Comparison of the Pathogen Purification Performance of Exgene™ Viral DNA/RNA to Other Commercial Kits

Experimental Conditions

Materials Required

- Exgene™ Viral DNA/RNA (128-150)
- 5 ml conical tube (for sample preparation)
- Vortex mixer
- Microcentrifuge (≤15,000 x g)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice (for maintenance normal state and freeze-thaw of Carrier RNA solution)

Sample Information

- Sample type: cultured virus and bacteria
  - Infectious bronchitis virus (IBV, $10^{3.5}EID_{50}$)
  - Rabies virus (RV, $10^{3.5}LD_{50}$)
  - Japanese encephalitis virus (JEV, $10^{5.0}TICD_{50}$)
  - *Mycoplasma gallisepticum* (MG, 1 x 10^{5}CCU)

- Extraction conditions
  - Sample amount: 200 µl
  - Elution volume: 100 µl
  - Extraction protocol: Viral_Normal (operation time: 29′ 35″)

Sample Preparation

1. Mix the all cultured viruses and bacteria medium to 5 ml conical tube and extract the 200 µl samples from the mixture.
2. One sample is according to Exgene™ Viral DNA/RNA protocol, the other samples are according to manual method of viral DNA/RNA extraction kits each from two different suppliers for comparison.

Protocol

Exgene™ Viral DNA/RNA Extraction Kit Protocol

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

1. Add 10 µl of Proteinase K solution (20 mg/ml) to 1.5 ml microcentrifuge tube.
2. Transfer the 200 µl of mixed samples and add 200 µl of Buffer BL to the tube.
3. Add 7 µl of Carrier RNA solution (1 µg/µl) to the tube and mix thoroughly by vortexing for 10 s.
4. Incubate the tube at 56 °C for 10 min and spin down briefly to remove any drops.
5. Add 400 µl of Buffer RB1 to the tube and vortex for 10 s.
6. Transfer the mixture to a Column Type S and centrifuge at ≥10,000 x g for 1 min at room temperature. Discard the pass-through and reinsert.
7. Add 500 µl of Buffer BW to the column and centrifuge at ≥10,000 x g for 1 min at room temperature. Discard the pass-through and reinsert.
8. Add 700 µl of Buffer TW to the column and centrifuge at ≥10,000 x g for 1 min at room temperature. Discard the pass-through and reinsert.
9. Centrifuge at full speed for 1 min at room temperature and transfer the column to a new 1.5 ml microcentrifuge tube.
10. Add 20–50 µl of Nuclease-free water to the center of the membrane in the column. Stand for 1 min and centrifuge at ≥ 10,000 x g for 1 min at room temperature.

Result

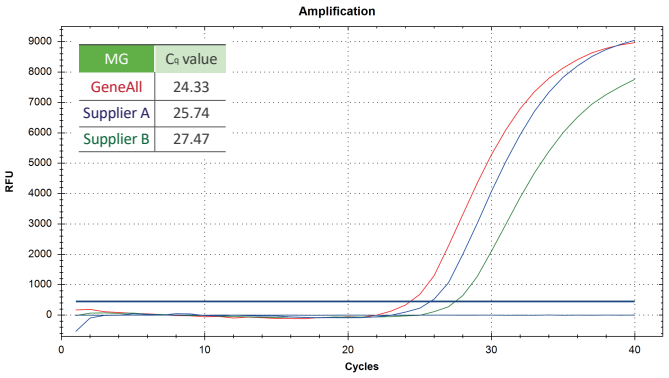

Figure 1. Comparison of C_q value of DNA template extracted from the *Mycoplasma gallisepticum* (MG).
The DNA templates were extracted from the *Mycoplasma gallisepticum* (MG) using Exgene™ Viral DNA/RNA (red line) and viral DNA/RNA extraction kits (manual method) each from two different suppliers (blue and green line). Eluted DNA templates were analyzed with a TaqMan-based real-time PCR assay using CFX-96.

