

Comparison of Automatic Nucleic Acid Extraction Kit and Manual Nucleic Acid Extraction Kit from Buccal Swab of Pathogen-infected Rooster

Experimental Conditions

Materials Required

- ♦ GENTi™³² Advanced Automatic Extraction System (GTI032A)
- ♦ GENTi™ Advanced Viral DNA/RNA Extraction Kit (902-096A)
- ♦ Extraction kit of manual type from two suppliers
- ♦ Cotton swab
- ♦ 1X PBS (Phosphate-buffered saline)
- ♦ Pipette & sterile pipette tips
- ♦ Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

- ♦ Sample type : Buccal swab of rooster infected with pathogen
 - Viral RNA : Infectious bronchitis virus (IBV)
 - Bacterial DNA : *Mycoplasma gallisepticum* (MG)
- ♦ Extraction conditions
 - Sample amount : 200 µl
 - Elution volume : 100 µl
 - Extraction protocol : Viral_Normal (operation time : 29' 35")

Sample Preparation

1. Collect the samples by rubbing the inside of the mouth of the pathogen-infected rooster with a cotton swab.
2. Transfer the cotton swabs to each 5 ml conical tube with 3 ml PBS and clip off the handle of the brush with a sterile sharp blade or medical scissors.
3. Vortex for 1 min and the next step is according to **GENTi™ Advanced Viral DNA/ RNA Extraction Kit protocol**.

Protocol

GENTi™ Advanced Viral DNA/RNA Extraction Kit protocol

* For more details and methods, please refer to [the handbook of GENTi™ Advanced Viral DNA/RNA Extraction Kit](#).

1. Add 7 µl of dissolved Carrier RNA (1 µg/µl) to 1st/7th well of plate type cartridge.
2. Add 200 µl of samples to 1st/7th well.
3. Load the plate type cartridge on the tray of GENTi™³² Advanced Automatic Extraction System.
4. Insert the magnetic rod cover to the end to strip bracket.
5. Select the correct extraction protocol and operate the extraction system.

Result

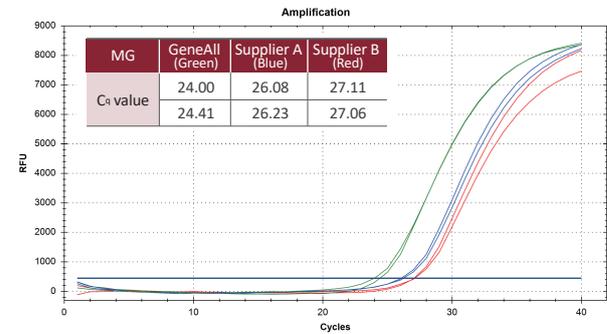


Figure 1. Real-time PCR performance comparing the C_t values of *Mycoplasma gallisepticum* extracted from buccal swab using kits from different manufacturers. Bacterial DNA were extracted from buccal swab taken from rooster infected with *Mycoplasma gallisepticum* (MG) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™³² Advanced Automatic Extraction System and kits (manual type) from supplier A and supplier B. Eluted DNA templates were analyzed with a TaqMan-based real-time PCR assay using CFX-96. Real-time PCR with each of the DNA eluate was performed in duplicate.

- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
- Target gene : None specific

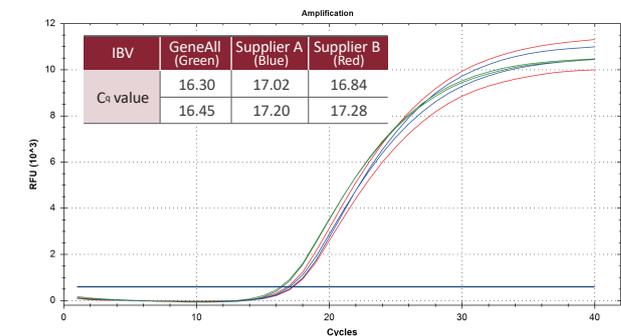


Figure 2. Real-time PCR performance comparing the C_t values of Infectious bronchitis virus extracted from buccal swab using kits from different manufacturers.

Viral RNA were extracted from buccal swab taken from rooster infected with Infectious bronchitis virus (IBV) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™³² Advanced Automatic Extraction System and kits (manual type) from supplier A and supplier B. Eluted RNA templates were synthesized to cDNA with reverse transcription; and then analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96. RT-qPCR with each of the synthesized cDNA was performed in duplicate.

- PCR instrument : CFX-96 (1855201)
- RT-qPCR kit : 2X 1 Step RT-qPCR Master Mix (for probe) (QRT1-XV-100R)
- Target gene : None specific