Instructions for use

InviScreen® Mycoplasma (Mg/Ms) Detection Kit

INVITEK diagnostics

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REF 6016003200 | Σ 100 tests | -15 ºC / -25 ºC | RUO

1. Intended use

InviScreen® Mycoplasma (Mg/Ms) Detection Kit is designed for the qualitative detection of *M. gallisepticum* and *M. synoviae* DNA in animal samples. The kit is intended for use by trained laboratory personnel in veterinary diagnostic laboratories or research institutions. The kit provides a reliable method for the detection of *M. gallisepticum* and *M. synoviae* in various animal specimens, including but not limited to tracheal swabs and tissue samples. Users should adhere to recommended biosafety protocols and handle all specimens, reagents, and materials following established laboratory practices to minimize the risk of contamination and ensure accurate results. Results obtained with this kit should be interpreted in conjunction with clinical observations and other relevant diagnostic information. It is important to follow the instructions provided in the kit manual carefully, including sample preparation, DNA extraction, and PCR setup, to ensure accurate and reliable results.

2. Product description

InviScreen® Mycoplasma (Mg/Ms) Detection Kit offers a reliable method for qualitatively determining the presence of *M. gallisepticum* and *M. synoviae* DNA animal specimens by targeting species-specific genes through a real-time reverse transcription and multiplex amplification strategy. The method utilizes a Taqman-based approach, where amplification detection relies on the degradation of a hydrolysis probe during the PCR reaction, resulting in the emission of fluorescence. This enables the sensitive and specific detection of Mycoplasma DNA in animal specimens, particularly tracheal tissues obtained by scraping the internal epithelial layer using a sterile blade or swab. To ensure the accuracy of the results and to monitor potential PCR inhibitors, an Internal Amplification Control (IAC) detection primer/probe set is included in the kit. The IAC serves as a process control, allowing for the identification of samples that may contain substances inhibiting the PCR reaction.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D44.01	Primer/Probe Mix[1]	Targeted detection	●	1 tube, 1000 µL
D44.02	qPCR Master Mix[1]	Amplification	●	1 tube, 1000 µL
D44.03	Negative Control	Negative Control	●	1 tube, 100 µL
D44.04	Positive Control	Positive Control	●	1 tube, 100 µL

[1] Reagents are supplied with a 5% of extra volume

4. Storage

Reagents should be stored sealed at -20 ± 5°C and may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles. Protect reagents from light exposure to prevent degradation.

5. Equipment and materials required (not provided)

- DNA extraction kit
- Real-Time PCR instrument
- Plates and/or tubes for qPCR
- 1.5 mL microcentrifuge tubes
- PCR cabinet
- Micropipettes (10, 200 and 1000 µL) and filter tips
- Vortex and microcentrifuge

6. Suitable test sample material

The test is applicable to biological samples of animal origin, specifically tissues from the trachea of birds collected by scraping the internal epithelial layer with a sterile blade or swab.

7. Test Procedure

a. PCR Reaction Preparation

Allow all reagents to thaw at room temperature, vortex and spin briefly to avoid drops on the vial cap. For each DNA sample prepare a reaction mixture according to the table below:

REAGENT	VOLUME
qPCR Master Mix	10 µL
Primer/Probe Mix	10 µL
Total Volume	**20 µL**

1. Homogenize the reaction mixtures and pipette 20 µL into individual wells according to the predicted PCR plate set-up.
2. Add 5 µL of DNA template to each well.

At least one positive control reaction and one negative control reaction must be included in the PCR run, replacing the sample in these wells with 5 µL of Positive Control and 5 µL of Negative Control, respectively.

It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone. The addition of the positive control and sample DNA should preferably be carried out in a separate room.

b. Amplification Protocol

The amplification conditions are as follows:

	STEPS	TEMPERATURE	TIME	CYCLES
1	Enzymatic Activation	95 ºC	10 min	1
2	Denaturation	95 ºC	15 sec	35
3	Hybridization/extension plate reading*	60 ºC	60 sec	

* Fluorescence data must be obtained during this step through FAM, HEX and Cy5 channels.

c. Results Interpretation

The results should be interpreted in accordance with the analysis software recommended by the Real-Time PCR instrument manufacturer. The software monitors DNA amplification through the detection of fluorescence emitted by each probe, attributing a Ct value for each reporter dye found in each individual sample. Target DNA amplification is monitored in FAM (Ms) and HEX (Mg) channels. The amplification control (IAC) is monitored in the Cy5 channel. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred bellow.

