

Certificate of Analysis for NR-3522

Kilbourne F54: A/turkey/Massachusetts/3740/1975 (HA) x A/Cambridge/1946 (NA) x A/Puerto Rico/8/1934 (H6N1)

Catalog No. NR-3522

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/turkey/Massachusetts/3740/1975 (HA) x A/Cambridge/1946 (NA) x A/Puerto Rico/8/1934 (H6N1)

Lot^{2,3}: 61001983 Manufacturing Date: 31MAY2012

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, Matrix, and Neuraminidase Coding Regions		
Hemagglutinin (280 nucleotides)	Consistent with A/turkey/Massachusetts/3740/ 1965 (H6N2) ⁴	99% identity with A/turkey/Massachusetts/3740/ 1965 (H6N2) ⁴ (GenBank: CY087752)
Matrix (913 nucleotides)	Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY105897)
Neuraminidase (638 nucleotides)	Consistent with A/Cameron/1946 (H1N1) ⁵	99% identity with A/Cameron/1946 (H1N1) ⁵ (GenBank: CY009598)
Titer by CEID ₅₀ Assay ^{6,7} in Embryonated Chicken Eggs ¹	Report results	8.9×10^8 CEID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth ⁸ , 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

¹9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

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²Derived from NIAID Catalog No. V-331-0E5034

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber

⁴There is no sequence information for influenza A virus, A/turkey/Massachusetts/3740/1975 (H6N2) in the NCBI database or any of the various influenza databases. The hemagglutinin gene sequence obtained for NR-3522 is 99% identical to the published sequence of influenza A virus, A/turkey/Massachusetts/3740/1965 (H6N2). A review of the literature suggests that these two strain designations likely represent the same virus isolate, and that the progenitor of this reassortant was originally isolated in 1965.

⁵There is no sequence information for influenza A virus, A/Cambridge/1946 (H1N1) in the NCBI database or any of the various influenza databases. The neuraminidase gene sequence obtained for NR-3522 is 99% identical to the published sequence of influenza A virus, A/Cameron/1946 (H1N1). A review of the literature and the databases indicates that A/Cameron/1946 is synonymous with A/Cam/46, and likely represents the original "CAM" isolate of the 1947 influenza "A prime" epidemic strain.



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⁶The 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.

⁷2 days at 35°C in a humidified chamber

⁸Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 08 APR 2013 Signature:

Title: Technical Manager, BEI Authentication or designee

Michael Q. Comple

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