LabTurbo

LabTurbo AIO Sexually Transmitted Diseases Panel PCR Detection Reagents User Manual

Catalog number: ASTD1309611

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

Intended use:

The LabTurbo AIO Sexually Transmitted Diseases Panel PCR Detection Reagents PCR Detection Reagents are intended for the presumptive qualitative detection of nucleic acid from the Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Herpes virus 1, Herpes virus 2, Treponema pallidum, Trichomonas vaginalis and Haemophilus ducreyi, in human urine 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 Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Herpes virus 1, Herpes virus 2, Treponema pallidum, Trichomonas vaginalis, and Haemophilus ducreyi, infection and should not be used as the sole basis for presence of the aforementioned pathogens.

The LabTurbo AIO Sexually Transmitted Diseases Panel 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:

Gonorrhea is a sexually transmitted disease (STD) caused by infection with the Neisseria gonorrhoeae bacterium. N. gonorrhoeae infects the mucous membranes of the reproductive tract, including the cervix, uterus, and fallopian tubes in women, and the urethra in women and men. N. gonorrhoeae can also infect the mucous membranes of the mouth, throat, eyes, and rectum. Gonorrhea is a very common infectious disease. CDC estimates that approximately 1.6 million new gonococcal infections occurred in the United States in 2018, and more than half occur among young people aged 15-24. Gonorrhea is the second most commonly reported bacterial sexually transmitted infection in the United States. However, many infections are asymptomatic, so reported cases only capture a fraction of the true burden.

Chlamydia is a common STD caused by infection with Chlamydia trachomatis. It can cause cervicitis, urethritis, and proctitis. In women, these infections can lead to pelvic inflammatory disease (PID), tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Lymphogranuloma venereum (LGV) is another type of STD caused by C. trachomatis. LGV is the cause of recent proctitis outbreaks among gay, bisexual, and other men who have sex with men (MSM) worldwide. CDC estimates that there were four million chlamydial infections in 2018.3 Chlamydia is also the most frequently reported bacterial sexually transmitted infection in the United States. It is difficult to account for many cases of chlamydia. Most people with the infection have no symptoms and do not seek testing. Chlamydia is most common among young people. Two-thirds of new chlamydial infections occur among youth aged 15-24 years. Estimates show that 1 in 20 sexually active young women aged 14-24 years has chlamydia. Disparities persist among racial and ethnic minority groups. In 2020, chlamydia rates for African Americans/Blacks were six times that of Whites. Chlamydia is also common among MSM. Among MSM screened for rectal chlamydial infection, positivity ranges from 3.0% to 10.5%. Among MSM screened for pharyngeal chlamydial infection, positivity has ranges from 0.5% to 2.3%.

Mycoplasma genitalium (or Mgen) is a sexually transmitted bacterium that can cause reproductive tract infections of the penile urethra or cervix. Mgen causes symptomatic and asymptomatic urethritis. It may also play a role in cervicitis, pelvic inflammatory disease (PID), preterm delivery, spontaneous abortion, and infertility. The 2017-2018 National Health and Nutrition Examination Survey estimates overall prevalence of urogenital Mgen to be 1.7% among people aged 14-59 years in the United States. The survey reported similar prevalence between males at 1.8% and females at 1.7%. Reported estimates of Mgen prevalence among clinic-based populations are higher. Among men presenting with urethritis in select STI clinics, 28.7% were positive for Mgen. Among women presenting in an STI clinic in Seattle, 26% had an Mgen infection. A large U.S. prospective multicenter study of a nucleic acid amplification diagnostic test for Mgen including male and female patients seeking care in diverse geographic regions found overall prevalence to be 10.3%. In this study, Mgen was more common among people ages 15 to 24 years than in people ages 35 to 39 years. The risk for Mgen was higher in Black participants than White participants and higher in non-Hispanic than in Hispanic participants. A meta-analysis of Mgen prevalence among gay, bisexual, and other men who have sex with men (MSM) found that urethral (5.0%) and rectal (6.2%) Mgen infections were more common than pharyngeal infections (1.0%).

