Certificate of Analysis for NR-3465

Kilbourne F19: A/swine/Nevada/101/82 (H1N1) Mutant, High (H) Yield (Animal Isolate)

Catalog No. NR-3465

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs[1] infected with a high (H) yield mutant (Kilbourne F19) of influenza A virus, A/swine/Nevada/101/82 (H1N1).

Lot[2,3]: 58411916 **Manufacturing Date: 21NOV2008**

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 Hemagglutinin gene (~ 450 nucleotides) Matrix (M) gene (~ 920 nucleotides)	 Influenza A virus Influenza A virus	 Influenza A virus Influenza A virus
Titer by $CEID_{50}$ Assay[4,5] in Embryonated Chicken Eggs[1]	Report results	2.8 X 10^9 $CEID_{50}$/mL
RT-PCR Assay of Extracted RNA[6] Hemagglutinin gene Matrix gene	 ~ 470 bp amplicon ~ 1030 bp amplicon	 ~ 470 bp amplicon ~ 1030 bp amplicon
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 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 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

[1]10 to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania
[2]Derived from NIAID Catalog No. V-331-0E4011
[3]Grown in the allantoic cavity of embryonated chicken eggs[1] for 2 days at 35°C in a humidified chamber
[4]The Chicken Embryo Infectious Dose 50% ($CEID_{50}$) 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $CEID_{50}$ provides a measure of the infectious titer (or infectivity) of a virus preparation.
[5]2 days at 35°C in a humidified chamber
[6]The H1 gene primers are described in Lee, M.-S., et al. "Identification and Subtyping of Avian Influenza Viruses by Reverse Transcription-PCR." J. Virol. Methods 97 (2001): 13-22. PubMed: 11483213; the M gene primers are described in Hoffmann, E., et al. "Universal Primer Set for the Full-Length Amplification of All Influenza A Viruses." Arch. Virol. 146 (2001): 2275-2289. PubMed: 11811679.
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 13 FEB 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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Biodefense and Emerging Infections Research Resources Repository
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
800-359-7370
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