

Glanders qPCR kit

Cat: LTQPCR-GLA

Quantity 50 tests

1. Purpose

Glanders is a contagious and fatal disease of horses, donkeys, and mules, caused by infection with the bacterium *Burkholderia mallei*. The pathogen causes nodules and ulcerations in the upper respiratory tract and lungs. The «Glanders qPCR kit» is intended to detect *Burkholderia mallei/pseudomallei* DNA in blood, tissues, swabs, feces and cultural material, which is rapid, accurate and easy-to-operate. The test kit «Glanders qPCR kit» is designed for multiplexed detection of the specific to *Burkholderia mallei/pseudomallei* DNA (aroA gene), according OIE validated protocol, and Internal Control (16 S) gene as sample processing control by real time polymerase chain reaction (PCR) with fluorescent detection of the analysis results.

2. Clinical significance

In horses, inflammatory pustules and ulcers develop in the nasal conchae and nasal septae, which give rise to a sticky yellow discharge, accompanied by enlarged firm submaxillary lymph nodes. Pyogranulomatous nodules are sometimes found in the liver and spleen. Discharges from the respiratory tract and skin are infective, and transmission between animals, which is facilitated by close contact, inhalation, ingestion of contaminated material. Glanders is transmissible to humans by direct contact with diseased animals or with infected or contaminated material. In the untreated acute disease, the mortality rate can reach 95% within 3 weeks.

3. Principle of analysis

The method is based on the isolation of total DNA from samples, and further amplification of specific regions of *Burkholderia mallei/pseudomallei* DNA (aroA gene) using specific oligonucleotide primers and the synthesis of complementary DNA chains using the enzyme Hot start Taq-DNA polymerase. Fluorescent detection of the analysis results is performed both in real-time (Real-time mode) or end point assay using two fluorescence detection channels (FAM525 nm, ROX580 nm), see table below:

Table1. Target specificity

Marker	Detection channel
B. mallei, aroA gene	FAM
Internal control, 18S gene	ROX

4. Kit contents:

Table 2. PCR reagents:

Component name	Quantity	Volume
B. mallei Master Mix (Lyophilized)	1 vial	
Glanders Positive Control (Lyophilized)	1 tube	

5. Storage and stability

The test kit reagents are stable for the shelf life indicated on the label (at least 12 months from manufacture date) if stored at room temperature.

6. Sample preparation

Blood, tissue, swabs and semen samples can be used directly for Nucleic Acids extraction according commercially available DNA extraction kit recommendations. We recommend using our Nucleic acids extraction kit compatible with automatic Nucleic extraction device LTEXT1.

7. Reagent preparation

7.1 Master Mix preparation: Add distilled water 0,5 mL directly to Lyophilized Master Mix, wait 5 min, mixing well and dispense 10 µL into PCR testing tubes.

7.2 Positive control preparation: Add distilled water 0,5 mL directly to Lyophilized Positive Control, wait 5 min, mixing well and use directly 10 µL for PCR or use 100 µL for Nucleic acid extraction (to control Nucleic acid extraction efficiency).

Note! Reagents and controls must be kept in a chilled stand. After use reagents and controls can be freeze at minus 20°C for storage. Several freezing-thawing cycles are allowed.

8. Assay procedure

8.1. Select the required amount of the PCR tubes or wells in the PCR plate.

8.2. Using pipette tips with an aerosol filter dispense Master mix 10 µL add 10 µL of DNA samples (Positive and Negative control samples).

8.3. Place testing tubes in the fluorescence PCR instrument, and set the negative/positive control and unknown samples parameters for PCR reaction according to the instrument operating instructions. Fluorescence is measured at 60°C on the FAM, and ROX channels. The PCR parameters are shown in the table 3:

Table 3. PCR run method parameters

Step	Temperature	Time	Repeats	Data collections
1	95 C	5 min	1	No
2	95 C	10 sec	45	No
	60 C	30 sec		Yes

8.4. Start run.

9. Result records and their interpretation is shown in the table 4:

Table 4. PCR results interpretation

Marker	Fluorescent channels	B. mallei Positive	B. mallei Negative
Glanders aroA gene	FAM	Ct≤ 45	Undetectable
Internal control gene 18S	ROX	Ct≤ 35	Ct≤ 35