Vibrio Cholerae
DNA Detection Kit
(Fluorescence PCR Method)

Σ P321H
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Σ P721H
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FOR RESEARCH USE ONLY!

User Guide

Version 1.0

For use with Real-time PCR Instruments compatible with Vibrio Cholerae DNA Detection Kit (Fluorescence PCR Method)

REF P321H/P721H

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Introduction

Vibrio cholerae is a gram-negative facultative anaerobic bacillus in the family Vibrioceae. Its ecological environment is mainly located in coastal waters and estuaries. According to the variation of its O antigen, more than 200 species of Vibrio cholerae have been identified so far. As the pathogen of cholera, a severe intestinal infectious disease, Vibrio cholerae can cause widespread severe infectious diseases and has caused many pandemics around the world. Therefore, it is listed as one of the Class A infectious diseases in international import and export quarantine. According to statistics, 3 million to 5 million people are infected with cholera every year, resulting in 100,000 to 120,000 deaths.

The ***Vibrio Cholerae DNA Detection Kit*** developed by TianLong Biotechnology is intended for the quick, accurate detection of Vibrio Cholerae nucleic acid, assists in the diagnosis and treatment of Vibrio cholerae patients and public healthcare management.

Intended Use

The TianLong ***Vibrio Cholerae DNA Detection Kit*** is intended for the qualitative detection of Vibrio Cholerae nucleic acid by Real-time Polymerase Chain Reaction (Real-time PCR) method.

The test is designed to detect DNA from Vibrio Cholerae in specimens such as human stool or anal swab samples collected from individual personnel based on clinical and/or epidemiological criteria.

Results from the test are for the identification of DNA from Vibrio Cholerae existence in samples tested. The Vibrio Cholerae DNA is mostly often detectable in human stool or anal swab samples collected during the acute phase of infection.

Positive test results are indicative of the presence of Vibrio Cholerae DNA; whereas clinical correlation with patient history and other diagnostic information is necessary for the determination of patient infection status. Positive results from this test do not rule out other bacterial infection or co-infection with viruses.

Negative test results from the test do not completely preclude Vibrio Cholerae carrying and should not be used as the sole basis for patient management decisions. Negative results must be used in combination with clinical observations, patient history, and epidemiological information for a medical decision.

The TianLong ***Vibrio Cholerae DNA Detection Kit*** is intended for use by qualified laboratory professionals trained in the techniques of Real-time PCR and in vitro diagnostic procedures. The TianLong ***Vibrio Cholerae DNA Detection Kit*** is for use in qualified labs which is in compliance with guidelines and regulations from corresponding professional organizations and government administrations.

The ***Vibrio Cholerae DNA Detection Kit*** is to be used with Real-time PCR instruments with 2 or more fluorescence detection channels, which the test performance of the kit has been validated on. These Real-time PCR thermal cyclers have appropriate fluorescence reading channels for FAM, Cy5 channels such as Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier Real-time PCR Systems.

Principles of the Assay

A pair of Vibrio Cholerae specific primers are selected and combined with specific probes. The probes can specifically bind to a DNA template in the middle of the primer amplification region. In the process of PCR extension, the exonuclease activity of Taq enzyme will cut off the 5'end fluorescent groups from the probes, freeing them in the reaction system, thus breaking away from the shielding of the 3'end fluorescence quenching groups, which can receive light stimulation and emit fluorescence that can be detected by the instrument, this kit uses FAM channel, so as to achieve the automatic detection of Vibrio Cholerae nucleic acid in closed reaction system.

This kit was designed with a synthetic, non-competitive sequence as an internal control that does not interfere with the target gene of Vibrio Cholerae. This sequence was entered into the NCBI website for BLAST comparison

analysis, which confirmed that this sequence could not be found in the NCBI nucleic acid library. The primer and probe were designed based on this internal control, and the internal control was detected at Cy5 wavelength, thus enabling monitoring of the detection process in a fully closed reaction system, which can effectively monitor the occurrence of false negatives.

Reagent Kit

Reagent Kit Components

Reagents for 25/50 tests (PCR reactions) are contained in one reagent kit box.

PCR Reagents	Volume (25 T/50 T)	In Tube (25 T/50 T)
REAG 1	375 µL/750 µL	1 tube/1 tube
REAG 2	250 µL/500 µL	1 tube/1 tube
Controls		
CONTROL +	40 µL/40 µL	1 tube/1 tube
CONTROL -	40 µL/40 µL	1 tube/1 tube

Note: Mix matching and use of the reagent components from different reagent lots should be avoided unless be specifically instructed to do so. The negative control could also be referred to as a "No Target Control" (NTC).

Reagent Storage, Shipment, and Handling

All reagents should be stored at the temperature between -25 °C to -15 °C in a non-frost-free freezer for use before the expiration date. Freeze/Thaw more than three times should be avoided during the kit usage period. The reagents should be shipped at the temperature between -25 °C to 8 °C.

