

Certificate of Analysis for NR-28614

Influenza A Virus, A/duck/Czechoslovakia/1956 (H4N6) (uncloned)

Catalog No. NR-28614

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A virus, A/duck/Czechoslovakia/1956 (H4N6)

Lot²: 61660346 Manufacturing Date: 19APR2013

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 (751 nucleotides) Matrix (954 nucleotides)	Consistent with A/duck/Czech Republic/1956 (H4N6) ³ Consistent with A/duck/Czech	99% identity with A/duck/Czech Republic/1956 (H4N6) (GenBank: CY045327) ³ 99% identity with A/duck/Czech
,	Republic/1956 (H4N6) ³	Republic/1956 (H4N6) (GenBank: CY045328) ³
Titer by CEID ₅₀ Assay ^{3,4} in Embryonated Chicken Eggs ¹	Report results	1.4×10^8 CEID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth ⁵ , 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

Date: 06 AUG 2013 Signature:

Title: Technical ivianager, bel Authentication of designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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²Grown in the allantoic cavity of embryonated chicken eggs¹ for 3 days at 34°C in a humidified chamber

³NR-28614 was deposited to BEI Resources as A/duck/Czechoslovakia/1956 (H4N6), but nucleotide sequence obtained from the same source material by the NIAID Influenza Genome Sequencing Consortium was deposited to NCBI as A/duck/Czech Republic/1/1956 (H4N6). Comparison of several published nucleotide sequences indicates that the two strain designations can be considered synonymous.

⁴The 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.

⁵³ days at 34°C in a humidified chamber

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.