SUPPORTING INFECTIOUS DISEASE RESEARCH

Kilbourne F174: A/turkey/Massachusetts/3740/1975 (HA) x A/Taiwan/1/1986 (NA) x A/Puerto Rico/8/1934 (H6N1), Reassortant X-94

Catalog No. NR-3642

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/turkey/Massachusetts/3740/1975 hemagglutinin (HA) x A/Taiwan/1/1986 neuraminidase (NA) x A/Puerto Rico/8/1934 (H6N1)

Lot^{2,3}: 61664695

Manufacturing Date: 04APR2013

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 (299 nucleotides) Matrix (952 nucleotides)	Consistent with A/turkey/Massachusetts/3740/ 1965 (H6N2) ⁴ Report results ⁵	99% identity with A/turkey/Massachusetts/3740/ 1965 (H6N2) ⁴ (GenBank: CY087752) 99% identity with A/Taiwan/1/1986 (H1N1) ⁵ (GenBank: DQ508876)
Titer by CEID ₅₀ Assay ^{6,7} in Embryonated Chicken Eggs ¹	Report results	5.0×10^8 CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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

²Derived from NIAID Catalog No. V-331-0E5528

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-3642 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.

⁵Except for the HA and NA genes, the origin of the genome segments in reassortant X-94 had not been determined prior to deposit. Although the matrix gene (RNA 7) of high yield reassortant influenza A viruses is usually donated by the A/Puerto Rico/8/1934 (H1N1) parent, the finding that this RNA segment of NR-3642 is derived from the A/Taiwan/1/1986 (H1N1) parent is not entirely unexpected. However, based on this result and additional sequencing data, it is possible that NR-3642 does not carry any A/Puerto Rico/8/1934 (H1N1)-derived genome segments.

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

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Certificate of Analysis for NR-3642

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Date: 23 JUL 2013

Signature: Michael Q. Cmla

Title:

Technical Manager, BEI Authentication or designee

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