Assay Procedures

Before Starting

- Check reagent components and supplies to ensure that there are enough materials ready for planned work.
- Check to ensure that equipment and instruments are ready for work.
- Follow the up-to-date instructions for use.
- Complete appropriate planning and calculations for coming testing.
- Finally, complete the testing procedures as outlined below.

Sample Requirements

The ***Vibrio Cholerae DNA Detection Kit*** is designed to detect DNA from Vibrio Cholerae nucleic acid in specimens such as human stool or anal swab samples collected from individuals based on clinical and/or epidemiological criteria.

Use the specimen collection, transportation, storage medium following reagent manufacturer instructions. Perform nucleic acid extraction following reagent manufacturer instructions.

Equipment and Instruments Required but not Provided

- Micropipette dedicated for assay setup (1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Refrigerated benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer.
- It is recommended to use a detection kit with Real-time PCR thermal cyclers with appropriate fluorescence reading channels for FAM and Cy5 dyes such as Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier Real-time PCR systems.

Note:
Equipment and instruments should be maintained and calibrated according to the manufacturer's recommendations.
Refer to manufacturer's manuals for operation procedures.

Nucleic Acid Extraction

TianLong ***Vibrio Cholerae DNA Detection Kit*** is compatible with DNA /nucleic acids of adequate quality prepared from intended samples using common DNA/nucleic acid extraction kits/methods. The prepared DNA/nucleic acids can be used directly as sample DNA/nucleic acid material, moved forward to the Real-time PCR reaction setup step. We recommend add 10 µL internal control (***RAGE 2***) to each 200 µL sample and extract together when extracting nucleic acid of samples.
Positive Control and Negative Control do not need to be extracted and tested directly in each Real-time PCR assay Run.
If under certain circumstances prepared DNA/nucleic acids need to be frozen stored for a later time testing, storage in a freezer of -70 °C or lower is recommended whenever possible for minimal nucleic acid degradation during storage.

Repeated Freezing/Thawing of prepared sample DNA/nucleic acids should be avoided whenever possible.

Real-time PCR Reaction Setup

1. Thaw the following reagents on ice: ***REAG 1***. Gently invert to mix the reagent, then briefly centrifuge (2000 rpm, 10 s) to let solutions be settled to the bottom of tubes before moving to next step.
2. Prepare Master MIX based on the planned number of samples to be tested.
   To calculate the volume of each reagent component required for Master MIX preparation, it needs to cover all the samples and controls to be tested in the assigned assay Run with reasonable extra set aside for operational tolerance.
   In many cases, preparing Premix with 10-20% extra volume is a good practice.
3. 96-well PCR reaction plates or PCR reaction tube stripes could be used for reaction setup. Evenly aliquot 15 µL of the prepared Premix into each PCR tube. Add 10 µL of each extracted DNA nucleic acid solution to the designated PCR tube. Add 10 µL of CONTROL + and CONTROL - to the respectively assigned tubes.
   At the end of setup, each PCR tube shall have a total volume of 25 µL.
   Then immediately close/cover the tubes and transfer the reaction setup tube stripes/plate into a Real-time PCR cycler for amplification reactions.

   Positive control and negative control must be run in each assay Run.

Thermal Cycler Settings

Real-time PCR cycling program:

Table 2 qPCR Cycling program

Stage	No. of cycles	Temperature	Duration
1	1	50 °C	2 min
2	1	95 °C	20 s
3	42	95 °C	2 s
		60 °C	12 s (fluorescence detection)

Assignment for Fluorescence Detection Channels:

- FAM channel for Vibrio Cholerae
- Cy5 channel for internal control

Result Analysis and Interpretation

For data analysis, a Fluorescence Threshold Setting needs to be assigned.

- Auto Setting: the instrument automatically sets the threshold value. Auto Setting is recommended for routine operations and data analysis.
- Manual Setting: in case the manual setting is desired under certain circumstances, the threshold value could be set just above the fluorescence baseline of the normal negative control.

Run Validity Check

All tests performed on one batch setup through the whole course of Real-time PCR considered in one Run. Only results from valid test runs are moved forward for analysis and interpretation.

Test Run is valid when

1) In the run, there is no Ct generated for FAM, Cy5 (IC) channels from the negative control.

2) Ct value for FAM channel from the positive control is less than 30 and no Ct curve for Cy5 (IC) channel.

3) The internal control Ct value of the test sample should be < 42; If the internal control (Cy5) detected no value during the test, the cause should be identified and the sample should be tested repeatedly.

Results from valid test runs could be further analyzed for reports.

If results from controls do not meet the validity criteria as indicated in 1), 2) and 3), then the test run is usually considered invalid, all samples involved in the test run need to be retested for reportable results.

If the target Ct values for FAM channel generated from negative control reactions repeatedly below to 42, it is implicated that amplicon contamination may be present in the working environment. Replacement of opened reagent components, and comprehensive working area cleaning and troubleshooting for contamination should be performed.

