SHIMADZU

Application Notes

DNA/ RNA Microchip Electrophoresis System (MultiNA)

H1N1 virus detection using Seeplex® FluA ACE subtyping kit

Introduction

H1N1 is currently the most striking issue in the public mind. Unlike seasonal influenza, H1N1 virus as the subtype of influenza A was classified by World Health Organization (WHO) as pandemic influenza as it has caused sustained community level outbreaks in multiple parts of the world**.** To overcome this issue, it is necessary to detect the exact subtypes of influenza A virus rapidly and reliably for the appropriate medicine to be given at early stage. Here we report on the application of MCE-202 (MultiNA) in combination with the Seeplex ® FluA ACE subtyping kit to detect five different influenza virus A using DPO™ (Dual Priming Oligonucleotide) based multiplex PCR method (Seeplex® PCR).

Materials

DNA-1000 Reagent Kit from Shimadzu Corporation, Seeplex® FluA ACE Subtyping kit reagent from Seegene Corporation, SYBR® Gold nucleic acid gel stain from Invitrogen Corporation, DNA 1000 ladder (phiX174DNA-HaeIII digest) from Promega and TE buffer from Nacalai Tesque

Method

Specimen Collection (e.g.: Nasopharyngeal swab)

Nucleic acid isolation and Reverse transcription

DPO based multiplex PCR amplification

Electrophoresis of PCR samples on MultiNA

Results & Discussions

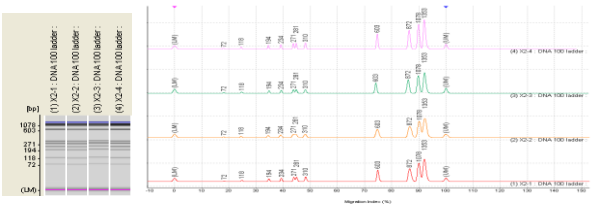

Fig. 1 Gel images and elecropherograms of phiX174DNA-HaeIII digest (Promega), conc. 10ng/µL

Figure 1 showed reproducibility of MultiNA separation using phiX174DNA-HaeIII digest as ladder (multiple injections) with DNA-1000 kit. The internal upper and lower markers virtually eliminate chip to chip variation.

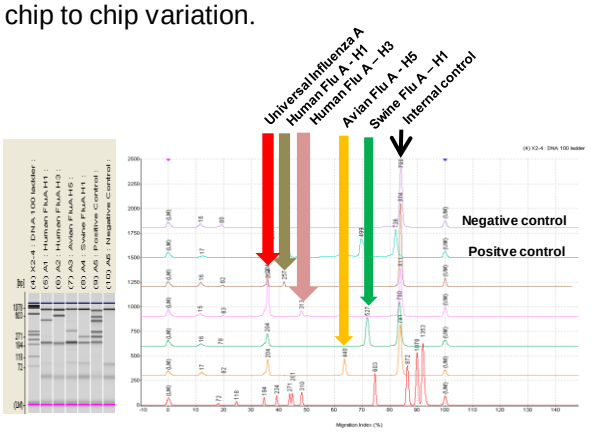

Fig. 2 Gel images and electropherograms of multiplex PCR products of five different influenza A virus using Seeplex® FluA ACE subtyping kit. DNA plasmid was used as the internal control. phiX174DNA-HaeIII digest (Promega) was used as the ladder.

Figure 2 showed that using the Seeplex/MultiNA, a panel of four different influenza virus A and one human common influenza virus were clearly detected and identified according to their subtyping, for example: 527 bp from Human FluA-H3, 440 bp from Human FluA-H1, 313 bp from Avian FluA-H5, 257 bp from Swine FluA-H1 and 204 bp from Universal Influenza A.

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