Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit (Fluorescence PCR Method)

User Guide

P726H

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FOR RESEARCH USE ONLY!

DO NOT USE IN DIAGNOSTIC PROCEDURES.

Version 1.1

For use with Real-time PCR Instruments compatible with
Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit (Fluorescence PCR Method)

REF P726H

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Introduction

Influenza viruses are divided into type A (A), type B (B) and type C (C), the first two viruses are most infected by humans. Influenza A Virus is the most common influenza virus in humans. It usually causes seasonal influenza and may also cause influenza pandemics. Influenza A Viruses can also infect animals, such as birds, pigs, and horses. Influenza B Virus infection is basically limited to humans and rarely causes epidemics. Influenza A Viruses can be further divided into different subtypes based on their two surface proteins-hemagglutinin (H) and neuraminidase (N).

A total of 16 subtypes of AIV have been identified, among which subtypes H5 and H7 are highly pathogenic, causing serious economic losses to the poultry industry, and also causing human infection and even death. In addition to the serious harm caused by the highly pathogenic subtype H5 and H7 avian influenza, the harm caused by the low pathogenic subtype H9N2 avian influenza also cannot be ignored.

Therefore, rapid and accurate diagnosis of Avian Influenza Virus IFVA/H5/H7/H9 is very important, the **Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit** developed by TianLong Biotechnology assist in the diagnosis Avian Influenza Virus IFVA/H5/H7/H9 and public healthcare management.

Intended Use

The TianLong ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** is intended for the qualitative detection of Avian Influenza Virus IFVA/H5/H7/H9 nucleic acid by real-time reverse transcription Polymerase Chain Reaction method.

This kit is used for qualitative detection of Avian Influenza Virus IFVA/H5/H7/H9 RNA in nasopharyngeal or oropharyngeal swabs collected from individual personnel based on clinical and/or epidemiological criteria.

Positive test results are indicative of the presence of Avian Influenza Virus IFVA/H5/H7/H9 RNA, whereas clinical correlation with patient history of the affected patients and other diagnostic information is necessary for the determination of the infection status of the affected patients. Positive results from this test do not rule out bacterial infection or co-infection with other viruses.

Negative test results from the test do not completely preclude Avian Influenza Virus IFVA/H5/H7/H9 infection and should not be used as the sole basis for management decisions. Negative results must be used in combination with clinical observations, the affected patients' history, and epidemiological information for a medical decision.

The TianLong ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** is intended for use by qualified clinical laboratory professionals who have received training in the techniques of Real-time PCR and *in vitro* diagnostic procedures. The TianLong ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** is intended for use in qualified clinical laboratories in accordance with applicable professional organization and government administration guidelines and regulations.

The TianLong ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** is designed for use with Real-time PCR instruments with 4 or more fluorescence detection channels, on which the kit test performance has been validated. These Real-time PCR thermal cyclers include appropriate fluorescence reading channels for FAM, HEX/VIC, Texas Red and Cy5 channels such as Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier Real-time PCR Systems.

Principles of the Assay

The highly conserved sequences in HA gene of Avian Influenza Virus IFVA/H5/H7/H9 were selected as target regions, and specific primers and TaqMan fluorescent probes were designed. The probes can specifically bind to a nucleic acid template in the middle of the primer amplification region. In the process of PCR extension reaction, the exonuclease activity of Taq enzyme will cut off the 5'-end fluorescent group from the probe, freeing it in the reaction system, thus breaking away from the shielding of the 3'-end fluorescence quenching group, which can receive light stimulation and emit fluorescence that can be detected by the instrument, so as to achieve the automatic detection of Avian Influenza Virus IFVA/H5/H7/H9 nucleic acid in closed reaction system.

Reagent Kit

Reagent Kit Components

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

RT-PCR Reagents	Volume	In Tube
REAG 1	750 µL	1 tube
Controls		
CONTROL +	80 µL	1 tube
CONTROL -	80 µL	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 and be used before the expiration date. Freezing/Thawing 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 there are enough materials ready for planned work.
- Check to ensure equipment and instruments are ready for work.
- Follow the up-to-date instructions for use.
- Complete appropriate planning and calculations for coming testing.

Sample Requirements

The ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** is designed to detect Avian Influenza Virus IFVA/H5/H7/H9 RNA in nasopharyngeal or oropharyngeal swabs collected from individual personnel based on clinical and/or epidemiological criteria.

Utilize the specimen collection, transportation, storage medium specified by the reagent manufacturer. Extraction of nucleic acid should be carried out according to the 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, HEX/VIC, ROX/Texas Red 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 ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** is compatible with nucleic acid of adequate quality prepared from intended samples using common nucleic acid extraction kits/methods. The prepared nucleic acid can be used directly as sample nucleic acid material, moved forward to the Real-time RT-PCR reaction setup step.
If under certain circumstances prepared nucleic acid needs 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 nucleic acid should be avoided whenever possible.

Real-time RT-PCR Reaction Setup

1. Thaw the following reagents on ice: ***REAG 1***. Gently invert to mix the reagent, then briefly centrifuge (2,000 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 Master Mix into each PCR tube. Add 10 µL of each extracted RNA 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 RT-PCR cycler for amplification reactions.