Genital herpes is a sexually transmitted disease (STD) caused by the herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2). Genital herpes infection is common in the United States. CDC estimated that there were 572,000 new genital herpes infections in the United States in a single year. Nationwide, 11.9 % of persons aged 14 to 49 years have HSV-2 infection (12.1% when adjusted for age). However, the prevalence of genital herpes infection is higher than that because an increasing number of genital herpes infections are caused by HSV-1. Oral HSV-1

infection is typically acquired in childhood; because the prevalence of oral HSV-1 infection has declined in recent decades, people may have become more susceptible to contracting a genital herpes infection from HSV-1. HSV-2 infection is more common among women than among men; the percentages of those infected during 2015-2016 were 15.9% versus 8.2% respectively, among 14 to 49 year olds. This is possibly because genital infection is more easily transmitted from men to women than from women to men during penile-vaginal sex. HSV-2 infection is more common among non-Hispanic blacks (34.6%) than among non-Hispanic whites (8.1%). A previous analysis found that these disparities, exist even among persons with similar numbers of lifetime sexual partners. Most infected persons may be unaware of their infection; in the United States, an estimated 87.4% of 14 to 49 year olds infected with HSV-2 have never received a clinical diagnosis. The age-adjusted percentage of persons in the United States infected with HSV-2 decreased from 18.0% in 1999–2000 to 12.1% in 2015-2016. 2

Syphilis is a sexually transmitted disease (STD) caused by the bacterium Treponema pallidum. Syphilis can cause serious health effects without adequate treatment. Syphilis case reports continue to increase since reaching a historic low in 2000 and 2001. During 2020, there were 133,945 new cases of syphilis (all stages). Men who have sex with men (MSM) are experiencing extreme effects of syphilis. They account for 43 percent of all primary and secondary syphilis cases in the 2020 STD Surveillance Report. They also account for 53 percent of all male P&S cases. However, case rates are increasing among heterosexual men and women in recent years. Congenital syphilis continues to be a concern in the United States. Congenital syphilis occurs when a pregnant person passes syphilis to their baby. Preliminary 2021 data show more than 2,100 cases of congenital syphilis.

Trichomoniasis (or “trich”) is a very common STD caused by infection with Trichomonas vaginalis (a protozoan parasite). Although symptoms vary, most people who have trich cannot tell they have it. In the United States, CDC estimates that there were more than two million trichomoniasis infections in 2018. However, only about 30% develop any symptoms of trich. Infection is more common in women than in men. Older women are more likely than younger women to have the infection.

Chancroid is caused by the bacterium Haemophilus ducreyi and results in painful, superficial ulcers, often with regional lymphadenopathy. Chancroid occurs in Asia, Africa, and the Caribbean, and is an important cofactor of HIV transmission. The genital ulcer from chancroid is painful, tender, and nonindurated. Symptoms usually occur 4-10 days after exposure. The lesion at the site of infection is, initially, a pustule that breaks down to form a painful, soft, ulcer with a necrotic base and irregular borders. Multiple lesions and inguinal adenopathy often develop. With lymph node involvement, fever, chills, and malaise may also develop. Other symptoms of chancroid include painful urination, vaginal discharge, rectal bleeding, pain with bowel movements, and dyspareunia.

Reference

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Warnings and limitations:

· LabTurbo AIO Sexually Transmitted Diseases Panel 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 Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Herpes virus 1, Herpes virus 2, Treponema pallidum, Trichomonas vaginalis, and Haemophilus ducreyi, 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 Sexually Transmitted Diseases Panel 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 Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Herpes virus 1, Herpes virus 2, Treponema pallidum, Trichomonas vaginalis, and Haemophilus ducreyi in urine specimens from human urine specimens. The BEEC is used as an extraction control to validate successful extraction nucleic acid from urine 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 Sexually Transmitted Diseases Panel PCR Detection Reagents for qPCR assay.

The LabTurbo AIO Sexually Transmitted Diseases Panel PCR Detection Reagents can be used with commercial extraction kits for nucleic extraction from human urine specimen and commercial real-time PCR systems with FAM, HEX or VIC, Texas Red or ROX, and Cy5.5 or Quasar 705 detection channels.

