Certified management system

ID Gene™ Influenza A Duplex

Ref: IDFLUA-50 / IDFLUA-100

50 / 100 tests

Real-time RT-PCR assay for the **qualitative** detection of the **Avian Influenza A virus**

Suitable samples: avian tracheal, oropharyngeal or cloacal swabs, organs and FTA® cards

(individual samples or pools of up to 5)

***In-vitro* Use**

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General information

▪ Characteristics

ID Gene™ Influenza A Duplex (IDFLUA) kit is a real-time RT-PCR kit that amplifies a target sequence in Avian Influenza A (FLUA) viral genome.

This kit is a qualitative duplex test. It simultaneously amplifies target RNA and an endogenous internal control.

The kit contains the target positive control (TPC-FLUA) which is to be extracted in the same condition as the samples to validate the extraction and amplification of the target.

This kit can be used to test avian tracheal, oropharyngeal or cloacal swabs, organs and FTA® cards (individual samples or pools of up to 5).

▪ Kit composition and storage conditions

The IDFLUA kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-FLUA	Target Positive Control	550 µl	Inactivated subtype H5 Influenza A virus, diluted in a chicken negative tracheal swab supernatant, freeze-dried and calibrated at between 1 and 10 times the method detection limit (MDL). Freeze-dried pellet to be reconstituted in 550 µl distilled or Nuclease-free water.
ARM-FLUA	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mix containing the reverse transcriptase, Taq polymerase and oligonucleotides for amplification and the detection of Avian Influenza A virus and of the endogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

▪ Required equipment, not provided in the kit

All material used should be of suitable quality for molecular biology.

Amplification Instrument:

- Real-time thermal cycler with channels capable of reading the following fluorophores: FAM, HEX or VIC and Cy5.
- Examples of compatible thermal cyclers: CFX96™, Chromo4™ (Biorad), LC®480 I, LC®480 II, LC®96 Roche, AB® 7500 and Rotor-Gene Q Qiagen. Please contact **info@id-vet.com** regarding suitability with other thermal cyclers.

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl
- Nuclease-free filtered tips
- 1.5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (with an optical quality compatible with the thermal cycler) and appropriate adhesive film or caps
- Refrigerated rack

Reagents:

- Distilled or Nuclease-free water (recommended)

Remarks and precautions

The material used contains less than 0.1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction and amplification controls

▪ Positive controls

The IDFLUA kit contains the following positive controls:

- Target Positive Control (TPC-FLUA):

This control consists of inactivated subtype H5 Influenza A virus, diluted in a chicken negative tracheal swab supernatant, calibrated at between 1 and 10 times the limit of detection method (MDL).
Freeze-dried pellet to be reconstitute in 550 µl distilled or Nuclease-free water.
This control validates the efficiency of the extraction and amplification process.
This control is prepared and extracted in the same way as the samples.

- Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively present in the cells of the sample to be tested. Its function is to validate (1) cell lysis and (2) amplification of a non-target gene. It also confirms the presence of cells, and gives an indication as to quality of the sample.

▪ Negative controls

It is recommended to include the following negative controls in each run:

- Negative extraction control (NEC)

This control should be prepared and extracted in the same way as samples, but should not contain any Influenza target RNA. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

- Negative control for amplification (NAC)

This control contains 8 µl of reaction mix (ARM-FLUA) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

▪ Extraction of viral RNA

The viral RNA must be extracted from the sample before being amplified by RT-PCR.
For this, IDvet Genetics offers a range of extraction kits that meet the French standard NF U47-600 requirements:

Description	*Product name*	*Product code*
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit ID Gene™ Mag Fast Extraction Kit	MAG192/MAG384 MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

Note: The RNeasy R Mini Kit (Qiagen) ref: 74104/74106 is also validated. Please contact **info@id-vet.com** for use with other extraction system.

▪ Extraction of the controls

The volumes of the control to be extracted are described in the table below:

Important:

- The volumes indicated are valid regardless of the extraction system.
- The controls must be extracted at the same time as the samples.

Control	*Volume*
TPC-FLUA	**50 µl**

Amplification protocol

▪ Preparation of the real-time PCR (RT-PCR) amplification reaction

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-FLUA) from the other samples.
2. Thaw the IDFLUA kit, ideally at 5°C (± 3°C) in a refrigerated rack. Thaw at room temperature 21°C (± 5°C) only if the mix is to be used immediately after thawing.
3. Homogenise the contents of the **ARM-FLUA** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-FLUA** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add the following to the reaction mix :
   - 5 µl of RNA extracted from each sample to be analysed
   - 5 µl of TPC-FLUA
   - 5 µl of extracted NEC
   - 5 µl of Nuclease-free water (NAC)
6. Cover the plate or strips with adhesive film or caps.

▪ Programming the amplification phase

1. Program the thermal cycler detectors to read the following wavelengths for each well:

Target	*Channel capable of reading*	*Quencher*
Influenza A virus FLUA	FAM	non-fluorescent*
Cell-specific sequence (NTPCen)	VIC/HEX	non-fluorescent (compatible VIC/HEX)*

Note: For devices requiring an internal reference for optical calibration, the amplification mix ARM-FLUA contains ROX.
**Using a TAMRA™ quencher can improve the data analysis with some instruments.*

2. Choose between the two 2 different amplification programs validated by IDvet Genetics:
   - standard program (allows for PCR kits from different vendors to be used in a single run)
     or,
   - rapid program

Step	Standard program	Rapid program	Number of cycles
(1) *Reverse transcription*	10 min at 45°C	10 min at 45°C	1
(2) *Polymerase activation*	10 min at 95°C	2 min at 95°C	1
(3) *DNA denaturation / elongation*	15 sec at 95°C 60 sec at 60°C	10 sec at 95°C 30 sec at 60°C	40

Note: The fluorescence is read at the end of the elongation phase at 60°C.

3. Enter one of these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single run, enter the largest volume on the plate.
4. Place the PCR plate, PCR strips or capillaries in the thermal cycler and start the program.

Validation and interpretation of results

Assay validation

The analysis of results is based on the Cq (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).
The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met**.

Control	Expected result	Acceptability criteria
TPC-FLUA	Detected in FAM and VIC/HEX	Presence of a characteristic curve Refer to the Cq value given in the quality control certificate of the corresponding batch
NTPCen	Detected in VIC/HEX in each sample	Presence of a characteristic curve
NEC	Nothing detected if water is used Detected in VIC/HEX if negative sample used	Absence of a characteristic curve Presence of a characteristic curve
NAC	No detection	Absence of a characteristic curve

Note: TPC-FLUA may be used to monitor variations in analytical sensitivity as it is calibrated at between 1 and 10 times the MDL.

Suggested interpretation of results

For each sample, results may be interpreted according to the following criteria:

Sample	FLUA signal	NTPCen signal	Interpretation
Individual or pooled	**Detected**	**Detected** or not detected	The sample is **detected** as positive for Avian Influenza A
	Not detected	**Detected**	The sample is **not detected** positive for Avian Influenza A
	Not Detected	Not detected	A problem occurred during sample distribution or extraction process PCR reaction was inhibited

Non-validated samples:

- If the NTPCen is not detected but the sample is detected positive for Avian Influenza A, consider the sample as positive.
- If the NTPCen is not detected:
- A problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again or,
- The PCR reaction was inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted RNA 10 times in Nuclease-free water.
2. Repeat the amplification reaction on 5 µl of this dilution.
3. If the NTPCen is detected, interpret the sample according to table above.
4. If the NTPCen is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: **info@id-vet.com**
For additional information, visit **www.id-vet.com**