A result is considered positive when Ct ≤ 32.

RESULT	Target DNA(FAM/HEX)	IAC (Cy5)
Positive	+	+/-
Negative	-	+
Inconclusive [2,3]	-/?	-

[2] PCR inhibitions may be due to the presence of excessive DNA and/or PCR inhibitors. It is recommended to dilute the RNA extracted from the sample 1:10 or 1:100 in DNAse/RNAse free water and repeat the Real-Time PCR reaction. When applicable, the LOD of the method should be adjusted in accordance with the dilution factor.
[3] The appearance and characteristics of the amplification curves should be thoroughly considered. Incomplete amplification curves often denote low amount of RNA template. In this case, the positivity of the result is dubious, and the Real-Time PCR reaction should be repeated using a superior amount of DNA template.

8. Quality Control

The test can only be considered valid under the following control conditions:

CONTROLS	Target DNA	IAC
Positive Control	+	+
Negative Control	-	+

If no amplification is observed for the positive control, the test results are invalid and must be repeated. The positive control template is expected to amplify before Ct 25. If amplification is observed for the negative control, it indicates that the reagents have become contaminated while setting up the run, invalidating test results.

9. Performance Characteristics

Specificity: InviScreen® Mycoplasma (Mg/Ms) Detection Kit was designed to specifically detect *M. gallisepticum* and *M. synoviae* DNA. The specificity was evaluated by analysing DNA extracted from Mycoplasma reference materials and cross reactivity tested using a large panel of infectious disease agents that include avian viruses and bacteria.

Reference material	Results		
	Target (Mg)	Target (Ms)	IAC
Avian orthoreovirus	Negative	Negative	Positive
Campylobacter coli	Negative	Negative	Positive
Campylobacter jejuni	Negative	Negative	Positive
Chlamydia psittaci	Negative	Negative	Positive
Clostridium perfringens type A	Negative	Negative	Positive
E.coli O157	Negative	Negative	Positive
Enterococcus faecalis	Negative	Negative	Positive
Infection bronchitis virus (IBV)	Negative	Negative	Positive
Infectious bursal disease virus (IBD)	Negative	Negative	Positive
Infectious laryngotracheitis (ILT)	Negative	Negative	Positive
Klebsiella pneumoniae	Negative	Negative	Positive
Legionella pneumophila	Negative	Negative	Positive
Newcastle disease virus	Negative	Negative	Positive
Ornithobacterium rhinotracheale (ORT)	Negative	Negative	Positive
Pseudomonas aeruginosa	Negative	Negative	Positive
Salmonella Typhimurium	Negative	Negative	Positive
Staphylococcus aureus	Negative	Negative	Positive
Poxvirus avium	Negative	Negative	Positive
Yersinia enterocolitica	Negative	Negative	Positive
Mycoplasma gallisepticum	Positive	Negative	Positive
Mycoplasma synoviae	Negative	Positive	Positive

Detection Limit and sensitivity: The limit of detection (LOD) is often matrix dependent, and the sensitivity of the analysis may be reduced depending on the total DNA extracted from the actual ingredient in test, but also its quality. This way, the LOD needs to be determined through in-house validation.
The LOD was determined using genomic DNA extracted from *M. gallisepticum* and *M. synoviae* vaccinal strains. Successive dilutions (base 10) were used, so that at the lowest dilution (highest concentration, $1x10^5$ copies/µL) all results are positive (for target gene) and at the last dilution (lowest concentration, 10 copies/µL) it is expected that all or most of the results will be negative. Under optimal conditions, the lowest amount of *M. gallisepticum* and *M. synoviae* DNA detected in 95% of the experiments was 500 copies/µL and 10^2 copies/µL, respectively.