Material and Equipment for Full Automation Method:

Equipment used for testing:

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

Kits used for testing:

Kits	
Nucleic acid extraction	LabTurbo stool DNA mini kit (LSD480-400)
PCR kit	LabTurbo AIO Sexually Transmitted Diseases Panel PCR Detection Reagents (ASTD1309611)
PCR reaction	8-strip PCR white tubes and clear caps

Kit Content:

LabTurbo STD DNA Testing Kit		
Component	Description	Quantity
Primer/Probe mixture ST1	For identification of Mycoplasma genitalium and Trichomonas vaginalis DNA	4 X 105 µl (20 X)
Primer/Probe mixture ST2	For identification of Neisseria gonorrhoeae and Chlamydia trachomatis DNA	4 X 105 µl (20 X)
Primer/Probe mixture ST3	For identification of Herpes virsu 1 and Herpes virus 2 DNA	4 X 105 µl (20 X)
Primer/Probe mixture ST4	For identification of Treponema pallidum and Haemophilus ducreyi DNA	4 X 105 µl (20 X)
PCR Master Mix (MM)	For polymerase chain reaction	8 X 1050 µl (2 X)
RNase-free water	For PCR master mix preparation	4 x 700 µl
BE Extraction Control (BEEC)	Extraction and internal control	4 x 20 µl
STD Positive Control (PC STD)	Positive control for PCR reaction qualification	4 x 205 µl
Negative Control (NC)	Negative control for PCR reaction qualification	4 x 205 µl

Detection target and fluorophore:

Well No.	Fluorophore	Pathogen type	Measurand
1	Cy5.5	Internal control	BEEC
	HEX	-	-
	FAM	Gram-positive bacteria	Mycoplasma genitalium
	TEXAS RED	Protozoan	Trichomonas vaginalis
2	Cy5.5	Internal control	BEEC
	HEX	Gram-negative bacteria	Neisseria gonorrhoeae
	FAM	Gram-negative bacteria	Chlamydia trachomatis
	TEXAS RED	-	-
3	Cy5.5	Internal control	BEEC
	HEX	double-stranded DNA virus	Herpes virus 2
	FAM	double-stranded DNA virus	Herpes virus 1
	TEXAS RED	-	-
4	Cy5.5	Internal control	BEEC
	HEX	-	-
	FAM	Gram-negative bacteria	Treponema pallidum
	TEXAS RED	Gram-negative bacteria	Haemophilus ducreyi

Controls used for LabTurbo AIO STD 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 extraction/internal control (EC/IC) BEEC gene is needed to validate the extraction procedure and is used to validate the performance of qPCR reaction.
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:

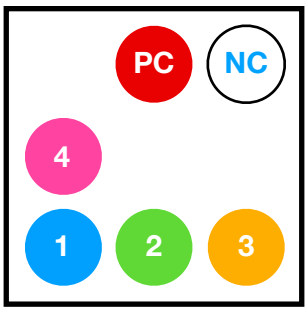
Follow the table below for preparing the 4 master mixes. All 4 master mixes use the same formula below.

Component	Multiplex STD 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	

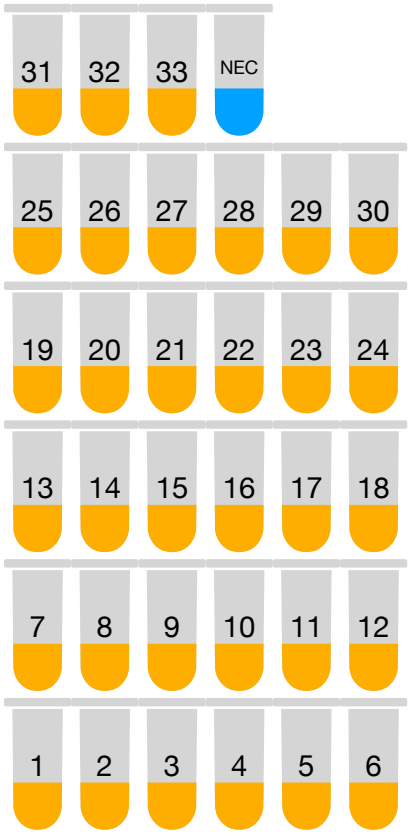
Prepare 2 additional reactions for system operating dead volume. Place the prepared master mixes in 1.5 ml screw cap tubes for the use on LabTurbo AIO 48 system.

