Instruction manual
ADL69Y1-AIV9_NO_(EN)_V01
03/2023

Adia X Lyo

AIV H9

Reference: ADL69Y1-100

Test for the detection of Avian Influenza Virus A subtype H9 by real time enzymatic amplification
PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Swab (cloacal, tracheal, oropharyngeal)	✓	10
Tissue	✓	10
Feather	✓	
Faeces	✓	
Environmental sample	✓	
FTA card	✓	
Culture/allantoic liquid	✓	

* Depending on the epidemiological case and on the quality of samples.

Kit composition

Content		ADL69Y1-100 Kit
		100 reactions
A6	Amplification solution	1 lyophilized vial with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)
H9 CTL+	Influenza virus subtype H9 positive control	1 tube with purple cap (To reconstitute)
EPC-Ext	Exogenous extraction or amplification control	1 lyophilized vial with yellow cap (To reconstitute)
NF-Water	Nuclease-Free Water	2 x 1000 µL tubes with white cap (Ready to use)

Revision history

Date	Version	Modifications
03/2023	V01	First version

Note: minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

Influenza viruses belong to the genus Influenza virus A of the family Orthomyxoviridae. Influenza A viruses of subtype H9, in particular H9N2, are widespread in the wild in many bird species worldwide, causing significant economic losses. There are numerous H9N2 lineages depending on geographical location (Carnaccini and Perez, 2020).

The ADIALYO™ AIV H9 test amplifies a sequence of the HA gene specific to H9 subtype influenza viruses. The kit detects G1-like and Y439 lineages, found in the Middle East and Europe.

B. Test principle

ADIALYO™ AIV H9 test is based on the reverse transcription (RT) of RNA into complementary DNA. Then, cDNA is amplified with a DNA polymerase using specific primers of Influenza A virus subtype H9. Both enzymatic reactions occur in the same tube (One-step RT-PCR). This test is intended to detect simultaneously, in one well:

- Influenza A virus subtype H9 (FAM labelled probe).
- Internal control of extraction and/or amplification specific from an exogenous RNA (HEX labelled probe or its equivalent).

C. Storage conditions

- Store the kit at a temperature below +2/8 °C after reception.
- Store away from sunlight and keep dry.
- After reconstitution, prepare aliquots and store them at a temperature below -15 °C until the expiration date.
- Do not thaw more than 3 times.

D. Material required but not provided

- Real-time Thermal cycler and device.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free water.
- Kit for nucleic acids extraction.

Additional kits for method adoption and PCR

- **Extraction Positive Control AIV H9 (Ref.: ADC69EPC).** Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 100x LOD_{Method})
- **LD_{PCR} Positive Control AIV H9 (Ref.: ADC69YLD)** Confirmation of performances – LOD_{PCR}

E. Warnings and precautions

- For veterinary *in vitro* use only.
- For animal use only.
- For professional use only.
- All instructions must be read before performing the test and strictly respected.
- Do not use reagents after the expiration date.
- Do not use reagents if the packaging is damaged.
- Do not open PCR wells or tubes after amplification.
- Do not mix reagents from different batches.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).

F. Nucleic acids extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The RNA/DNA purification kits listed below are recommended by Bio-X Diagnostics:

Product name	Extraction system	Number of tests and reference
ADIAMAG™	Magnetic beads	200 tests: ref. NADI003 800 tests: ref. NADI003-XL
ADIAPURE™ Lysis Flex	Direct lysis from avian swabs	500 mL: ref. ADPLF1-500

For the extraction, consult the user manual version, available on the website, indicated on the certificate of analysis included in the PCR kit of interest.

Other purification kits can be used if they have been validated by the user.

After extraction, nucleic acid extracts can be kept on ice or at +2/8 °C for a few hours or until use. For long term storage, they must be kept at a temperature below -15 °C or -65 °C.

2. Controls

Using controls allow to verify the reliability of the results. Controls can be included.

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
H9 CTL+	Influenza target amplification	5 µL CTL+ in a well per run
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100X LOD_{Method}) per run

G. Procedure

1. Amplification solution A6 preparation

- Add **1000 µL** of **« Rehydration buffer »** per A6 tube.
- Homogenize tube contents using a mixer, such as vortex, at least 20 seconds.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use the A6, please refer to § « Amplification », Step 1.

2. Preparation of controls

a. Use of EPC-Ext

EPC-Ext must be added to each sample and controls.

- Add **1000 µL** of **« NF-Water »** per tube.
- Homogenize the tube contents using a shaker such as a vortex, > 20 seconds.

- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.
- For use, 2 solutions are possible:
  - Either add **5 µL** of EPC-Ext in the first lysis buffer during the extraction of nucleic acids using magnetic beads or silica columns.
  - Or add **0.5 µL** of EPC-Ext to each PCR well (if using direct lysis extraction). See § "Amplification", Step 1.

b. Use of CTL+

- Add **200 µL** of **« NF-Water »** per tube.
- Homogenize the tubes using a mixer, such as vortex, > 20 seconds and until dissolution of the blue pellet.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.
- For each assay, use **5 µL** of CTL+ in one of the dedicated wells (see § « Amplification », Step 2).

3. Amplification

Warning:

- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15 °C after use.