- Red line: GeneAll® Exgene™ Viral DNA/RNA
- Blue line: viral DNA/RNA extraction kit (supplier A)
- Green line: viral DNA/RNA extraction kit (supplier B)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391A)
- Target gene: none specific

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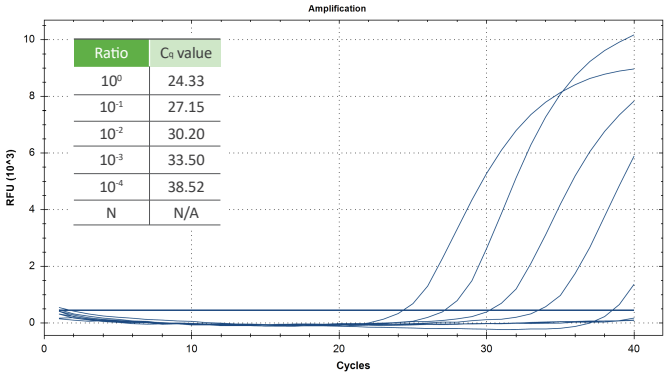

Figure 2. Analysis of extraction sensitivity on serial dilutions of *Mycoplasma gallisepticum* (MG).
The DNA templates were extracted from a 10-fold serial dilution of *Mycoplasma gallisepticum* (MG) using Exgene™ Viral DNA/RNA. All eluates were analyzed with a TaqMan-based real-time PCR assay using CFX-96.

- N: negative control (nuclease-free water)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391AT)
- Target gene: none specific

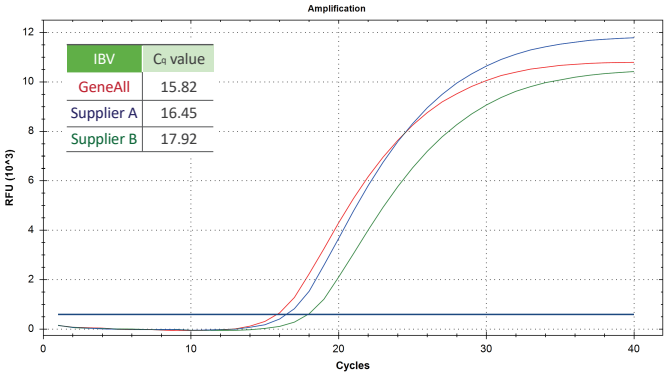

Figure 3. Comparison of C_q value of DNA template extracted from the Infectious bronchitis virus (IBV).
The RNA templates were extracted from the Infectious bronchitis virus (IBV) using Exgene™ Viral DNA/RNA (red line) and viral DNA/RNA extraction kits (manual method) each from two different suppliers (blue and green line). Eluted RNA templates were synthesized to cDNA with reverse transcription; and then analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96.

- Red line: GeneAll® Exgene™ Viral DNA/RNA
- Blue line: viral DNA/RNA extraction kit (supplier A)
- Green line: viral DNA/RNA extraction kit (supplier B)
- Real-time PCR system: CFX-96™ System (1855201)
- RT-qPCR kit: GeneAll® HyperScript™ 2X One-step RT-PCR Master Mix (602-110)
- Target gene: none specific

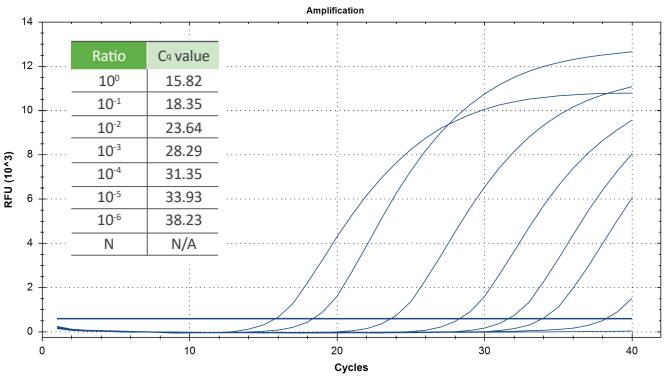

Figure 4. Analysis of extraction sensitivity on serial dilutions of Infectious bronchitis virus (IBV).
The RNA template were extracted from a 10-fold serial dilution of Infectious bronchitis virus (IBV) using Exgene™ Viral DNA/RNA. All elutes were analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96.

