Certificate of Analysis for NR-3172

Influenza A Virus, A/Japan/170/62 (H2N2)

Catalog No. NR-3172

This reagent is the tangible property of the U.S. Government.

Product Description: Pooled allantoic fluid from embryonated chicken eggs infected with influenza A virus, A/Japan/170/62 (H2N2).

Lot: V301-041-000 **Manufacturing Date: 15AUG1963**

TEST	SPECIFICATIONS	RESULTS (AUG2008)
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 (~ 236 nucleotides)	Consistent with influenza A virus	Consistent with influenza A virus
Titer by $CEID_{50}$ Assay[2,3] **in Embryonated Chicken Eggs**[1]	Report results	2.81 X 10^6 $CEID_{50}$/mL
Functional Activity by RT-PCR Assay[4,5]	~ 1030 bp amplicon	~ 1030 bp amplicon ~ 500 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth[6], 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]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.
[3]48 hours at 35°C in a humidified chamber
[4]BM-M1 and BM-M-1027R primers; Obenauer, J. C., et al. "Large-Scale Sequence Analysis of Avian Influenza Isolates." Science 311 (2006): 1576-1580. PubMed: 16439620.
[5]RT-PCR of NR-3172 produced 2 bands, one of the expected size (~1030 bp), in addition to a second band at ~ 500 bp.
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 15 SEP 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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