Influenza A RNA Test Kit

BioChek qPCR assays

Catalogue Number MP101

Description of test

The Influenza A RNA Test Kit (InfA qPCR) will detect the presence of RNA from Influenza A strains in extracts from swine (respiratory tract tissue, oropharyngeal or nasal swabs) and avian (tracheal/oropharyngeal or cloacal swabs) samples. Primers and probe are specific for InfA; the probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. After extraction of the RNA, samples are added to wells along with the dedicated Reaction Mix. The prepared wells are placed in the qPCR cycler for reverse transcription, amplification and detection.

Method used: qPCR

The InfA qPCR assay enables the simultaneous detection of:

- Influenza A (InfA; detected in FAM channel)
- Internal Control (IC; detected in CY-5 channel)

Reagents and materials provided

InfA qPCR (catalogue number MP101) contains reagents for 100 25 µl PCR reactions.

1. **InfA qPCR Primer/Probe mix with Internal Control (PP/IC),** 2 vials, liquid (412.5 µl) (yellow cap).
2. **RNA Mastermix I,** 2 vials, liquid (675 µl) (black cap).
3. **Reverse Transcryptase Enzyme (RT enzyme),** 2 vials, liquid (20 µl) (white cap).
4. **qPCR Negative Control (NC),** 4 vials, molecular grade water (60 µl) (blue cap).
5. **InfA qPCR Positive Control (PC),** 2 vials, diluted Influenza A plasmid with cloned target sequence standardized to represent significant amounts of Influenza A target (60 µl**)** (red cap).

Storage conditions: Upon receipt, store at -20 ^{o}C

Materials and equipment required (not provided with kit)

- qPCR thermocycler (detection channels for FAM, CY-5)
- RNA extraction method
- Heating block (optional)
- Vortex mixer (x2)
- Mini-centrifuge (x2)
- PCR plate-spinner (recommended)
- Pipettes & disposable filter-tips for volumes of 1 – 1000 µl
- Single, 8 or 12 channel pipettes
- DNase/RNase free tubes for preparation of reaction mix
- Plates, strips (+caps) or microtubes for RNA extraction
- Recommended plates for PCR reaction (suitable for use with your qPCR thermocycler)
- Heat resistant sealers for plate
- Disposable powder free gloves

Warnings and precautions

1. Wear disposable powder free gloves at any stage of running the assay and/or sample preparation.
2. Handle all reagents with care.
3. Treat all biological materials as potentially biohazardous, including all field samples.
4. Never pipette anything by mouth. There must be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
5. This kit is for *in vitro* use only.
6. For veterinary research only.
7. Strict adherence to the test protocol will lead to achieving best results.
8. Dedicate one airspace for kit storage/reagent preparation (Room 1, clean room) and another airspace (Room 2) for running the assay and sample preparation. A third airspace is optional (Room 3) for dedicated PCR amplification/running the assay.
9. Never move any materials from Room 2 or 3 to Room 1.
10. Decontaminate PCR laboratories with bleach (or alternative DNA decontaminant) and UV light (optional) after testing.
11. Assays must be performed by qualified laboratory personnel only.

Recommended work flow protocol

When running complete assay including RNA extraction in 1 day

1. Start in Room 1 with reagent preparation.
2. Go to Room 2 for RNA extraction and running assay.
3. Never go from Room 2/3 to Room 1 during the same day.

When doing RNA extraction first

Day 1

1. Start in Room 2 with RNA extraction.

Day 2

1. Start in Room 1 with reagent preparation.
2. Go to Room 2/3 for running the assay.
3. Never go from Room 2/3 to Room 1 during the same day.

Preparation of samples

Sample: Avian – tracheal/oropharyngeal or cloacal swabs; Swine – respiratory tract tissue, oropharyngeal or nasal swabs.

RNA extraction from sample:

Before running the PCR RNA must be extracted from the sample.

The extraction method chosen to be used with the BioChek InfA kit must be suitable for RNA extraction.

Recommended are spin column type extraction methods such as:

1) GeneAid Viral nucleic acid extraction kit II
2) Qiagen cador Pathogen mini kit

Extracted RNA can be stored at -20 ℃ prior to running the PCR.

Handle RNA extracts with great care due to the fragile nature of RNA.

It is recommended to validate the InfA PCR and chosen extraction method combined internally prior to generating results.

Test protocol InfA qPCR

Reagent preparation

Room 1

1. Defrost reagents at room temperature.
2. Vortex reagents thoroughly and briefly spin to remove any residues from the lid.
3. Calculate total volumes of RNA Mastermix I, PP/IC and RT enzyme required for all reactions (Reaction Mix). Do not forget to include reactions for controls (minimum one positive and one negative), and to compensate for dead volume, + 1 reaction for instance.

Reaction Mix	
RNA Mastermix I	12.25 µl
PP/IC	7.5 µl
RT enzyme	0.25 µl

4. Place the total volume of required RNA Mastermix I, PP/IC and RT enzyme into a clean microtube.
5. Vortex microtube to mix thoroughly, and briefly spin to remove any residues from the lid.

Assay preparation

Room 1

1. Take a suitable qPCR plate and record location of samples on template.
2. Add 20 µl of Reaction Mix as prepared above for every sample. The plate does not need to be cooled.
3. Add 5 µl of Negative Control into control well. This is a reagent and environment control (optional in Room 1)
4. Cover plate and take into Room 2.