The analysis and interpretation of test results

1. If the Ct values of FAM channel are ⩽38, **the result can be considered Vibrio Cholerae nucleic acid Positive**.
2. If the Ct values of the FAM channel are >42, **the result can be considered Vibrio Cholerae nucleic acid Negative**.
3. If the Ct value of FAM channel is between 38 and 42, **the sample should be retested**.
   If the retest result shows that the Ct values are still between 38 and 42, **the result can be considered Vibrio Cholerae nucleic acid Positive**. Otherwise, **the result can be considered Vibrio Cholerae nucleic acid Negative.**

Table 3 The interpretation of the test results with Vibrio Cholerae DNA Detection Kit

Condition	FAM	Cy5 (IC)	Result
1	Ct⩽38	Ct＜42	**Result can be considered Vibrio Cholerae nucleic acid Positive**
2	NO Ct Value or Ct=42	Ct＜42	**Result can be considered Vibrio Cholerae nucleic acid Negative**
3	38<Ct<42	Ct＜42	**Retested** If retest result indicates target of FAM Ct<42, the **result can be considered Vibrio Cholerae nucleic acid Positive**.
4	NO Ct Value or Ct=42	NO Ct Value or Ct=42	**Invalid** Need to be reviewed and retested.

Assay Performance Characteristics

The following performance characteristics of the TianLong ***Vibrio Cholerae DNA Detection Kit*** have been established as described below.

Non-clinical Studies

- Limit of detection:
  500 copies/mL.
- Specificity:
  No cross-reactivity with other common pathogens and human genomes that share the same site of infection or have similar infection symptoms.
- Precision:
  The assay was used to respectively detect the precise reference specimens of high and low concentrations in different time ranges for 10 times, and the precision values of intra and inter Ct values were all <5%.

Quality Control

In accordance with the ISO 13485:2016 Medical devices— Quality management systems and TianLong ***Vibrio Cholerae DNA Detection Kit*** Quality Control Program, each batch of the ***Vibrio Cholerae DNA Detection Kit*** is tested against predetermined specifications to ensure consistent product quality.

Limits and Precautions

Limits

- All reagents in the kit are intended for in vitro diagnostic use as indicated.
  The test should be carried out by professionals adequately trained in IVD lab practices. It is the user's responsibility to verify/validate the testing system performance in their respective laboratory settings. Expired reagents should not be used.
- Strict compliance with the IFU is required for optimal results. Deviation from standard procedures during sample collection, preservation, transportation, processing, and testing could lead to false negative or false positive testing results.
- Theoretically, variations in the target sequences of Vibrio Cholerae arise from natural mutations that could potentially influence testing performance and result in false testing results. Up to today, results from bioinformatics analysis and comprehensive laboratory studies indicate that, partially due to the emphasis on mutation tolerance concept during assay design and development, this kit could tolerate currently known Vibrio Cholerae mutations without obvious compromise on assay performance.
- Test results should be used in combination with clinical and epidemic information for medical decisions.

Laboratory Precautions

Laboratories using the assay should be ISO 15189 qualified and/or in compliance with local regulations.

Use extreme caution to prevent:

- **RNase/DNase contamination that may cause degradation of the template RNA/DNA.**
- **Amplicon contamination that may result in false positive test results.**

The following are recommended for desirable test performance:

- Use DNase/RNase-free disposable pipette tips, tubes, and supplies as appropriate.
- A standard PCR Lab Suite under workflow and air pressure control would be desirable for testing use. If not available, separated/segregated working areas could be used with precaution for contamination control:
  1) Reagent preparation area: preparing the reagents for amplification;
  2) Sample preparation area: extraction and separation of the RNA/nucleic acids from samples and controls;
  3) Amplification area: amplification and detection of the nucleic acid target.
- Perform regular decontamination practice and cleaning of working areas, equipment, and instruments. Commercially available cleaning products containing sodium hypochlorite, 75% alcohol, and ultraviolet light could be applied for the purpose of cleaning and decontamination.
- Nucleic acid samples should be stored at -70 °C or lower for long term storage.
- Equipment such as micropipette needs to be calibrated per the manufacturer's recommendation.
- The Real-time PCR instrument needs calibration per manufacturer's schedule.
- The handling and management of samples and lab wastes should be in compliance with relevant guidelines recommended by professional organizations and regulations imposed by authorities.

Symbols

The following table describes the symbols that may appear on the labeling or in this document.

REF	Catalog number
LOT	Batch code
Σ <N>	Contains reagents sufficient for <N> tests
	Use-by date
	Date of manufacture
	Caution
	Temperature limit
	Manufacturer
CE	Conformed with EU standard
EC REP	Authorized representative in the European Community
	Consult instructions for use
	Keep away from sunlight
	Fragile handle with care
CONT	Content of the Kit
REAG 1	P321H/P721H Rx Sol (Vibrio Cholerae Reaction Mixture)
REAG 2	P321H/P721H IC (Internal Control)
CONTROL +	P321H/P721H PC (Vibrio Cholerae Positive Control)
CONTROL -	P321H/P721H NC (Negative Control)
21 PAP	Recycling symbol PAP21: non corrugated cardboard

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