Step 1:

If use of EPC-Ext at the extraction step:

Dispense **10 µL** of amplification solution (A6) in each well.

If no used of EPC-Ext at the extraction step:

Place (n+1) x 10 µL of amplification solution (A6) in a microtube and add (n+1) x 0,5 µL of EPC-Ext. Dispense **10 µL** of the mixture into each well.

Step 2: Dispense **5 µL** of nucleic acids extracts and **5 µL** of controls in each dedicated well.

Use NF-Water for the No Template Control (NTC).

Step 3: Cover the wells with an appropriate optical film or caps.

Step 4: Set up the amplification program.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantiStudio5, Step-one…) from Applied Biosystems, for Mx3000, Mx3005P and AriaMx from Agilent, for LightCycler from Roche Diagnostics, for Rotor-Gene Q from Qiagen, for CFX96 and Chromo 4 from Biorad and for MIC from BioMolecular System.

DNA/RNA Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	

*Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	538	554
ROX	575	602

Note: The Quencher is non-fluorescent. The A6 solution contains a passive reference read in the same spectra as ROX for ABI machines.

For other thermal cycler instruments**,** please contact your sales representative or the customer relations department.

H. Reading and interpretation

Display all curves and position the threshold line for each fluorochrome.

1. Test validation

Amplification is valid if the following results are obtained.

Expected Ct (Threshold Cycle*)* values for the CTL+ are indicated on the certificate of analysis of the kit.

Controls	Amplification		Validation of
	FAM	HEX or equivalent	
No Template Control (NTC)	No	No/Yes*	Absence of amplification contamination
H9 CTL+	Yes	No/Yes*	Target amplification
Extraction negative control	No	Yes	Absence of extraction contamination
Extraction positive control	Yes	Yes	Extraction and amplification steps

*According to the addition or not of EPC-Ext during the amplification step.

2. Results interpretation

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM and/or HEX or equivalent.

Amplification		Interpretation
FAM	HEX or equivalent	Influenza virus subtype H9
No	Yes	Undetected
Yes	Yes	Detected
Yes	No	Detected
No	No	Undetermined

« Undetermined »: no characteristic amplification curve.

Possible causes:

Defective PCR due to inhibitors, set up error, absence of samples, degraded samples and/or issue with nucleic acids extraction (loss or destruction of nucleic acids).

Recommendations:

Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water;

If the assay is inconclusive, perform a new nucleic acids extraction.

Bibliography

- Silvia Carnaccini and Daniel R. Perez. H9 Influenza Viruses: An Emerging Challenge. Cold Spring Harb Perspect Med 2020.

Symbols

Symbole	Signification
REF	Catalog number
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
Σ	Contain sufficient for "n" tests
VET	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight
	Keep dry

Notes

1 Extract nucleic acids with

Adia X Mag →

Scan me to discover Adiamag™

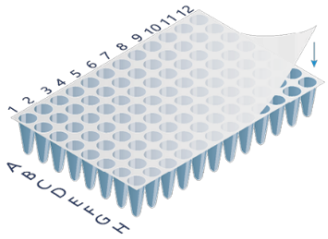
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

If using the EPC at the extraction step:

3 Distribute **10 µL** of **A6** amplification solution

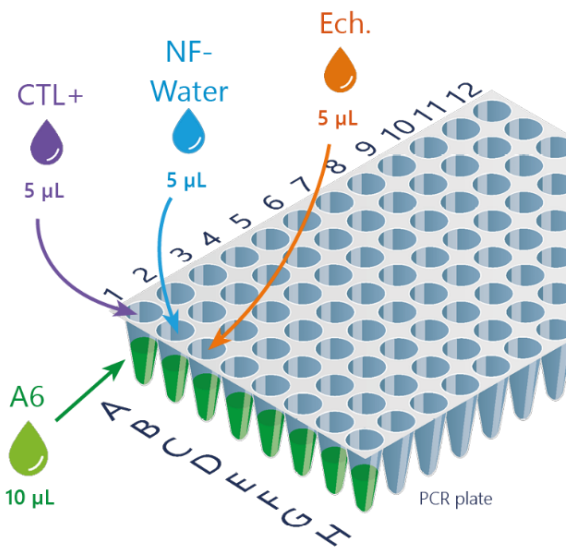
4 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**

5 Seal the wells

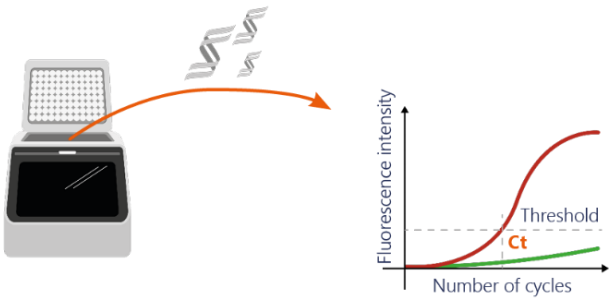


If not using the EPC at the extraction step:

3 Prepare a premix of **10 µL** of **A6** amplification solution + **0,5 µL** of EPC
Dispense **10 µL** of the premix



6 Start PCR analysis



*The notes do not replace the instructions for use of which they are a summary.

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