Comparison of Exgene™ Viral DNA/RNA kit to other commercial kits for pathogen nucleic acid extraction from rooster stool

Experimental Conditions

Materials Required

- Exgene™ Viral DNA/RNA (128-150)
- Buffer FL, 70 ml (115-910)
- 2 ml Glass Bead Tube (406-893)
- 1.5 ml microcentrifuge tube
- Microcentrifuge (≤14,000 x g)
- Vortex mixer
- Pipette & sterilized pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice

Sample Information

Pathogen	*Mycoplasma Gallisepticum* (MG)	Infectious Bronchitis Virus (IBV)
Target	Bacterial DNA	Viral RNA
Sample	Pathogen-infected rooster stool	
Sample amount	200 mg	
Elution volume	50 µl	

Protocol

Exgene™ Viral DNA/RNA Protocol

*** For more details and methods, please refer to the handbook of Exgene™ Viral DNA/RNA.**

Preparation of Proteinase K and Carrier RNA Solution

- **Proteinase K solution**

  To obtain a 20 mg/ml Proteinase K solution, add 650 µl of PK Storage Buffer to the tube of lyophilized 13 mg of Proteinase K, and mix carefully to avoid foaming.

- **Carrier RNA solution**

  To obtain a 1 µg/µl Carrier RNA solution, add 370 µl of Nuclease-free water to the tube containing lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots.

Sample Preparation

- **Pathogen-infected rooster stool**
  1. Add 200 mg of each rooster stool infected with pathogen to each 2 ml Glass Bead Tube.
  2. Add 1 ml of Buffer FL and 25 µl of Proteinase K solution (20 mg/ml) to the tube. Vortex for 1 min or until the stool samples is thoroughly homogenized.
  3. Incubate for 5 min at 65 ˚C.
  4. Centrifuge at 10,000 x g for 10 min at room temperature.
  5. Transfer 200 µl of supernatant to the each 1.5 ml microcentrifuge tube.
  6. The subsequent protocol follows **step 3 on page 10 of protocol in the Exgene™ Viral DNA/RNA handbook.**

Result

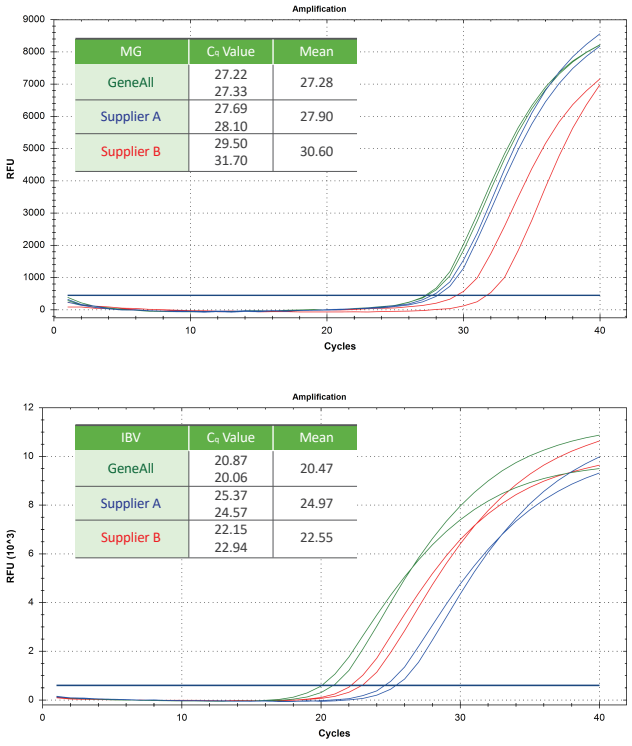

Figure 1. Comparison of bacterial DNA and viral RNA detection by qPCR analysis of rooster stool samples obtained from Exgene™ Viral DNA/RNA kit and competitor kits.
Nucleic acids were extracted from stool samples of two types of pathogen-infected roosters using GeneAll's Exgene™ Viral DNA/RNA kit ('Green') and competitor kits ('Blue' and 'Red'), with each extraction performed in duplicate.
Real-time PCR was performed on following extraction of bacterial DNA and viral RNA. Each pathogen nucleic acid was amplified with target-specific primers and probes. The PCR data shows that Exgene™ Viral DNA/RNA kit is more efficient in extracting and detecting the nucleic acids of interest from the rooster stool samples.
- Real-time PCR system: CFX96™ System (1855201, supplier B)
- qPCR kit: RealAmp™ 2X qPCR Master Mix (801-020)
- RT-qPCR kit: HyperScript™ One-step RT-PCR Master Mix (602-110)