RNA amplification

Room 2

1. Add 5 µl of Positive Control into control well, the plate does not need to be cooled.
2. Add 5 µl of Negative Control into control well. This is an environment control.
3. Add 5 µl of RNA extract into each sample well.
4. Cover plate with heat resistant sealer.

5. Spin plate for 30-60 seconds at 200-1000 x g.
6. Place plate in qPCR thermocycler and run using the specified thermal cycler program in the table (qPCR program at normal ramp speed).

Note: Do not use fast mode

Temperature	Time	No. Of cycles
48 °C	10 min	1
95 °C	3 min	1
95 °C	15 sec	40
60 °C	60 sec	
Data collection (@ 60 °C): FAM = Influenza A CY-5 = Internal Control		

Alternative channel names for the reporter dyes:
FAM: no alternative name
CY-5: Quasar 670
When the run is finished, remove the plate from the qPCR instrument and discard it without removing the seal.

Validation and interpretation

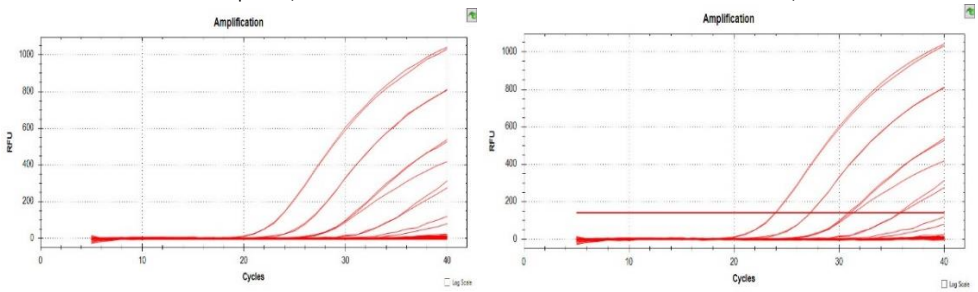
Analysis settings

Bio-Rad CFX96™	Applied Biosystems® 7500	Stratagene Mx3005P™	Qiagen Rotor-Gene Q
Fluorescence drift correction: Yes	Passive reference : no	Amplification-based threshold	Dynamic tube on
Cycles to analyse: 5 – 40	Baseline cycles: 3 – 12	Adaptive baseline	Slope correct on
			Ignore first 5 cycles

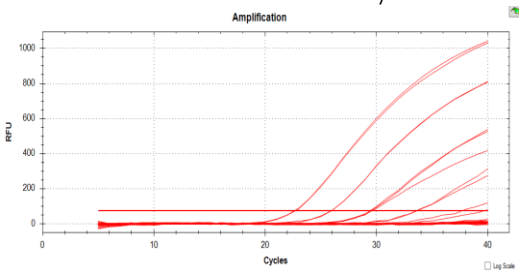
Other PCR machines can be used, contact BioChek for further information regarding suitable PCR thermocyclers.

Setting thresholds in the cycler software

Go to the part of the software where you can see the amplification curves.
Select all wells on the plate, select linear view and select the FAM channel, turn off other channels.



Depending on the amount of samples the linear curves should look like the ones in the picture above on the left.
To set the threshold look at which cycle the first curve starts to form, in this case around cycle nr 20.
Look at which cycle the first formed curve goes up in straight line, in this case around cycle 24, see picture above on the right. The threshold is placed for demonstration purposes at the point where the curve becomes a straight line.
The threshold should be set halfway between the fluorescence of cycles 20 and 24, see picture below.



Repeat this process for the CY-5 channel.

Validation of assay run

The following must apply for the PCR run to be valid:

	Influenza A (FAM) Cq values	IC (CY-5) Cq values	Interpretation
Positive Control	22.0-33.0	Not considered	Valid Control
Negative Control	N/A* or >38.0	<34.0	Valid Control

* No Cq value

Validation and interpretation of sample results: Always check validity of the amplification curves

Influenza A (FAM) Cq values	IC (CY5) Cq values	Interpretation
<38.0	Not considered	Positive sample for Influenza A
N/A*	<34.0	Negative sample
N/A*	N/A* or >34.0	Invalid well**

* No Cq value

** The assay is invalid for the particular sample and should be repeated with a new RNA extract.

NOTE: For sample results with a Cq between 38 and 40, it is recommended to check the amplification curve.

For final diagnosis qPCR positive results should be considered presumptive and confirmed by standard reference methods or alternative tests for Influenza A.

KI/MP101REV04

EC REP

BioChek B.V.
Fokkerstraat 14
2811 ER Reeuwijk
The Netherlands
tel: +31 182 582 592
fax: +31 182 599 360
E-mail: info@biochek.com
Website: www.biochek.com

BioChek (UK) Limited
Unit 5
Kings Ride Park
Kings Ride
Ascot
Berkshire SL5 8BP
United Kingdom

Symbols Glossary

REF	Catalogue number		Expiry date
EC REP	Authorised representative in the European Community	LOT	Batch number
	Manufacturer	SN	Serial number
	Date of manufacture	IVD	In vitro diagnostic
	Consult instructions for use		Lower limit of temperature
CONTROL -	Negative control	CONTROL +	Positive control