Certificate of Analysis for NR-29027

Influenza A Virus, A/Puerto Rico/8-9VMC2/1934 (H1N1)[1]

Catalog No. NR-29027

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs[2] infected with influenza A virus, A/Puerto Rico/8-9VMC2/1934 (H1N1)

Lot[3]: 60300875 **Manufacturing Date: 06OCT2011**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs[2]		
Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species- and Strain-Specific Regions		
Hemagglutinin (947 nucleotides)	Consistent with A/Puerto Rico/8-9VMC2/1934 (H1N1) (GenBank: CY083990)	Identical to A/Puerto Rico/8-9VMC2/1934 (H1N1) (GenBank: CY083990)[4]
Matrix gene (885 nucleotides)	Consistent with A/Puerto Rico/8-9VMC2/1934 (H1N1) (GenBank: CY083991)	Identical to A/Puerto Rico/8-9VMC2/1934 (H1N1) (GenBank: CY083991)
Titer by $CEID_{50}$ Assay[5,6] in Embryonated Chicken Eggs[2]	Report results	1.6×10^{10} $CEID_{50}$/mL
Sterility (21-day incubation)		
Harpo's HTYE broth[7], 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]Deposited and labeled as influenza A virus, A/Puerto Rico/8-34-9VMC2/2010 (H1N1)
[2]10- to 11-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania
[3]Grown in the allantoic cavity of embryonated chicken eggs[2] for 2 days at 35°C in a humidified chamber
[4]Nucleotide sequencing of this region of the hemagglutinin gene of NR-29027 confirmed the presence of the point mutation responsible for the E246G substitution.
[5]The Chicken Embryo Infectious Dose 50% ($CEID_{50}$) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the inoculated embryonated chicken eggs, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $CEID_{50}$ provides a measure of the infectious titer (or infectivity) of a virus preparation.
[6]2 days at 35°C in a humidified chamber
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 07 JUN 2012 **Signature:** Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

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