

Certificate of Analysis for NR-28621

Influenza A Virus, A/Hong Kong/1/1968-1 Mouse-Adapted 12 (H3N2)

Catalog No. NR-28621

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A virus, A/Hong Kong/1/1968-1 mouse-adapted 12 (H3N2)

Passage History: MKX/MK2E2/E1M2ML12M2E2/E1 (Isolating laboratory/WHO World Influenza Centre, London/Laboratory CDC, Health Canada, Ottawa/BEI); E# = Number passages in eggs; M# = Number passages in MDCK cells; MK# = Number passages in monkey kidney cells; ML# = Number passages in mouse lung; X = unknown

Lot²: 62097495 Manufacturing Date: 16OCT2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (695 nucleotides) Matrix (942 nucleotides)	Consistent with A/Hong Kong/1- 1-MA-12/1968 (H3N2) ³ Consistent with A/Hong Kong/1- 1-MA-12/1968 (H3N2) ³	100% identity with A/Hong Kong/1-1-MA-12/1968 (H3N2) (GenBank: CY033505) ³ 99% identity with A/Hong Kong/1-1-MA-12/1968 (H3N2) (GenBank: CY033506) ³
Strain Identification by Sequencing of PB2 Coding Region	Identity confirmed ⁴	Identity confirmed ⁴
Titer by CEID ₅₀ Assay ^{5,6} in Embryonated Chicken Eggs ¹	Report results	8.9 × 10 ⁸ CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic 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) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania ²Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber

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³NR-28621 was deposited to BEI Resources as A/Hong Kong/1/1968-1 mouse-adapted 12 (H3N2), but nucleotide sequence obtained from the same source material by the NIAID Influenza Genome Sequencing Consortium was deposited to NCBI and IRD as A/Hong Kong/1-1-MA-12/1968 (H3N2).

⁴The PB2 gene (RNA segment 1) of influenza A/Hong Kong/1-1-MA-12/1968 (H3N2) (GenBank: CY033512) contains a G to A transition at nucleotide 1587 relative to the parental wild type A/Hong Kong/1/1968 (H3N2) virus. This point mutation is unique to this isolate and serves to confirm the identity of the strain.

⁵The Chicken Embryo Infectious Dose 50% (CEID₅₀) 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the CEID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁶2 days at 35°C in a humidified chamber

⁷Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Date: 22 APR 2014 Signature: Millar Q. Gyylla

Title: Technical Manager, BEI Authentication or designee

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