

Certificate of Analysis for NR-3467

Kilbourne F6: A/New Jersey/10/76 (H1N1) Mutant, High (Hx) Yield

Catalog No. NR-3467

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with a high (hx) yield mutant (Kilbourne F6) of influenza A virus, A/New Jersey/10/76 (H1N1).

Lot^{2,3}: 58405661 Manufacturing Date: 31OCT2008

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 Species-Specific Region (~ 450 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	2.8 X 10 ⁸ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA ⁶	~ 470 bp amplicon	~ 470 bp amplicon
Sterility (BacT/ALERT® 3D Microbial Detection System) 14-day incubation of NR-3467: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic 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 Sheep blood agar, 37°C, aerobic Sheep 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

¹10 to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 22 JAN 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E3914

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

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.

⁵2 days at 35°C in a humidified chamber

⁶The primers are described in Lee, M.-S., et al. "Identification and Subtyping of Avian Influenza Viruses by Reverse Transcription-PCR." <u>J. Virol. Methods</u> 97 (2001): 13-22. PubMed: 11483213.

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