Workflow for running LabTurbo Sexually Transmitted Diseases Panel using LabTurbo SP-qPCR AIO 48 system:

1. On the system monitor, select the sample number and the Sexually Transmitted 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 4 master mix vials and the PC and NC vials on the LabTurbo 9-well rack matching the following layout:



5. Place the urine specimen on the worktable and key in the specimen barcode. Place the samples on the system thermal block following the layout below:



6. Click “Start” to initiate the Sexually Transmitted Diseases Panel pathogen nucleic acid extraction and PCR detection.

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 (ºC)	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	1	NEC	PC	NC								
B	1	NEC	PC	NC	1	NEC	PC	NC								
C																
D																
E																
F																
G																
H																
I																

PCR well layout on LabTurbo AIO software for 33 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	1	2	3	4	5	6	7	8
B	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16

C	17	18	19	20	21	22	23	24	17	18	19	20	21	22	23	24
D	25	26	27	28	29	30	31	32	25	26	27	28	29	30	31	32
E	33	NEC	PC	NC	1	2	3	4	33	NEC	PC	NC	1	2	3	4
F	5	6	7	8	9	10	11	12	5	6	7	8	9	10	11	12
G	13	14	15	16	17	18	19	20	13	14	15	16	17	18	19	20
H	21	22	23	24	25	26	27	28	21	22	23	24	25	26	27	28
I	29	30	31	32	33	NEC	PC	NC	29	30	31	32	33	NEC	PC	NC

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. Extraction control/Internal Control (EC/IC): The recombinant BEEC DNA spiked into each sample serves as an extraction/internal control for each specimen. The Primer/Probe mixture reagent should react with the BEEC spiked into the urine specimen to generate **a positive Cy5.5 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.
4. Positive Control (PC): The PC is the positive control provided in the LabTurbo AIO STD 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:

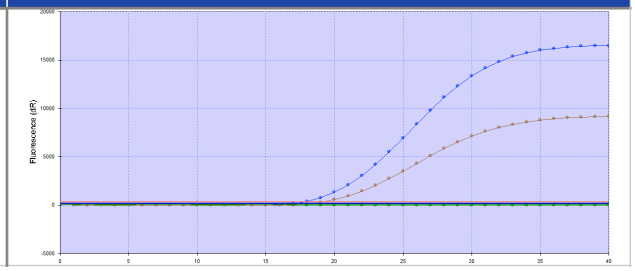
Control type	Control Target	Used to monitor	Expected Ct values
Negative Extraction Control (NEC)	-	Non-specific signal and Cross-contamination during DNA extraction, qPCR reaction setup and qPCR	Not detected Or >37.00 Ct for all pathogenic targets. Detected for internal control target.

