPV-16 and HPV-18 in a significant proportion of cervical cancers nationwide. The prevalence of these high-risk HPV types may vary across different demographics and age groups. Therefore, public health initiatives have emphasized the importance of vaccination and screening programs to reduce the incidence of cervical cancer associated with these HPV strains.

In the United States, HPV-associated cancers also affect men, particularly oropharyngeal cancers (cancers of the back of the throat, including the base of the tongue and tonsils). These cancers are a significant health concern, with approximately 46,711 new cases diagnosed each year in the U.S. In this context, HPV is responsible for about 37,000 of these cancer cases, with 25,689 among women and 21,022 among men.

HPV-Associated Cancers:

HPV-associated cancers are specific cellular types of cancer commonly diagnosed in regions of the body where HPV is frequently found. These areas include the cervix, vagina, vulva, penis, anus, rectum, and oropharynx (back of the throat, including the base of the tongue and tonsils). They encompass various cellular types, such as carcinomas of the cervix and squamous cell carcinomas of the vagina, vulva, penis, anus, rectum, and oropharynx. Early detection through HPV testing can significantly impact the prevention and management of these cancers.

Number of HPV-Associated and Estimated Number of HPV-Attributable Cancer Cases per Year

Cancer site	Average number of cancers per year in sites where HPV is often found (HPV-associated cancers)	Percentage probably caused by any HPV type	Estimated number probably caused by any HPV type
Cervix	11,869	91%	10,800
Vagina	875	75%	700

Cancer site	Average number of cancers per year in sites where HPV is often found (HPV-associated cancers)	Percentage probably caused by any HPV type	Estimated number probably caused by any HPV type
Vulva	4,238	69%	2,900
Penis	1,364	63%	900
Anusb	7,560	91%	6,900
Female	5,150	93%	4,800
Male	2,410	89%	2,100
Oropharynx	20,805	70%	14,800
Female	3,557	63%	2,300
Male	17,248	72%	12,500
TOTAL	46,711	79%	37,000
Female	25,689	84%	21,500
Male	21,022	74%	15,500

Symptoms:

Infection with HPV-16 and HPV-18 typically does not result in immediate symptoms. Most HPV infections, including these high-risk types, are asymptomatic. However, persistent infection with HPV-16 and HPV-18 can lead to changes in cervical cells that are precancerous. If left untreated, these changes can progress to cervical cancer. While the presence of HPV-16 or HPV-18 does not produce immediate symptoms, they significantly elevate the risk of developing cervical or other anogenital cancers. Regular screening, early detection, and vaccination are crucial for reducing the potential impact of these high-risk HPV types on women's health in the United States.

Reference

1. HPV and Cancer – Centers for Diseases Control and Prevention
2. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 90: Human papillomaviruses. Lyon, France: International Agency for Research on Cancer, World Health Organization; 2007.
3. Shiels MS, Kreimer AR, Coghill AE, Darragh TM, Devesa SS. Anal cancer incidence in the United States, 1977–2011: distinct patterns by histology and behavior. Cancer Epidemiology, Biomarkers and Prevention 2015;24:1548–1556.

Warnings and limitations:

· LabTurbo AIO Human papillomavirus (HPV) PCR Detection Reagents are for research use only.
· This test has not been FDA cleared.
· This test is only for the detection of nucleic acid from the HPV-16 and HPV-18, not for any other viruses or pathogens.
· Negative results do not preclude infection with the aforementioned pathogens and should not be used as the sole basis for bacterial identification.
· Positive detections are indicative of the presence of nucleic acids of the aforementioned pathogens.
· Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
· All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The laboratory personnel should receive professional training (including sample processing, reagent preparation, instrument operation, and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratories issued by local regulatory agencies.
· The laboratory should have dedicated reagent preparation area, sample preparation area, and PCR area. Each area should be used solely for its own purpose to avoid contamination.
· In all test procedure, well laboratory and system operation training for laboratory personnel are essential to guarantee the test accuracy and safety. Please read the manual carefully before the experiment.
· Specimen sampling and processing should be performed according to the recommendation of local regulation. Sample processing shall be carried out in the biosafety cabinet to protect the safety of operators and prevent environmental contamination.
· RNase / DNase free pipette tips and water are recommended in all test procedure.
· Avoid swallowing or contacting skin and eyes with Proteinase K/Lysis/Wash Buffer. In case of accidental swallowing or contact, please rinse with tap water and seek medical care.

Principle and Methods:

The LabTurbo AIO Human Papillomavirus (HPV) PCR Detection Reagents are for multiplex real-time polymerase chain reaction (qPCR) test. The primer and probe set is designed to detect DNA from the Human papillomavirus (HPV) type 16 and 18 in human vaginal/genital/oral specimens. The endogenous control RNaseP is used as an extraction control to validate successful extraction nucleic acid from specimens and is used as an internal control to validate successful qPCR reaction in each well.