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

Thermal Cycler Settings

Real-time RT-PCR cycling program:

Table 1 qPCR Cycling program

Stage	Number of cycles	Temperature (°C) *	Time (min: sec)
1	1	50	10:00
2	1	95	00:20
3	45	95	00:02
		60	00:20** (collect fluorescence)

*: It is recommended to use Tianlong Gentier series real-time fluorescent PCR instrument with a heating rate of 6 °C/s and select other instruments according to the specific performance of the instrument.

**: For other instruments such as ABI7500, the collection fluorescence is set to 31 s, which has no effect on the results.

Assignment for Fluorescence Detection Channels**:**

- FAM channel for IFVA H9
- HEX/VIC channel for IFVA H5
- ROX/Texas Red channel for IFVA
- Cy5 channel for IFVA H7

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 RT-PCR are 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, HEX/VIC, ROX/Texas Red and Cy5 channels from the negative control.

2) Ct value for FAM, HEX/VIC, ROX/Texas Red and Cy5 channels from the positive control is less than 30.

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

If the results of the controls do not meet the validity criteria outlined in 1) and 2), the test run is usually considered invalid. All samples involved in the test run need to be retested for reportable results.

In uncommon cases of extremely high target viral load in samples, the efficiency of RT-PCR reaction for internal control could be negatively influenced. This may result in delayed IC Ct in companion with a highly advanced target Cts. In this case, the validity of the test run, as well as the interpretation of positive test results could be confirmed with no need for a further retest of samples.

If the target Ct values for FAM and/or HEX/VIC and/or ROX/Texas Red and/or Cy5 Channels generated from negative control reactions repeatedly reach 37 or below, 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

Target Gene		**Interpretation of Results**
Influenza A virus H9 subtype (FAM)	Ct≤37	Positive Influenza A virus H9 subtype
	37<Ct<45	Retest***
	No Ct value or Ct value=45	Negative Influenza A virus H9 subtype
	No Ct value or Ct value=45	Invalid****
Influenza A virus H5 subtype (HEX/VIC)	Ct≤37	Positive Influenza A virus H5 subtype
	37<Ct<45	Retest***
	No Ct value or Ct value=45	Negative Influenza A virus H5 subtype
	No Ct value or Ct value=45	Invalid****
	Ct≤37	Positive Influenza A virus H7 subtype
	37<Ct<45	Retest***
	No Ct value or Ct value=45	Negative Influenza A virus H7 subtype

Influenza A virus H7	No Ct value or Ct value=45	Invalid****
IFVA (ROX/Texas Red)	Ct≤37	Positive IFVA
	37<Ct<45	Retest***
	No Ct value or Ct value=45	Negative IFVA
	No Ct value or Ct value=45	Invalid****

***: The test result of the sample to be tested is 37<Ct<45. At this time, the sample should be tested again. If the Ct value of the repeated test result is less than 45, the curve is S-shaped and there is an obvious exponential growth period, it is judged as positive, otherwise it is negative.

****: This test is invalid and needs to be checked and re-tested.

Assay Performance Characteristics

The following performance characteristics of the TianLong ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** have been established as described below.

Non-clinical Studies

- Limit of detection:
  500 copies/mL.
- Specificity:
  There was no cross-reaction between this kit and Influenza Virus B, adenovirus type 3/7, respiratory syncytial virus, parainfluenza type 1/2/3, enterovirus 71, coxsackie virus 16, *Mycoplasma pneumonia*, *Chlamydia pneumonia*, rhinovirus, human cytomegalovirus, human metapneumovirus, human coronavirus OC43/229E, human coronavirus NL63/HKU1, EB virus, measles virus, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus salivarius*, *Streptococcus pyogenes*, *Neisseria meningitidis*, *Bordetella pertussis* and other relevant pathogens (above the viral concentration of 10^5 pfu/mL and bacteria of 10^6 cfu/mL).
- Precision:
  In a Precision Study with reference specimens of high and low concentrations, multiple operators and instruments were utilized.
  The within run and between run Precision of test results, as represented by the Ct value CV%, are all less than 5%.
- Disruptor:
  0.2 mg/L beclomethasone, 0.15 mg/L dexamethasone, 0.4 mg/L budesonide, 0.5 mg/L fluticasone, 5 mg/L zanamivir, 75 mg/L tobramycin and 10 g/L mucin have no influence on the detection results on the test result of this kit.

Quality Control

In accordance with the ISO 13485: 2016 Medical Devices—Quality management systems and TianLong ***Avian Influenza Virus IFVA/H7/H5/H9 Detection Kit*** Quality Control Program, each batch of the ***Avian Influenza Virus IFVA/H7/H5/H9 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 Avian Influenza Virus IFVA/H7/H5/H9 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 Avian Influenza Virus IFVA/H7/H5/H9 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 contamination that may cause degradation of the template RNA.**
- **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.

	Catalog number
	Batch code
	Contains reagents sufficient for <N> tests
	Use-by date
	Date of manufacture
	Caution

	Temperature limit
	Manufacturer
	Consult instructions for use
	Keep away from sunlight
	Fragile handle with care
CONT	Content of the Kit
REAG 1	P726H Rx Sol (A/H7/H5/H9 Master Mix)
CONTROL +	P726H PC (A/H7/H5/H9 Assay Positive Control)
CONTROL -	P726H NC (A/H7/H5/H9 Assay Negative Control)
	Recycling symbol PAP21: non corrugated cardboard

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   Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline; Clinical & Laboratory Standard Institute (CLSI): EP17A

Contact Information

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