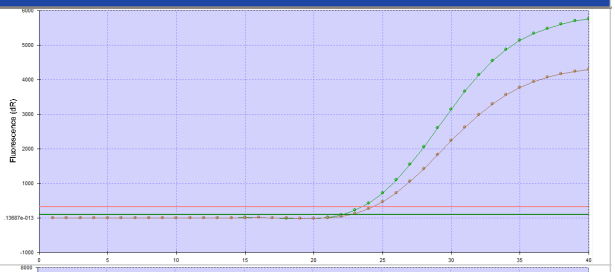
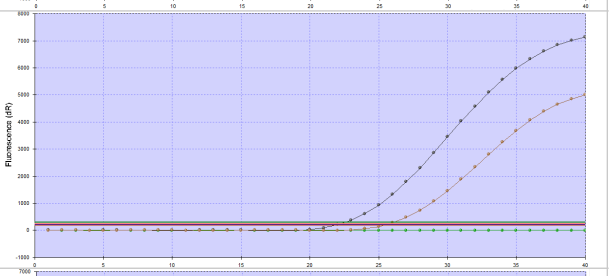
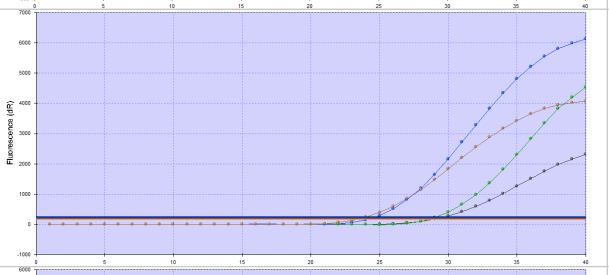
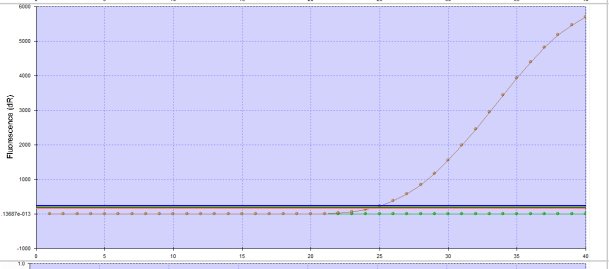
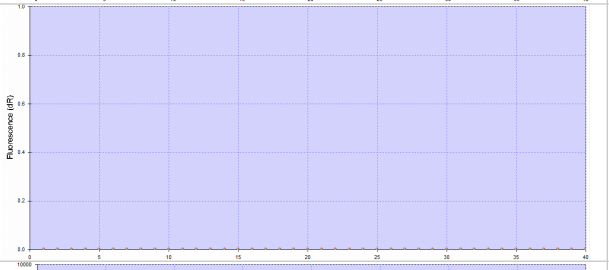
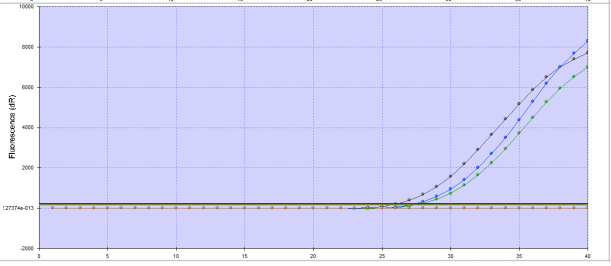
Control type	Control Target	Used to monitor	Expected Ct values
Extraction/Internal control (EC/IC)	BEEC	Correct extraction and qPCR efficiency	<37.00 Ct
Negative control (NC)	-	Non-specific signal and Cross-contamination in qPCR reaction setup	Not detected Or >37.00 Ct
Positive Control (PC)	Each pathogen target of LabTurbo AIO STD testing method	Correct PCR reagent, PCR setup, and qPCR performance	<37.00 Ct

Expected sample results:

Pathogen targets (FAM, HEX, or Texas Red)	Internal/ Extraction contro BEEC (Cy5.5)	Negative extraction control and negative control (NEC & NC)	Result	Interpretation and action
+ (Ct < 37)	+ (Ct < 37)	- (Ct > 37)	Valid	**One or multiple STD pathogen(s) detected**
- (Ct > 37)	+ (Ct < 37)	- (Ct > 37)	Valid	**STD 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 STD pathogen(s) detected. The pathogen signal may out-compete the pathogen signals for qPCR.**

Sample results example:

Fluorefore	Positive Pathogen detection
FAM+Cy5.5	

Fluorefore	Positive Pathogen detection
HEX+Cy5.5	
Texas Red+Cy5.5	
Multi detection	
NEC	
NC	
PC	

Contact information

Manufacturer:
LabTurbo Biotech Corporation
Address: 3F.-1, No. 150, Sec. 4, Chengde Rd., Shilin Dist., Taipei City 111, Taiwan
TEL: +886-2-28891136
FAX: +886-2-28891146
Website: https://labturbo.com
Email: info@labturbo.com

US branch:
LabTurbo Biotech, LLC
Address: 12 Roszel Rd., B102, Princeton, NJ 08540
TEL: +1-609-619-2889
FAX: +1-609-913-2889
Website: https://labturbo.com
Email: info@labturbo.com

Germany office:
LabTurbo Biotech
Address: Johannstraße 37, 3. Etage 40476, Düsseldorf, Germany
TEL: +49 17622867631
Website: https://labturbo.com
Email: info@labturbo.com