The full automation method was established by using LabTurbo AIO SP-qPCR System (LabTurbo AIO), LabTurbo stool DNA Mini kit (LSD480-400) for nucleic acid extraction and LabTurbo AIO Human Papillomavirus (HPV) PCR Detection Reagents for qPCR assay.

The LabTurbo AIO Human Papillomavirus (HPV) PCR Detection Reagents can be used with commercial extraction kits for nucleic extraction from human vaginal/genital/oral swab specimen and commercial real-time PCR systems with FAM, HEX/VIC, and Cy5 detection channels.

Material and Equipment for Full Automation Method:

Equipment:

Nucleic acid extraction	PCR reaction setup liquid-handling	Real-time PCR
LabTurbo SP-qPCR All-in-one system (LabTurbo AIO 48)		

Kits:

Kits	
Nucleic acid extraction	LabTurbo stool DNA mini kit (LSD480-400)
PCR kit	LabTurbo AIO Human Papillomavirus (HPV) PCR Detection Reagents (AHPV1309611)
PCR reaction	8-strip PCR white tubes and clear caps

Kit Content:

LabTurbo AIO Human Papillomavirus (HPV) PCR Detection Reagents (AHPV1309611)		
Component	Description	Quantity
Primer/Probe mixture	For recognization of the specific HPV nucleic acid targets.	1 X 250 µl (20 X)
PCR Master Mix (MM)	For polymerase chain reaction	1 X 1250 µl (2 X)
RNase-free water	For PCR master mix preparation	1 x 1000 µl
HPV Positive Control (PC HPV)	Positive control for PCR reaction qualification	1 x 50 µl
Negative Control (NC)	Negative control for PCR reaction qualification	1 x 50 µl

Detection target and fluorophore:

Well No.	Fluorophore	Pathogen type	Measurand
	Cy5	Internal control	RNaseP

Well No.	Fluorophore	Pathogen type	Measurand
1	HEX	DNA virus	HPV-18
	FAM	DNA virus	HPV-16

Controls used for LabTurbo AIO HPV testing method:

1. A “no template” negative extraction control (NEC) is needed to validate reactions from cross contamination and non-specific signal during extraction and qPCR and is used in each batch of qPCR reaction.
2. An endogenous internal control (IC) RNaseP gene is used to validate the extraction and qPCR reaction performance and is used to validate the integrity of the specimen.
3. A “no template” (negative) control (NC) is needed to validate reactions from cross contamination and non-specific signal in qPCR procedure and is used for troubleshooting when NEC is invalid in testing.
5. A positive template control (PC) is needed to validate the correctness of reagent in qPCR procedure.

Reagent preparation guide:

Extraction reagent preparation:

For the use of LabTurbo LSD480-400 kit on LabTurbo AIO SP-qPCR 48 system, follow the step below for extraction reagent preparation.

Buffer LW1: add 230 ml of absolute ethanol to 175 ml of concentrated LW1 buffer bottle. Mix by inverting 10 times.

All the other reagents provided in the kit are ready to use.

Master mix preparation:

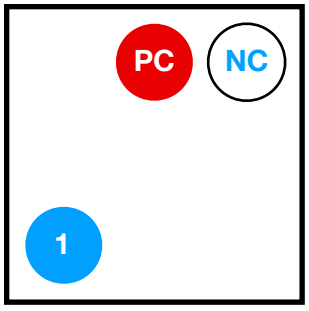
Follow the table below for preparing the master mixes.

Component	Multiplex HPV pathogen detection	Volume for n samples
2X PCR Master Mix	12.5 µl	(n+2) x 12.5 ul
Primer/probe mixture	2.5 µl	(n+2) x 2.5 ul
Nuclease-free water	4.0 ul	(n+2) x 4.0 ul
Total Target Tube I Mixture	19.0 µl	(n+2) x 19.0 ul
Extract volume added to each reaction	6.0 ul	
Total volume for each reaction	25 ul	

Prepare 2 additional reactions for system operating dead volume. Place the prepared master mixes in 1.5 ml screw cap tubes for the use on LabTurbo AIO 48 system.