- N: negative control (nuclease-free water)
- Real-time PCR system: CFX-96™ System (1855201)
- RT-qPCR kit: GeneAll® HyperScript™ 2X One-step RT-PCR Master Mix (602-110)
- Target gene: none specific

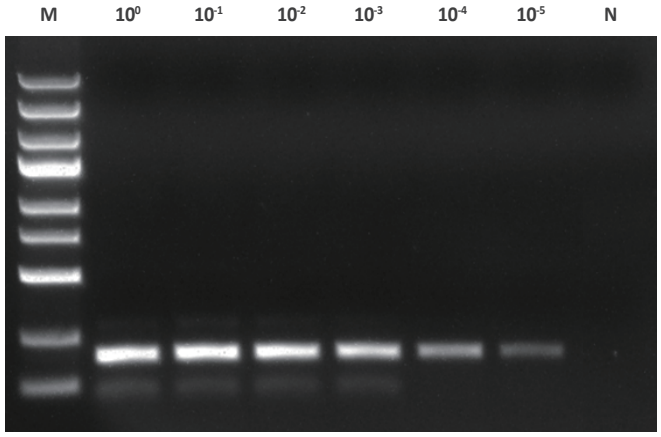

Figure 5. Analysis of extraction sensitivity on dilutions of Rabies virus (RV).
The RNA template were extracted from a 10-fold serial dilution from 10^0 to 10^{-5} of known positive Rabies virus (RV) samples using Exgene™ Viral DNA/RNA. All eluates were analyzed using conventional reverse transcription PCR (one-step RT-PCR) assay.

- M: GeneAll® GENESTA™ 250 bp DNA ladder (GA-025)
- N: negative control (nuclease-free water)
- Target gene (PCR product size): Jecom (100 bp)
- PCR system: MultiGene™ Optimax thermal cycler (TC9610, supplier L)
- RT-PCR kit: GeneAll® HyperScript™ One-Step RT-PCR Master Mix, 0.5 ml x 2 (602-125)
- Electrophoresis conditions: 1.2% agarose, 110 V, 30 min, 10 µl

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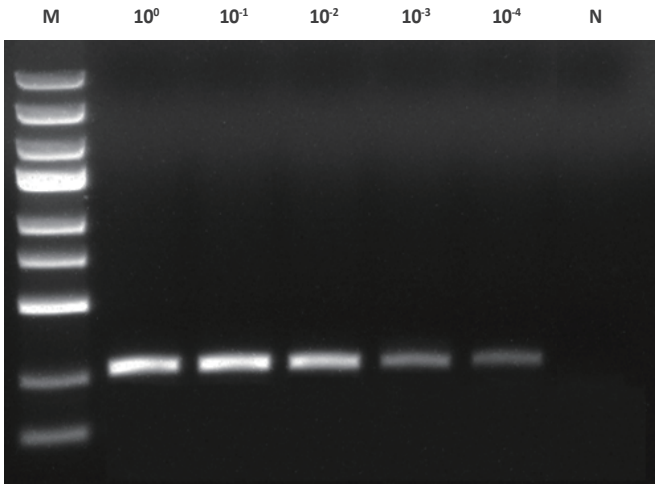

Figure 6. Analysis of extraction sensitivity on dilutions of Japanese encephalitis virus (JEV).
The RNA template were extracted from a 10-fold serial dilution from 10^0 to 10^{-4} of known positive Japanese encephalitis virus (JEV) samples using Exgene™ Viral DNA/RNA. All eluates were analyzed using conventional reverse transcription PCR (one-step RT-PCR) assay.

- M: GENESTA™ 250 bp DNA ladder (GA-025)
- N: negative control (nuclease-free water)
- Target gene (PCR product size): omRABV (192 bp)
- PCR system: MultiGene™ Optimax thermal cycler (TC9610, supplier L)
- RT-PCR kit: GeneAll® HyperScript™ 2X One-step RT-PCR Master Mix (602-110)
- Electrophoresis conditions: 1.2% agarose, 110V , 30 min, 10 µl