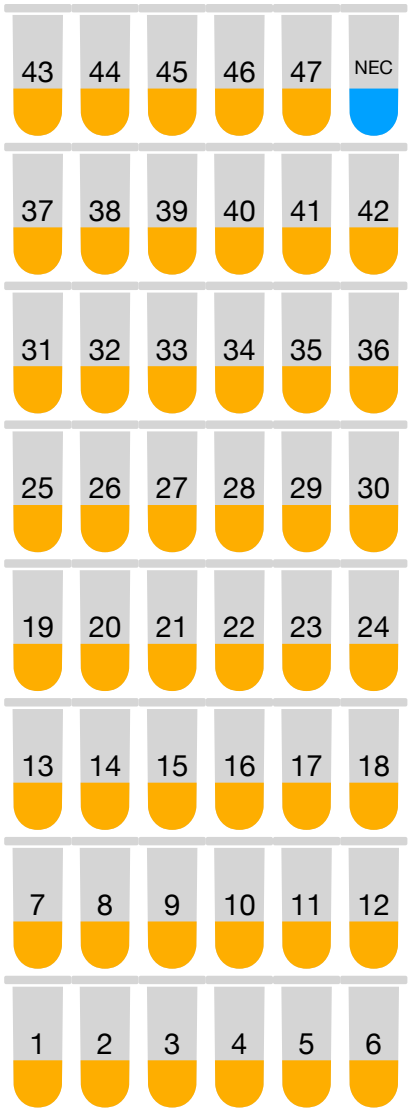
Workflow for running LabTurbo Human papillomavirus (HPV) PCR Detection Reagents using LabTurbo SP-qPCR AIO 48 system:

1. On the system monitor, select the sample number and the LabTurbo HPV Diseases Panel procedure.
2. Place filtered tips, 6-trip sample tubes, 6-strip column sets, 6-strip elution tubes, 8-strip PCR caps and tubes on the system worktable following the instruction on the monitor.
3. Place extraction reagents RLL, CCEB, EtOH and reconstituted LW1 on the LabTurbo AIO 48 system door, and place the Proteinase K vial on the PK rack on the worktable.
4. Place the prepared master mix vial and the PC and NC vials on the LabTurbo 9-well rack matching the following layout:



5. For each dry swab specimen, add 1 ml of buffer CCEB into the swab and vortex for 30 seconds. For swab with preservation medium, transfer 400 ul of the medium for the testing.
6. Place the swab specimen on the worktable and key in the specimen barcode. Place the samples on the system thermal block following the layout as shown on the right.
7. Click “Start” to initiate the Sexually Transmitted Diseases Panel pathogen nucleic acid extraction and PCR detection.

LabTurbo AIO48 sample layout:



Extraction setting:

Urine specimen input volume: 400 ul
Elution volume: 160 ul

PCR setup liquid-handling setting:

Master mix volume: 19 ul
Extracted DNA volume: 6 ul

PCR Thermal profile setting:

	Activation	Denature	Anneal & elongation
Temperature (⁰C)	95	95	60
Time (sec)	60	10	20
Cycle (s)	1	45	

PCR well layout on LabTurbo AIO software for 1 sample:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	1	NEC	PC	NC												
B																
C																
D																
E																
F																
G																
H																
I																

PCR well layout on LabTurbo AIO software for 47 samples:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	1	2	3	4	5	6	7	8								
B	9	10	11	12	13	14	15	16								
C	17	18	19	20	21	22	23	24								
D	25	26	27	28	29	30	31	32								
E	33	34	35	36	37	38	39	40								

F	41	42	43	44	45	46	47	NEC								
G	PC	NC														
H																
I																

Results interpretation:

The NEC control is specifically designed for validating the whole procedure of the assay. If the NEC is invalid, the provided PC and NC can be used for further trouble-shooting in order to inspect all of the possible compromises that could happen in the assay.

1. Negative Extraction Control (NEC): The NEC is the RNase-free water used in molecular biology applications. One reaction is needed for each batch of the assay. All amplification curves in NEC reactions should be negative or with Ct value higher than 37 except the internal control signal. The internal control signal should have positive Cy5.5 detection. If NEC is invalid, it might be due to cross-contamination during handling, improper setup of qPCR assay or degradation of probe. The batch of assay is considered invalid and the whole batch should be repeated from extraction.
2. Endogenous Internal Control (IC): The endogenous human RNaseP gene in each sample serves as an internal control for each specimen. The Primer/Probe mixture reagent should react with the RNaseP gene in each specimen to generate **a positive Cy5 detection for each well** (Ct value should be below 37). If IC is invalid, re-extract the specimen.
3. Negative Control (NC): The NC is the RNase-free water used in molecular biology applications. NC is needed in each batch of testing. All amplification curves in NC reactions should be negative or with Ct values higher than 37. If NC is invalid, it might be due to cross-contamination during handling, reagent preparation, improper setup of qPCR assay or degradation of probe. The batch of assay is considered invalid and the whole batch should be repeated from PCR.
5. Positive Control (PC): The PC is the positive control provided in the LabTurbo AIO HPV testing kit. It is needed for each batch of the assay. The Primer/Probe mixture reagent should react with the PC and generate a positive detection for each target (Ct values should be below 37). If the PC generates a positive result, the reagents and RT-qPCR setup are correct.

Expected control results:

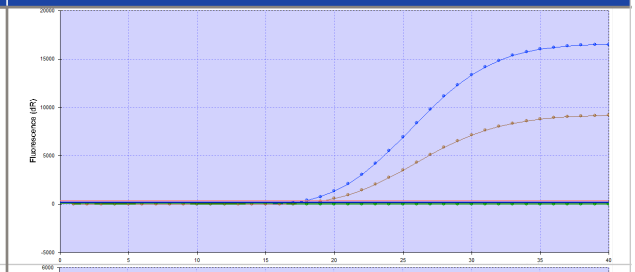
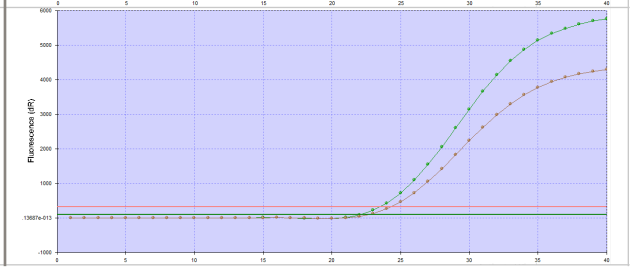
Control type	Control Target	Used to monitor	Expected Ct values
Negative Extraction Control (NEC)	-	Non-specific signal and Cross-contamination during DNA extraction, qPCR reaction setup and qPCR	Not detected Or >37.00 Ct for all pathogenic targets. Detected for internal control target.
Endogenous internal control (IC)	RNaseP	Correct extraction and qPCR efficiency	<37.00 Ct
Negative control (NC)	-	Non-specific signal and Cross-contamination in qPCR reaction setup	Not detected Or >37.00 Ct

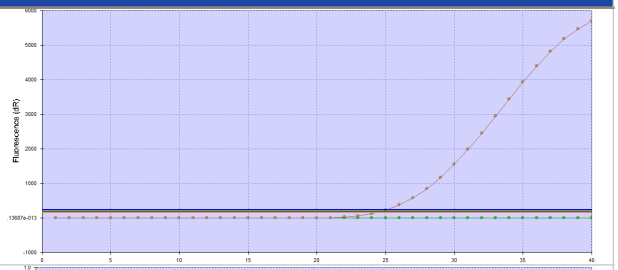
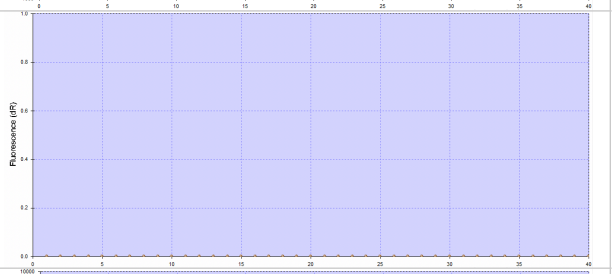
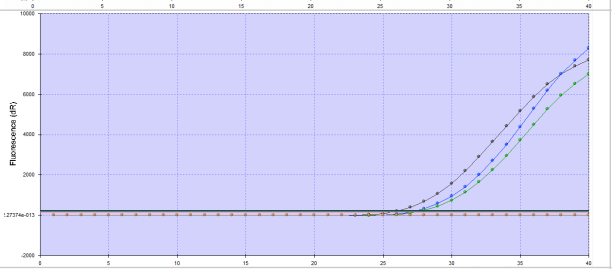
Control type	Control Target	Used to monitor	Expected Ct values
Positive Control (PC)	HPV-16, HPV-18 and RNaseP	Correct PCR reagent, PCR setup, and qPCR performance	<37.00 Ct

Expected sample results:

Pathogen targets (FAM or HEX)	Endogenous internal control (Cy5)	Negative extraction control and negative control (NEC & NC)	Result	Interpretation and action
+ (Ct < 37)	+ (Ct < 37)	- (Ct > 37)	Valid	**One or multiple HPV pathogen(s) detected**
- (Ct > 37)	+ (Ct < 37)	- (Ct > 37)	Valid	**HPV pathogen not detected**
- (Ct > 37)	- (Ct > 37)	- (Ct > 37)	Invalid	**Repeat extraction and qPCR for the failed sample.**
+ (Ct < 37)	- (Ct > 37)	- (Ct > 37)	Valid	**One or multiple HPV pathogen(s) detected. The pathogen signal may out-compete the pathogen signals for qPCR.**

Sample results example:

Fluorefore	Positive Pathogen detection
FAM+Cy5	
HEX+Cy5	

Fluorefore	Positive Pathogen detection
NEC	
NC	
PC	

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